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Effects of salinity and adult extract on settlement of the oligohaline barnacle *Balanus subalbidus*

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Abstract *Balanus subalbidus* (Henry) has the most oligohaline distribution of three congeneric barnacles in Chesapeake Bay and tolerates prolonged exposure to fresh water. We studied larval settlement (i.e., permanent attachment and metamorphosis) of *B. subalbidus* in the laboratory, over a 3 yr period, May 1989 to March 1992, under the following conditions: (1) across an array of salinities at 25°C in the presence and absence of settlement factor consisting of adult *B. subalbidus* extract; (2) in the presence of conspecific or congeneric settlement factors; and (3) cyprids which were, and were not, induced to delay metamorphosis were compared in their capacities to settle in a range of salinities. Discrepancies between salinity profiles of larval settlement in the laboratory and adult oligohaline distribution in the estuary were striking, and there was a significant interaction between salinity and settlement factor. Averaging results of four different batches of larvae, although peak settlement (87±9%) of *B. subalbidus* occurred at 2 ppt salinity in the presence of adult cue, substantial settlement also occurred at higher salinities: >70% at 5, 10 and 15 ppt; and 47% at 20 and 25 ppt. In addition, settlement in the absence of settlement factor was relatively high (>50%) and peaked at mid-salinity ranges (e.g. 56±10% at 15 ppt). Variation observed in settlement among larval batches reflected detailed differences in settlement between adjacent test salinities. No difference in settlement occurred between replicate aliquots of cyprids within a batch. Cyprids of *B. subalbidus* settled most abundantly in the presence of settlement factor extracted from conspecifics, followed in decreasing order by settlement factor extracted from *B. improvisus* and *B. eburneus*. Delay of metamorphosis produced by keeping *B. subalbidus*

cyprids for 8 d at 5°C resulted in a decreased level of settlement, but settlement frequency patterns of delayed and non-delayed cyprids were indistinguishable relative to salinity. These results indicate that the oligohaline distribution of adult *B. subalbidus* is probably not determined by larval behavior at settlement. We suggest that pre-settlement behavior, resulting in larval retention in low saline waters, could be an important factor in determining distribution of this species.

Introduction

The significance of larval behavior in determining spatial distributions of sessile marine invertebrates is receiving increasing attention (Keough and Downes 1982; Caffey 1985, review by Connell 1985; Roughgarden et al. 1985; Underwood and Fairweather 1989; Minchinton and Scheibling 1991). For larval barnacles in particular, pre-settlement phenomena such as retention mechanisms ((Bousfield 1955), location in the water column (Grosberg 1982; Shanks 1986), relative supply (Gaines et al. 1985; Roughgarden et al. 1988) and predation on the larvae (Gaines and Roughgarden 1987) can have direct effects on spatial patterns of settlement. Field studies of barnacle settlement have demonstrated non-random responses to chemical cues (Strathmann et al. 1981; Bushek 1988; Raimondi 1988; Johnson and Strathmann 1989), physical cues (Crisp and Barnes 1954; Wethey 1986; Chabot and Bourget 1988) or both (Hudon and Bourget 1983; Le Tournex and Bourget 1988; but see Strathmann et al. 1981).

After the fundamental concept of gregarious settlement of cypris larvae in response of proteinaceous "settlement factor" extracted from adults had been established (Knight-Jones 1953; Crisp and Meadows 1962), larval biologists studied barnacle settlement under controlled laboratory conditions from several perspectives. These included: stimulation by purified extract, cyprid "footprints", biochemically fractionated barnacle odor and bacterial films

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(Larmon and Gabbott 1975; Rittschof 1985; Yule and Walker 1985; Maki et al. 1990); inhibition by natural products as well as bacterial films (Standing et al. 1984; Rittschof et al. 1985, 1986a; Maki et al. 1988, 1990); and strength of temporary adhesion (Crisp 1955; Walker and Yule 1984; Yule and Walker 1985; Eckman et al. 1990). In our laboratory, we have been assessing larval settlement of three congeneric barnacles (*Balanus eburneus*, *B. improvisus*, and *B. subalbidus*) distributed along an estuarine gradient in Chesapeake Bay by examining the combined effects of adult settlement factor and salinity (an environmental variable that might affect settlement). Ionic stimulation has been shown to induce metamorphosis in some invertebrates, but not in barnacles (Rittschof et al. 1986b). In a recent study of the interaction between salinity and adult chemical cue in the mesohaline barnacle, *B. improvisus*, we demonstrated that cyprids of this species settled in a salinity regime corresponding to the adult field distribution (Dineen and Hines 1992). We also demonstrated that other factors, such as settlement factor from congeneric species, and delay of metamorphosis could influence larval settlement of *B. improvisus*. Here, we present results of a parallel laboratory study of another estuarine barnacle, *B. subalbidus*, which we undertook to determine the responses of larval settlement to these factors in correspondence with adult distribution in the field.

