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Avoidance of dried biofilms on slate and algal surfaces by certain spirorbid and bryozoan larvae

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The effect of aerial drying of biofilmed surfaces to simulate a tidal emersion upon the settlement preferences of spirorbid and bryozoan larvae was investigated using choice experiments with still water conditions carried out in the laboratory. Aerial drying of biofilmed slates and pieces of *Fucus serratus* for 1 h at 20°C negated their usual settlement-inducing properties to larvae of *Spirorbis spirorbis*, *S. tridentatus* and *Flustrellidra hispida*. Such larval settlement preference may contribute to observed variations in the natural distribution of these species in the intertidal.

Benthic marine invertebrate larvae are capable of responding to a variety of physico–chemical cues during substratum exploration and settlement. Chemical cues are often of biological origin and may originate from adult, juvenile or larval conspecifics, prey organisms, or biofilms (Pawlik, 1992). Biofilms may induce, inhibit or have no apparent effect upon larval settlement (Wieczorek & Todd, 1998). Larvae frequently respond positively to cues that indicate that a substratum is likely to be suitable for settlement and post-settlement survival. It has been suggested that surface biofilms may provide this information to settling larvae because they reflect the physical regime which has acted on that surface over time (Wieczorek & Todd, 1998).

We have found that aerial drying of biofilmed slate surfaces for as little as 1 h, to simulate a tidal emersion, negates the previous settlement-inducing effect of the biofilms to larvae of the serpulid polychaete *Pomatoceros lamarkii* (Hamer et al., in press). Furthermore, the negative settlement effect was shown to last for 5–10 d after a single drying event. We speculate that such behaviour may have real ecological significance in explaining the distribution of this species, which occurs in the low intertidal region. The purpose of the present laboratory study was to determine if the larvae of selected intertidal spirorbid and bryozoa species, already known to settle readily on either biofilmed slate surfaces or *Fucus serratus*, also avoid settling on dried biofilms under still water laboratory conditions.

Spirorbis tridentatus (Polychaeta: Spirorbidae) larvae were obtained from adults collected from the Menai Strait, North Wales by inducing artificial liberation of larvae (see de Silva, 1962). *Spirorbis spirorbis* and *Flustrellidra hispida* (Bryozoa) larvae were obtained by inducing the liberation of larvae from adults on *Fucus serratus* plants also collected from the shores of the Menai Strait. *Spirorbis spirorbis* and *S. tridentatus* larvae are photopositive immediately after liberation and were collected at a point-light source. Larvae of *Flustrellida hispida* were collected by eye, using a pipette. The larvae of these species are competent to settle immediately after being released; larvae that were used in the settlement assays were collected for up to 1 h after larval liberation was initiated.

All assays were conducted in 300 ml Pyrex glass dishes containing 100 ml of UV-irradiated and filtered (0.2 μm) seawater; surfaces to be assayed were placed randomly together in the centre of each of 12 replicate dishes. Dishes were kept at 19°C in a constant environment cabinet in the dark during the 1 h assay period. For each assay, ten larvae of the species being assayed were added to each of the 12 replicate dishes. An assay was repeated three times on different occasions for each of the larval species.

To determine the effect of drying biofilmed slates on the settlement of spirorbid larvae, drying took place for 1 h at 20°C immediately prior to an assay. The control in each dish was a non-dried biofilmed slate. All slates (25×25×4 mm; cut from Welsh slate by Inigo Jones, North Wales; polished with 600 grade wet and dry paper) were submerged in the laboratory running seawater system and allowed to accumulate a biofilm for at least eight weeks prior to an assay. The dried and non-dried slates were then placed together in each dish and larvae added. The number of settled individuals was counted on each slate after the 1 h period allowed for settlement. This first experimental series was carried out separately for *S. spirorbis* and *S. tridentatus* larvae.

