Foundations of gregariousness

SIR — Most benthic marine invertebrates have a pelagic larval phase, during which they may disperse widely. Once ready to metamorphose, the planktonic larvae of many species (for example mussels, oysters, barnacles, sand dollars) settle preferentially on or near conspecific adults, forming monospecific aggregations[1]. Gregarious settlement has many advantages, most notably enhanced adult reproductive success[2], but at the cost of increased intraspecific competition. Aggregations must initially develop from a two-step process: solitary larvae first colonize an uninhabited substratum, then gregarious settlement occurs on or near these 'founders'. To date, research has focused on the latter step[1]. It has been suggested that founders settle because they are unable to locate conspecifics and are incapable of prolonging their planktonic lives[3,4], a concept we term the 'desperate larva' hypothesis. One alternative, however, is that larvae differ in their substratum preferences and that founders are somehow distinct. We have found that females of a tube-dwelling polychaete worm produce larvae that settle in two different ways: one type colonizes uninhabited substrata (founders), whereas the other settles only in response to cues associated with conspecifics (aggregators).

We conducted still-water, single-substratum, laboratory settlement assays with larvae of *Hydroides dianthus*, a common member of epibenthic fouling communities along the east coast of North America. Three experiments were performed, each on a population of 90,000 larvae (pooled from the spawns of about 25 females) cultured in three 4-litre glass jars. We used two types of settlement substrata to assess larval responses: 'biofilmed' slides and 'adult' slides. Biofilmed slides were made by placing etched glass microscope slides into running unfiltered seawater for at least 5 days, so that they became coated with an organic/microbial film. Adult slides were treated similarly, but five small conspecific worms were attached by gluing their tubes to each slide. Two types of settlement assay were conducted until larval cultures were depleted: sample and whole-population assays. For sample assays, a sample of larvae was removed from culture jars and exposed to experimental substrata: for whole-population assays, all remaining larvae were exposed to substrata. Twelve replicate sample assays were run simultaneously in Petri dishes containing a single biofilmed or adult slide and 25 competent larvae. Assay dishes were examined for settlers after 24 h. For whole-population assays, the entire population of larvae was filtered down into a small volume and split evenly among nine replicate glass dishes, each containing a single biofilmed or adult slide. Dishes were placed on a shaker-table rotating at 50 r.p.m. for 1 h, and slides then examined for settlers. Larval types were defined by their responses: founders settled on biofilmed slides, and aggregators settled on adult slides.

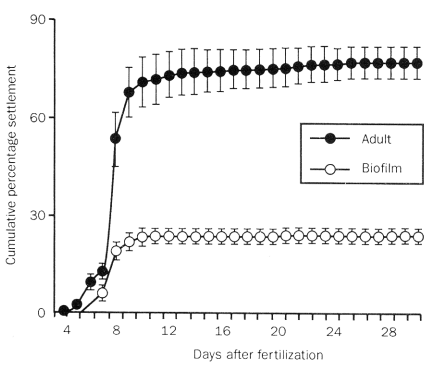

FIG. 1 Mean cumulative percentage settlement (n = 9, ±1 s.e.) of *H. dianthus* when the whole population of larvae was exposed to biofilmed and adult slides daily.

In the first experiment, the whole population was exposed to both biofilmed and adult slides daily after day 3; therefore, all larvae had access to both substrata throughout development. Settlement on both experimental substrata began simultaneously as larvae became competent (Fig. 1), contrary to the prediction of the desperate larvae hypothesis. The bulk of settlement on both biofilmed and adult slides occurred soon after competence, with settlement occurring 7–8 days post-fertilization. Larvae continued to settle on adult slides in low numbers through day 26, but no larvae settled on biofilmed slides after day 14.

In a second experiment, the whole population was exposed to biofilmed slides daily (day 4 through day 14, weekly thereafter), then samples were exposed to adult and biofilmed slides. Under these constraints, only the larvae exposed to adult slides had the opportunity to settle gregariously, whereas the remainder of the population were forced to delay settlement, or settle on biofilmed slides. Surprisingly, virtually all founders settled between 5 and 12 days after fertilization, with the remaining larvae settling only in response to conspecifics (in sample assays) throughout a seven week period (Fig. 2). These results indicate the existence of a distinct subpopulation of larvae that responded to biofilmed substrata; once these individuals were removed, the remaining aggregators delayed settlement in the absence of acceptable conspecific-associated cues. In a similar experiment (not presented here) in which daily exposure of the whole larval population to biofilmed slides was omitted, sample assays revealed the same patterns as seen in Fig. 2 over a period of 70 days, after which, larvae began to die in culture rather than settle in the absence of conspecifics.

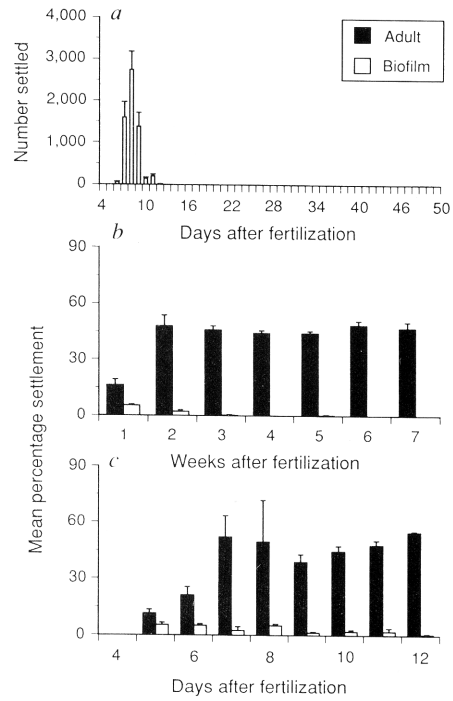

FIG. 2 Settlement of *H. dianthus* when the whole population was exposed to biofilmed slides daily, but only samples were exposed to adult slides. *a*, Number of founding settlers (n = 3, + 1 s.e.) from daily whole-population assays of biofilmed slides. *b*, Weekly mean percentage settlement (n = 12, + 1 s.e.) in sample assays of biofilmed and adult slides. Sample assays were conducted daily for the first 14 days, and weekly thereafter. *c*, Daily mean percentage settlement for the first week, a weekly mean of which is shown in *b*.

The desperate larva hypothesis was advanced on the basis of observations of the development of non-feeding pelagic larvae that exhaust a yolk reserve as they age in the plankton[3,4]. If reduced substratum selectivity is a function of energetic desperation, rather than age alone, it should be possible to test this by starving the larvae of a planktotrophic (feeding) species, such as *H. dianthus*, and monitoring larval responses to substrata. We performed a third experiment, like the second, in which half the population of larvae was starved during days 10–14, and fed and starved subpopulations were

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assayed separately. We found that settlement of founders could not be induced by starvation. Moreover, starvation resulted in a developmental reversion to a precompetent state, a condition that was reversed when larvae were again provided with food. An ontogenetic shift of this kind was recently described for the larvae of another species of marine polychaete[5].

Besides random settlement, the desperate larva hypothesis is, to our knowledge, the only published explanation for the colonization of new habitats by larvae of gregarious species. The results of our study, a full version of which will be presented in a forthcoming paper, provide an alternative explanation for the process by which monospecific aggregations develop on hard substrata previously uninhabited by conspecifics.

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