The interactive effects of environmental cues upon larval settlement may be particularly critical in low salinity zones of estuaries, where the cues may be distributed along steep horizontal and vertical gradients, conditions fluctuate rapidly, and prevailing surface currents are likely to carry larvae away from suitable habitats. There are few experimental studies of the larval biology of invertebrates which are restricted to the low salinity zones of estuaries (but see Bousfield 1955; Cain 1972; Hopkins et al. 1973; Cronin 1982). *Balanus subalbidus*, a member of the *Balanus-amphitrite* complex, was previously referred to as *Balanus amphitrite pallidus* until designated a new species (Henry 1973). This barnacle is characteristically found in oligohaline waters of the eastern coastal United States from Massachusetts to Florida, the Gulf of Mexico, and has also been reported from Trinidad (Henry and McLaughlin 1975) and tolerates prolonged exposure to fresh water (Poirrier and Partridge 1979; Dineen personal observation). Within its geographic range, *B. subalbidus* is the only barnacle occurring at salinities of 0.1 to 3.5 ppt, and at sites of increasing salinity the size and abundance of *B. subalbidus* decreases (Poirrier and Partridge 1979; Dineen personal observation). On oyster beds in Chesapeake Bay, *B. subalbidus* was found at salinities ranging from 0.8 to 17.9 ppt, but was most abundant in low salinity waters in upper tributaries and upper bay locations (Kennedy and DiCosimo 1983). The latter two studies also both indicate that adults of *B. subalbidus*, *B. improvisus* and *B. eburneus* are distributed sequentially along a horizontal gradient of increasing salinity. Other than morphological descriptions of the naupliar stages of *B. subalbidus* (Lang 1979), the larval biology of this low-salinity species has not been studied previously.

Materials and methods

Adult barnacles

Adult *Balanus subalbidus* (Henry) were obtained from submerged tree branches in the Middle River (upper Chesapeake Bay), near Baltimore, Maryland, and from settlement plates deployed in Muddy Creek, the primary fresh water tributary to the Rhode River, a sub-estuary of central Chesapeake Bay, in Edgewater, Maryland. The latter site has a seasonal salinity range of 0 to 14 ppt, and *B. subalbidus* was the only barnacle to settle on plates deployed at this location from 1989 to 1991. If not ovigerous at the time of sampling, adult barnacles were induced to breed in the laboratory by supplemental food [*Artemia* sp. and phytoplankton, *Isochrysis galbana* (Tahitian strain)] and a warm temperature (25 °C) for approximately 4 to 6 wk, thereby providing larvae out of the normal breeding season. Experiments commenced in the spring of 1989 and continued through the spring of 1992. *B. subalbidus* was induced to breed aseasonally in the laboratory on three occasions: February 1990, early April 1991, and March 1992. *B. improvisus* and *B. eburneus*, used in the preparation of congeneric settlement factor, were obtained, respectively, from the Rhode River and the York River, Gloucester Point, Virginia.

Larval cultures

Nauplii were either obtained directly by crushing adults that contained ripe egg masses, or by removing egg masses and incubating them in 0.1- μ m filtered estuarine water (4 to 5 ppt) for a few days until hatching. Nauplii were placed in 4 liters of aerated, 0.1- μ m filtered estuarine water (4 to 5 ppt salinity) at 25 °C and fed daily on a mixed algal diet of *Isochrysis galbana* (Tahitian strain) and *Phaeodactylum tricornutum*. Sodium Penicillin G (21.9 mg l⁻¹) and Streptomycin sulphate (36.5 mg l⁻¹) were used as antibiotics. Cyprids developed in 5 to 7 d and were collected on a 200- μ m mesh nylon sieve.

Settlement experiments

Settlement factor

Conspecific (*Balanus subalbidus*) and congeneric (*B. improvisus* and *B. eburneus*) settlement factors were prepared by crushing whole adult barnacles in distilled water, removing solids by centrifugation, and boiling the resulting supernate. The crude extract was applied to slate substrata at a concentration of 50 μ g protein ml⁻¹ (determined using the BCA™ Protein Assay, Pierce, Inc.) (Rittschof et al. 1984; Dineen and Hines 1992).