The second experimental series aimed to determine if the larvae of *S. spirorbis* and *F. hispida* avoid settling on pieces of *Fucus serratus* that had been dried. Two centimetre long sections of *F. serratus* fronds were cut from the middle portion of plants freshly collected from the shore. Each section was cut down the midrib to give two pieces of equal area. Again, one of these was dried for 1 h at 20°C and the other remained immersed in seawater. The two pieces were then placed in each replicate dish and larvae of the relevant species added. The number of settled larvae on each piece of alga was counted after the 1 h period allowed.

The larvae of all three species strongly avoided biofilmed surfaces that had been dried prior to the assay and settled preferentially on non-dried biofilmed surfaces (Tables 1 & 2). No larvae of *S. spirorbis* or *S. tridentatus* settled on the dried slates in any of the experimental repeats (Table 1). Settlement of *S. spirorbis* and *Flustrellidra hispida* larvae was significantly higher on the non-dried pieces of *Fucus serratus*, although some settlement did occur on the dried *Fucus* (see Table 2).

The non-random distribution of adult *S. spirorbis* and *S. tridentatus*, on algal and rock surfaces respectively, is well documented. Seed et al. (1981) reported the non-random distribution of *S. spirorbis* (=*borealis*) on *F. serratus* plants in Strangford Lough, Northern Ireland and also that variations in recruitment of *Flustrellidra hispida* occurred between plants and even within

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Table 1. *Percentage settlement (mean ±SE; N=12) of* Spirorbis spirorbis *and* S. tridentatus *larvae on non-dried and dried (1 h at 20°C) biofilmed slates after 1 h still water, choice assay. Data for all three experimental repeats (A–C) are shown. Percentage settlement was significantly different between treatments for all assays for both species (χ^2; P<0.05).*

Species	Experimental repeat	Non-dried biofilmed slate	Dried biofilmed slate
	A	29.10 ±5.41	0
Spirorbis spirorbis	B	30.00 ±6.40	0
	C	49.10 ±4.96	0
	A	56.70 ±5.23	0
Spirorbis tridentatus	B	58.30 ±5.32	0
	C	52.50 ±5.59	0

Table 2. *Percentage settlement (mean ±SE; N=12) of* Spirorbis spirorbis *and* Flustrellidra hispida *larvae on non-dried and dried (1 h at 20°C) pieces of* Fucus serratus *after 1 h still water, choice assay.* t*-test statistic (*t*) and level of significance at 11 df are shown.*

Species	Experimental repeat	Non-dried *Fucus*	Dried *Fucust*	*t*	Significance level
	A	75.83 ±4.17	0.80 ±0.81	17.65	$P<0.001$
Spirorbis spirorbis	B	71.74 ±5.70	7.50 ±2.50	10.23	$P<0.001$
	C	54.29 ±5.00	5.06 ±2.61	8.72	$P<0.001$
	A	28.04 ±5.50	1.70 ±1.70	5.43	$P<0.001$
Flustrellidra hispida	B	33.35 ±4.71	2.50 ±1.34	6.37	$P<0.001$
	C	25.03 ±3.44	3.32 ±1.46	5.92	$P<0.001$

individual plants (Seed & Wood, 1994). In the intertidal zone, *S. tridentatus* is most abundant on the undersides of rocks and in dark and shaded places (Nelson-Smith & Gee, 1966). Wood & Seed (1980) reported that in the Menai Strait, *F. hispida* is less abundant on higher shore *Fucus serratus* plants and attributed this observation to the increased desiccation higher on the shore. The larval behaviour described here may help to explain how larval settlement preferences may be important factors that account for the adult distributions of these species.

The nature of the settlement-inducing cue from a biofilm or the inhibitory effect of drying a biofilm to the larvae of these species is not known. Certainly, bacteria and/or their extracellular products are frequently reported to play an important role in inducing settlement of a wide range of marine invertebrate larvae (see Wiekzorek & Todd, 1998) and bacteria have already been shown to induce settlement of the spirorbid *Janua brasiliensis* (Kirchman et al., 1982).

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