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Random and Aggregative Settlement in Some Sessile Marine Invertebrates

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ABSTRACT: Settlement patterns of 4 sessile invertebrates are statistically analysed. In the field, the colonial ascidians *Botryllus schlosseri* and *Diplosoma listerianum* settle randomly on perspex panels. Laboratory experiments with *D. listerianum* substantiate these results and demonstrate that larvae are not attracted towards already attached juveniles. Two solitary species, the barnacle *Elminius modestus* and the ascidian *Molgula complanata* settle aggregatively, but the latter less strongly than the former. Species differ in their preference for horizontal or vertical panel orientation. The settlement pattern of each species is consistent and not influenced by panel orientation or by the presence of other species.

INTRODUCTION

Settlement responses in marine invertebrates are often complex as they may be determined by both biological and environmental parameters (see Crisp, 1976 for review). Environmental heterogeneities, e. g. crevices, may give rise to aggregated distribution patterns, as may the presence of already attached conspecifics. Solitary animals which are attracted to members of their own species ('gregariousness') thereby will increase the chances of cross-fertilization. By forming single-species stands they may also effectively reduce interspecific competition, particularly with colonial species (Schmidt, 1982). Colonial species capable of lateral expansion over the substrate through asexual reproduction would not need to settle close to conspecifics. Indeed, such response would considerably increase intraspecific competition for space. Sessile colonial invertebrates would therefore be expected to settle randomly, but solitary forms to aggregate (Jackson, 1977; Woodin, 1979; Scheltema et al., 1981). However, some colonial ascidians have been observed to settle at unexpectedly high densities (Sabbadin, 1978; van Duyl et al., 1981; Schmidt, unpubl.); however as yet statistical analyses of settlement patterns are lacking for colonial species.

It was therefore decided to analyse settlement patterns of 2 colonial and also 2 solitary species on perspex panels in the field. In addition, a laboratory study was carried out for 1 of the 2 colonial species to compare field an laboratory settlement patterns.

MATERIALS AND METHODS

Field Experiments

The study site was Langstone Harbour, a marine, shallow, natural harbour bordering the northern shore of the eastern Solent (near Portsmouth, southern England).

Black perspex panels, 0.25 × 0.25 m square, were fixed to steel frames and suspended from a raft within 1.5 m of the surface and about 5 m from the seabed. Three panels were horizontally and another 3 vertically aligned, and submerged for 4 wk during June, 1981. The positions of all attached individuals of *Botryllus schlosseri*, *Diplosoma listerianum*, *Molgula complanata*, and *Elminius modestus* were then determined for each panel over an area of 0.16 × 0.24 m. This was achieved with the aid of a grid ruled on a thin sheet of a 0.25 × 0.25 m transparent perspex and placed over the settlement panel. The sizes of the squares of the grid were 10 × 10 mm. To determine the distribution patterns of each species, numbers of individuals per 20 × 20 mm and 40 × 40 mm respectively were counted. The observed distributions were compared with Poisson and negative binomial distribu-

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Table 1. *Botryllus schlosseri, Diplosoma listerianum, Mogula complanata, Elminius modestus*. Numbers of individuals which had settled on a panel area of 0.16 × 0.24 m during a 4 wk period (June, 1981). For horizontal lower sides (3 replicates), each value is listed. For vertical sides (6 replicates), the median and range (in brackets) are given

Panel side	Species: *B. schlosseri*	*D. listerianum*	*M. complanata*	*E. modestus*
Horizontal lower	191	35	106	70
	328	57	174	73
	473	63	176	93
Vertical				
median	157	38	4	143
(range)	(68–402)	(7–61)	(1–6)	(53–256)

tions fitted to the observed data using the method of Maximum Likelihood (Meyer, 1970; Ross, 1980).

Laboratory Experiments

The experiments were conducted in September 1981 at the Stazione Idrobiologica di Chioggia (University of Padova, Italy). Larvae of *Diplosoma listerianum* were obtained from freshly collected colonies placed into 1 l aquaria. The colonies released larvae which accumulated at the side illuminated by a 60W overhead table lamp. Twentyfive to 30 larvae were pipetted into each of 3 Petri dishes containing filtered sea water. Pilot experiments had shown that larvae readily attach to black plastic sheet. Discs of 83 mm diameter were cut from the plastic material, and one per Petri dish was floated on the water surface. Even illumination from above resulted in the majority of larvae settling on the underside of the plastic within one centimeter from the edge of the disc. This pattern was attributed to the tendency of the larvae to settle in dimly illuminated rather than the darkest parts (cf. Crisp and Ghobashy, 1971). In order to eliminate such light response Petri dishes were kept in light-proof plastic bags for the entire duration of the experiments. Experiment 1 started when all larvae were in the 3 replicate Petri dishes with a floating piece of plastic, and lasted for 22 h. Nearest neighbour distances of animals which had settled on the undersides of the plastic discs were then measured to the nearest mm. These values were used to test for Poisson (random) distribution (Wratten and Fry, 1980). After changing the water in the dishes a second batch of 25 to 30 larvae was introduced and the plastic discs with the attached and scored juveniles were added one per Petri dish. Experiment 2 then continued as described above (Experiment 1).