Settlement assay procedures

Depending on the assay, 100 or 200 cyprids were placed in polystyrene dishes (4 cm deep, 10 cm diameter) containing 0.1- μ m filtered estuarine water at a salinity similar to the larval culture (4 to 5 ppt). Slate substrata (3 \times 6.5 cm) were held in coarsely filtered estuarine water (4 to 5 ppt) for 24 h prior to an experiment and were either adsorbed with settlement factor or untreated, and placed in dishes prior to introducing larvae. All experiments were conducted at 24 to 25 °C on a 15 h light:9 h dark schedule. Substrata were examined for metamorphosed cyprids at 24-h intervals for 144 h. Three sets of experiments were conducted to assess: (1) the interactive effects of salinity and adult chemical cue; (2) the taxonomic specificity of adult chemical cue; and (3) the effects of delaying metamorphosis.

Salinity and adult chemical cue

After larvae were placed into settlement assay dishes, salinity was adjusted sequentially, over a period of 6 h, with either distilled water or a saline solution (40 ppt) to produce eight salinities (2, 5, 10, 15, 20, 25, 30, and 35 ppt) in combination with and without settlement factor for a total of 16 treatments. (Although this is a rapid adjustment for those larvae going through the extremes of the salinity range, it is an ecologically relevant adjustment for estuarine larvae which may migrate vertically through a pycnocline over the time course of a tidal cycle during spring when low surface salinities over-

lay a salinity wedge on the bottom.) No settlement or mortality occurred during the salinity adjustment period, and cyprid behavior was normal. Final volume in all settlement culture dishes was approximately 300 ml, and the experimental trials began after the adjustment period. This experiment was conducted on four different batches of larvae. For two of these batches, cyprids were partitioned into two replicate aliquots. To test for variation in settlement within a batch of larvae, we compared effects of salinity and settlement factor treatments among aliquots of cyprids. Each aliquot was then partitioned into the 16 treatments described above, and the frequencies of settled barnacles after 144 h were compared using log-linear models (see below) comparing main effects of replicate and salinity-settlement factor treatment, as well as interaction effects. To test for differences in settlement among batches of larvae, we compared effects of salinity and settlement factor treatments among batches of cyprids. For each of the four separately cultured batches of larvae (including the two batches cultured for the test of replicated aliquots above), we partitioned the larvae into the 16 treatments. As before, frequencies of settled barnacles after 144 h were compared using log-linear models comparing main effects of batch and treatment, as well as interaction effects. Each of the 96 trials (i.e., 16 treatments times six batches/aliquots) received 200 cyprids.

Congeneric conspecific chemical cue

Slate substrata adsorbed with settlement factor extracted from *Balanus subalbidus*, *B. improvisus*, or *B. eburneus*, or untreated were placed in assay dishes as above at a salinity of 5 ppt. This resulted in four treatments which were replicated three times, 100 cyprids per trial.

Metamorphic delay

Balanus subalbidus cyprids from the same larval cohort were divided into two groups, one of which was used in settlement assays the following day, while the other was induced to delay metamorphosis by placing larvae in 450 ml of filter-sterilized 5-ppt river water, slowly cooling to 5 °C and storing in the dark for 8 d. Prior to the experiment, larvae were gradually warmed to room temperature over a 2-h period. Settlement assays were then conducted as above, at four salinities (5, 15, 25, and 35 ppt) with substrata adsorbed with conspecific settlement factor, replicated three times, 100 cyprids per trial.

Statistical methods

Data were treated categorically. Logistic regression analysis (PROC CATMOD with maximum likelihood estimation, SAS Institute 1985) was used to analyze settlement frequencies in experiments investigating: (1) the effects of salinity, the presence and absence of conspecific settlement factor and their interaction; as well as (2) the effects of salinity, metamorphic delay and their interaction. Settlement frequencies in the presence of conspecific and congeneric settlement factor were compared using two-way contingency tables. Unplanned multiple comparisons controlling for experiment-wise Type I error were subsequently used to distinguish differences among frequencies [Simultaneous Test Procedures (STP), Sokal and Rohlf 1981, p 728].

Results

The time course of cypris settlement followed a similar pattern in all of the experimental trials (Fig. 1). Little or no settlement occurred in the first 24 h; about 50 per cent of maximum settlement occurred within 72 h (slightly slower in the absence of settlement factor); an asymptotic level of maximal settlement was approached at 120 h; and no further settlement occurred after 144 h. Maximum set-

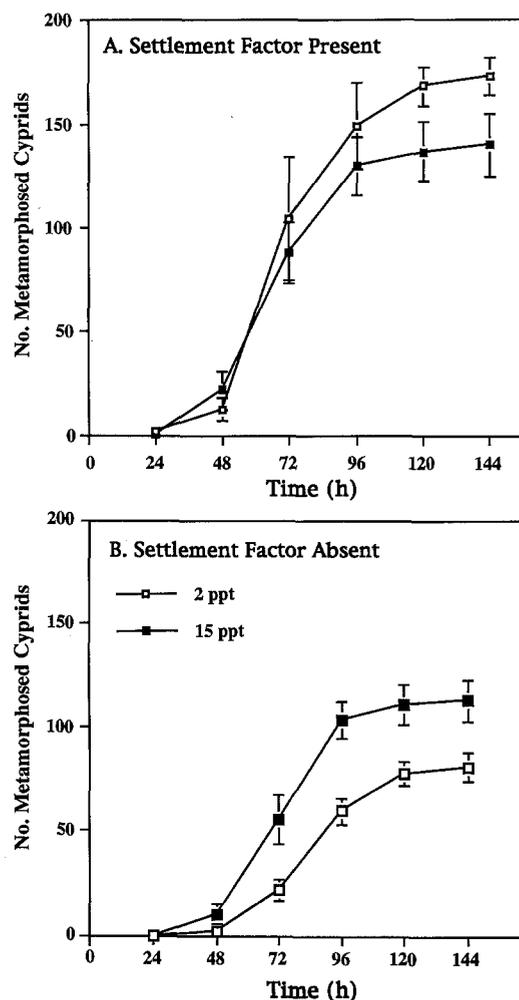


Fig. 1 *Balanus subalbidus*. Time course of cypris settlement in the **A** presence and **B** absence of settlement factor at 2 (□) and 15 (■) ppt. Values plotted are means (\pm SE) or four batches of larvae, 200 cyprids per trial. Values at 144 h are extracted from data presented in Fig. 2 C

tlement levels ranged from $95\pm 2\%$ in the congeneric extract experiment, to $87\pm 10\%$ in the optimal treatment of the salinity \times settlement factor experiment, and to $41\pm 5\%$ in the metamorphic delay experiment (see below). While maximum settlement levels also varied significantly among treatments within experiments (Fig. 1 and see below), this pattern of asymptotic settlement at 144 h was observed for all treatments in all three experiments.

Mortality of non-metamorphosed cyprids was low in all experiments. Typically not more than 10 per cent mortality of cyprids occurred after 144 h in each experiment, even those in which maximal settlement was relatively low. Mortality in the salinity \times settlement factor experiment was higher at the highest salinity levels than the optimum conditions, but variability in combination with low values does not allow us to draw conclusions. Larval mortality in the other two experiments did not exhibit any notable pattern among treatments. Thus, the majority of non-settled larvae were alive and swimming at the 144-h observation. Ac-

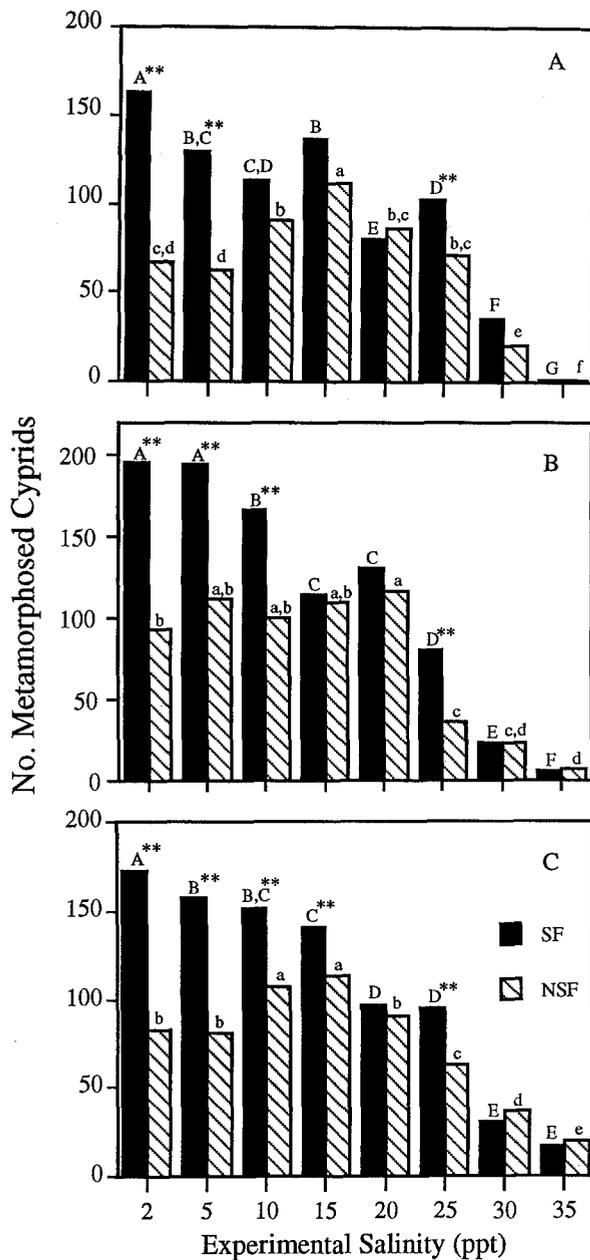


Fig. 2 *Balanus subalbidus*. A and B cypris settlement of two different batches of larvae and C average of four batches after 144 h in the presence (SF) and absence (NSF) of conspecific settlement factor across an array of salinities. Identical letters above bars indicate settlement frequencies that did not differ significantly (upper case letters for slate substrata treated with settlement factor, lower case letters for untreated substrata). Significant differences in settlement frequencies between settlement factor treatments within each salinity level designated as (**), $p < 0.01$. Bars indicate number of settled barnacles out of 200 cyprids per trial

cordingly, we report experimental results as number of cypris larvae which have settled, i.e., permanently attached and metamorphosed to juvenile barnacles.

When *Balanus subalbidus* cyprids were assayed for settlement in the presence and absence of settlement factor across an array of salinities, settlement frequencies did not differ significantly between replicate aliquots within

batches of cyprids (log-linear model, $\chi^2=2.8$, 15 df, $p > 0.09$), nor were there significant interaction effects of replicate \times treatment ($\chi^2=0.02$, 1 df, $p > 0.8$). Settlement frequencies did differ significantly among the four batches of larvae ($\chi^2=319.3$, 3 df, $p < 0.001$), and there were significant interaction effects of batch \times treatment ($\chi^2=207.0$, 15 df, $p < 0.001$). However, when we examined settlement in the presence and absence of settlement factor across the array of salinities, we observed similar patterns among batches (e.g. Fig. 2 A, B). For each batch of larvae, salinity and settlement factor had significant interactive effects upon settlement frequencies ($\chi^2 > 15$, 7 df, $p < 0.05$), such that settlement frequencies in the presence of settlement factor were highest at low salinities (2 to 10 ppt), while in the absence of settlement factor, frequencies were highest at mid salinities (10 to 20 ppt). The significant settlement differences among batches of larvae were explained by detailed differences in settlement frequencies between test salinities but not in basic pattern across the array of salinities (Fig. 2 A, B). Thus, we averaged settlement frequencies across all four batches (Fig. 2 C). In parallel with the analyses of each individual batch, settlement frequencies of the combined batches showed significant interactive effects of salinity and settlement factor (log-linear model, $\chi^2=485.5$, 7 df, $p < 0.001$).

Combined settlement frequencies differed significantly among salinities for substrata treated with settlement factor ($\chi^2=2901.0$, 7 df, $p < 0.001$) and for substrata without treatment factor ($\chi^2=1052.0$, 7 df, $p < 0.001$). Settlement frequencies in the presence of settlement factor were highest at 2 ppt (unplanned multiple comparisons, STP Tests; $\chi^2=18.5$, 7 df, $p < 0.01$) and decreased with increasing salinities. Settlement frequencies in the absence of settlement factor were significantly higher at mid-salinities (10 and 15 ppt), and settlement frequencies did not differ significantly among salinities of 2, 5 and 20 ppt in the absence of settlement factor (unplanned multiple comparisons, STP Tests; $\chi^2=18.5$, 7 df, $p < 0.01$). When settlement was compared within each salinity level, significantly higher settlement frequencies occurred in the presence of settlement factor at salinities of 2, 5, 10, 15 and 25 ppt (pairwise *G*-tests; $p < 0.001$, experiment-wise error rate=0.01, 7,1 df, $\chi^2=10.2$). No significant differences in settlement frequencies occurred at salinities of 20, 30 or 35 ppt with and without settlement factor.

Source of settlement factor (conspecific as opposed to congeneric) significantly influenced settlement frequency at 5 ppt salinity ($\chi^2=420.9$, 3 df, $p < 0.001$) (Fig. 3). Settlement frequencies of *Balanus subalbidus* were highest in the presence of conspecific settlement factor, followed in decreasing order by settlement factor extracted from *B. improvisus* and *B. eburneus*. No significant difference in settlement frequency was seen between slate substrata untreated or adsorbed with settlement factor extracted from *B. eburneus* (unplanned multiple comparisons, STP tests; $\chi^2=9.2$, 2 df, $p < 0.01$).

Settlement frequencies of *Balanus subalbidus* cyprids differed significantly among salinities for both non-delayed cyprids ($\chi^2=176.0$, 3 df, $p < 0.001$) and those which

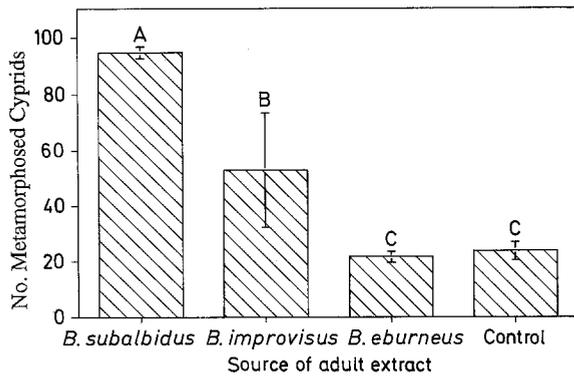


Fig. 3 *Balanus subalbidus*. Cypris settlement after 144 h in the presence of conspecific and congeneric settlement factors adsorbed to slate substrata at an experimental salinity of 5 ppt. Significant differences in settlement frequency among treatments designated by different letters, $p < 0.01$. Bars indicate means (\pm SE) of three replicate trials with 100 cyprids per trial

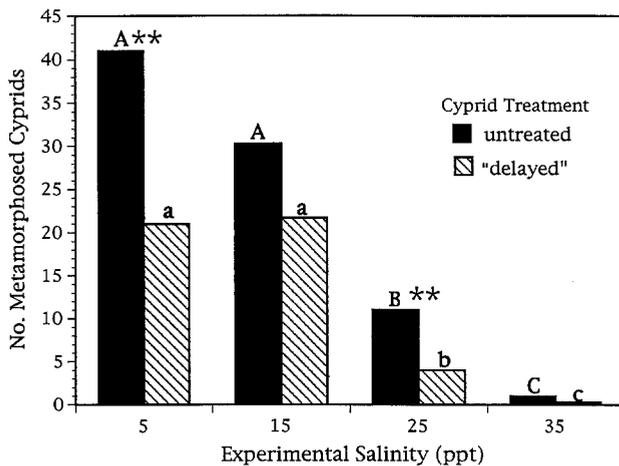


Fig. 4 *Balanus subalbidus*. Cypris settlement after 144 h of non-delayed cyprids (untreated) and those induced to delay ("delayed") metamorphosis by maintenance at 5 °C for 8 d, in the presence of conspecific settlement factor at various salinities. Identical letters above bars indicate settlement frequencies that were not significantly different (upper case letters for newly metamorphosed cyprids and lower case letters for cyprids induced to postpone metamorphosis). Significant difference in settlement frequencies between settlement factor treatments with in each salinity level designated as (**), $p < 0.01$. Bars indicate means of three replicate trials with 100 cyprids per trial

were induced to postpone metamorphosis ($\chi^2 = 104.3$, 3 df, $p < 0.001$), but there were no significant interactive effects ($\chi^2 = 4.5$, 3 df, $p > 0.21$) (Fig. 4). More untreated cyprids settled than did those which were metamorphically delayed (20.8 compared to 11.8%) when averaged over the salinity treatments. Cyprids settled in greater abundance at 5 and 15 ppt salinity regardless of treatment. In pairwise comparisons, settlement levels of non-delayed cyprids were significantly higher than "delayed cyprids" at 5 and 25 ppt, but not at 15 and 35 ppt salinity (pairwise G -tests; $p < 0.01$; experiment-wise error rate = 0.05, 3.1 df, $\chi^2 = 8.6$) (Fig. 4).

Discussion

Settlement across salinities

The pattern of *Balanus subalbidus* settlement across a wide range of salinities in the laboratory contrasts with the narrower salinity distribution of adults in the field. In Chesapeake Bay and other estuaries, *B. subalbidus* recruits abundantly at salinities of 0.5 to 10 ppt and occurs rarely up to 18 ppt, but has never been documented at higher salinities (Poirrier and Partridge 1979; Kennedy and DiCosimo 1983; Dineen personal observation). When laboratory-reared cyprids were tested in an array of salinities in the presence of conspecific settlement factor, peak settlement occurred at 2 ppt ($87 \pm 8.9\%$ after 144 h), but substantial settlement occurred over a broad range of salinities up to 25 ppt ($48 \pm 11\%$ after 144 h) (Fig. 2). In addition, cyprids of *B. subalbidus* settled abundantly in the absence of settlement factor in a range of mid-salinities, with a peak at 15 ppt ($57 \pm 9.9\%$ after 144 h). Differences in settlement between test salinities obtained for the four larval batches in the present study could be due to subtle differences in larval culturing conditions (Holm 1990) and/or due to variation in environmental conditions during embryogenesis (Dineen and Hines 1992). Nevertheless, all four batches of our test larvae consistently showed a broad, relatively indiscriminate pattern of settlement in the laboratory. In contrast, laboratory settlement of *B. improvisus* cyprids exposed to a similar array of salinities with conspecific settlement factor coincided with its mesohaline adult distribution, and settlement in the absence of settlement factor was low and showed no discernable pattern relative to experimental salinity (Dineen and Hines 1992). Similarly, laboratory settlement of a third estuarine barnacle, *B. eburneus*, coincided with its polyhaline adult distribution in the presence of conspecific settlement factor (Dineen and Hines in press). Thus, in comparison with congeners, *B. subalbidus* settlement appears less dependent on salinity.

Larval retention in the upper estuary could markedly limit adult distribution independent of specific cues for settlement but spatial distributions of *Balanus subalbidus* larvae in an estuary have never been documented. Although larvae of *B. improvisus* exhibited a progression of developmental stages while entrained within the Miramichi Estuary, with early stages carried away from, and advanced stages eventually returning to parental stocks (Bousfield 1955), other larval species do exhibit distinct retention in upper zones of estuaries. For example, larvae of the xanthid mud crab, *Rhithropanopeus harrisi*, are retained in low to medium salinity ranges of the upper Newport River Estuary (Cronin 1982), and veligers of the low salinity bivalve, *Rangia cuneata*, also appear to be restricted to low salinities where adults occur in estuaries (Cain 1972; Hopkins et al. 1973).

Despite many studies on the induction of metamorphosis in larvae, few have tested the interactive effects of two or more factors on settlement (Pawlik 1992). In variable

environments like estuaries, the interaction of factors might serve as a more reliable cue for good settlement habitat than any single factor. Previous studies with *Balanus improvisus* indicate that this could be the case because larvae were stimulated to settle at a salinity regime indicative of adult distribution only in the presence of conspecific settlement factor. In contrast, present results with *B. subalbidus* suggesting relative independence from settlement cues tested, both in a qualitative (Fig. 2) and temporal (Fig. 1) sense, could indicate that even interactive cues may not be sufficient to explain the distribution of this species in the highly fluctuating oligohaline zone. Larval retention in this zone, on the other hand, might be more likely to produce effective habitat selection than interactive cues. In addition, if larvae of *B. subalbidus* are not dispersing far from adult populations in low saline waters, the adaptive value of utilizing conspecific settlement factor as a settlement cue would not be as critical as it would be for species like *B. improvisus* with larvae that are initially carried away from parental stocks.

Post-settlement osmotic/ionic stress (possibly resulting in mortality or slower growth rates of newly metamorphosed juveniles) was not investigated in our study and could potentially be a factor in influencing adult salinity distribution of *Balanus subalbidus*. However, when adult *B. subalbidus* were transplanted to mesohaline regions, these barnacles survived over a year and reproduced (Dineen unpublished data). Alternatively, it could be argued that post-settlement mortality from predation and competition could be influencing horizontal distribution of estuarine barnacles. However, in Chesapeake Bay, flatworms and bryozoans, the chief predators and competitors of barnacles in this estuarine system (at least in mesohaline regions) (Branscomb 1976), are unlikely to distinguish preferentially among congeneric barnacles thereby eliminating *B. subalbidus* while allowing *B. improvisus* to persist at mesohaline salinities.

Conspecific versus congeneric settlement factor

The present study as well as others have shown that stimulation of gregarious cypris settlement is, not surprisingly, greatest in the presence of conspecific settlement factor compared to settlement factor from congeners or more distantly related species (Knight-Jones 1953, 1955; Crisp 1990; Dineen and Hines 1992). In some cases, a correlation between degree of settlement stimulation and phylogenetic affinity of the cue species and receptor species has been implied (Knight-Jones 1955). This relationship has been demonstrated at the family level (Knight-Jones 1955, Crisp 1990) but is probably not a good indicator of affinity at or below the generic level. For instance, cyprids of *Balanus amphitrite* settled in higher numbers in the presence of *Conopea galeatus* as opposed to *B. eburneus* settlement factors with equivalent protein concentration (Crisp 1990). Furthermore, we recently demonstrated that *B. improvisus* settlement is greater in the presence of settlement factor derived from *B. subalbidus* than from

B. eburneus (Dineen and Hines 1992), although *B. improvisus* is more closely allied to *B. eburneus* within the *Balanus amphitrite*-complex (Henry and McLaughlin 1975). Similarly, the present study shows greater stimulation of *B. subalbidus* settlement in the presence of *B. improvisus* settlement factor (Fig. 3), even though *B. subalbidus* is more closely allied to *B. eburneus* (Henry and McLaughlin 1975). It is not known whether the differential response of *B. subalbidus* cyprids to congeneric extracts could have ecological implications in the field. However, it is interesting to note that *B. subalbidus* was stimulated to settle more in the presence of extract obtained from the congener with which it would more likely co-occur in the field (*B. improvisus*).

Settlement of metamorphically delayed larvae

Delayed metamorphosis of larvae in the absence of appropriate cues or habitat has been documented in several marine invertebrate groups, including barnacles (review by Pechenik 1990). Barnacle larvae retained within an estuary probably often encounter inappropriate habitat. As a consequence of delay, larvae sometimes settle less discriminately (Rittschof et al. 1984; Crisp 1988). For example, there were fewer differences in settlement in different test salinities when cyprids of *Balanus improvisus* were induced to postpone metamorphosis by keeping them at low temperature (Dineen and Hines 1992). However, when delayed metamorphosis was imposed on *B. subalbidus* cyprids by lowering temperature in the present study, subsequent settlement did not differ among test salinities relative to the untreated group (in the presence of conspecific settlement factor). Although overall settlement levels were lower for metamorphically delayed cyprids, patterns of settlement as a function of salinity were similar for both delayed and non-delayed larvae. This similarity again could reflect relatively low dependency on salinity as a cue for metamorphosis of *B. subalbidus* cyprids. Because delaying metamorphosis appears detrimental for metamorphic completion without apparently increasing the likelihood of settlement at "appropriate" salinities, postponing metamorphosis may not be a good strategy for *B. subalbidus* cyprids.

The causes and effects of metamorphic delay are complex (Pawlik 1992; Pechenik et al. 1993). Although delaying metamorphosis by lowering temperature probably does not produce an effect of aging larvae but rather suspends development, we used this method for two reasons. First, temperature is an ecologically relevant factor for delaying development and settlement in the field. At our Chesapeake study site, larvae hatch in late April to early May, when salinities are low and variable, and when water temperatures are warming rapidly from ca. 8 °C. Settlement at our collecting site typically begins in early May (14 °C) and peaks in mid to late May (22 °C). In years when cold persisted longer than normal (e.g. 1992), water temperature was only 10 °C in early May and settlement occurred at least 2 wk later than usual. Although we are not sure whether hatch-

ing was late, whether larval development was slowed, or both, "early" larvae will often encounter temperatures lower than 14 °C. While no settlement occurs in the laboratory or field at temperatures as cold as 5 °C, *Balanus subalbidus* larvae along the northern portion of its geographic range (extending to Massachusetts) are likely to encounter large variation in temperatures during the critical spring period of development. Second, this low temperature treatment provides an interesting comparison with experimental studies using the same technique for other species (Rittschof et al. 1984, 1986b; Maki et al. 1988; Crisp 1990; Maki et al. 1990; Dineen and Hines 1992; Pechenik et al. 1993). These studies demonstrate that low temperature does not appear to have any confounding deleterious effects on the larvae that many other factors do (e.g. starvation for some types of larvae). Moreover, Pechenik et al. (1993) showed that three experimental techniques of delaying metamorphosis in *Balanus amphitrite* (cold temperature, silanized surfaces, and over-crowding) produced settlement results that did not differ significantly from each other, but all produced similar differences from "non-delayed" larvae.

In conclusion, the present study has tested the interactive effects of two settlement cues for larvae. Cyprids of *Balanus subalbidus* are capable of settling, with and without adult settlement factor, in meso- and polyhaline salinities where adults rarely, if ever, occur in the estuary. Our results suggest that pre-settlement behavior, leading to larval retention in oligohaline head waters, may be the underlying mechanism that ultimately accounts for the adult distribution of *B. subalbidus*.

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