Table 2. *Botryllus schlosseri* and *Diplosoma listerianum*. Numbers of juveniles were counted over a panel area of 0.16 × 0.24 m using regularly spaced quadrats of 40 × 40 mm size (see Methods). Poisson distributions were fitted to the data and compared with the observed distributions by a Chi-square test. Only 1 value is significant at the 5 % level; it is marked with an asterisk. Dregrees of freedom (D. F.) are given in brackets

Chi-squared values (D.F.) *B. schlosseri*: Vertical sides	*B. schlosseri*: Horizontal lower sides	*D. listerianum*: Vertical sides
13.16 (7)	11.66 (11)	1.33 (5)
20.87* (11)	9.89 (13)	6.72 (6)
12.18 (12)	21.03 (23)	12.71 (7)
17.80 (13)		
16.27 (17)		
22.75 (22)		

RESULTS

Ninety percent or more of the panel surfaces had remained unoccupied during the 4 wk exposure time. Horizontal and vertical panels had collected different assemblages of species and different numbers of individuals (Table 1). However, this did not affect the outcome of the settlement patterns which were consistant for each species. The settlement intensity of *Diplosoma listerianum* was low at this time of the year; numbers sufficient for statistical analyses were found on only 3 panel sides.

The observed distribution patterns of *Botryllus schlosseri* and *Diplosoma listerianum* fitted calculated Poisson distributions indicating that both species had settled randomly (Table 2). Fig. 1 is an example of observed and calculated random distributions of *B. schlosseri* for one side of a vertically aligned panel. In contrast, the distributions of the solitary ascidian *Molgula complanata* and the barnacle *Elminius modestus* departed significantly from random ($P < 0.001$) but fitted calculated negative binomial distributions.

In both species, the values of the defining parameter k were positive indicating that departures from random were due to animals being aggregated in groups (Ross, 1980). As k tends towards ∞ for random distributions, large k values are found with random and small k values with aggregated distributions. Thus, compared to the small k values of the aggregated patterns of *M. complanata* and *E. modestus*, values for the randomly settled *B. schlosseri* were high and variable ranging from 7.58 to 45.58 (absolute numbers) with a median of 13.24. For each of the solitary species the k values of all distributions were used to compute the weighted mean and its approximate standard error ('JOIN directive' in Ross, 1980). For *M. complanata*, additional data from 3 horizontally aligned panels submerged during 4 wk in May 1981 were included for these calculations (Schmidt, 1982). The results (Table 3) show that while both solitary species had lower k values when the smaller quadrat size was used, *E. modestus* was overall more strongly aggregated than *M. complanata*. Data were consistent for numbers of individuals ranging from 70 to 256. Fig. 2 gives an example of aggregated settlement of *E. modestus*, and should be compared to the random distribution pattern of *B. schlosseri* (Fig. 1).

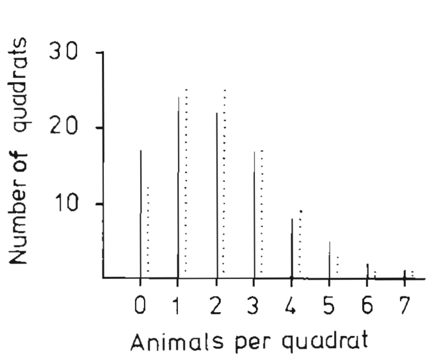

Fig. 1. *Botryllus schlosseri*. Distribution pattern on one side of a vertically aligned panel which had been submerged in the sea for 4 wk. The observed data (solid bars) fitted calculated Poisson (random) distribution (dotted bars). On an area of 0.24 × 0.16 m 195 individuals were scored. Chi-squard for Poisson distribution: 4.13 on 7 degrees of freedom. *K* value: 7.58

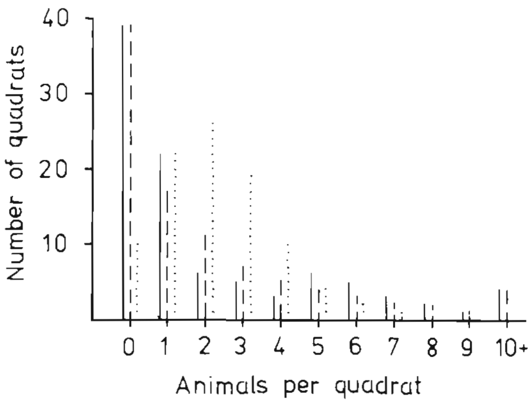

Fig. 2. *Elminius modestus*. Distribution pattern on one side of a vertically aligned panel which had been submerged in the sea for 4 wk. The observed pattern (solid bars) departed significantly from calculated Poisson (random) distribution (dotted bars; $P < 0.001$), but fitted negative binomial distribution calculated from the observed data (broken bars). On an area of 0.24 × 0.16 m 215 individuals were scored. Chi-squared of Poisson fit: 173.33 on 13 degrees of freedom; negative binomial: 19.09 on 12 degrees of freedom. *K* value: 0.55

The results of the laboratory experiments with *Diplosoma listerianum* are summarised in Table 4. Apart from one instance, which showed aggregated settlement related in some way to the procedures followed, settled juveniles were randomly distributed. All animals were of one zooid size. After the introduction of a second batch of larvae to dishes which contained the attached juveniles of the first batch, all distribution patterns were random.

Table 3. *Molgula complanata* and *Elminius modestus*. Calculated negative binomial distributions compared to observed distributions by a Chi-square test. The k values (defining parameter) of all replicates (n) were used to compute the weighted mean and its approximate standard error (S. E. in brackets)

Species	n	Weighted mean k values (S.E.) Quadrat size 40 × 40 mm	Quadrat size 20 × 20 mm
M. complanata	6	1.71 (0.21)	0.84 (0.07)
E. modestus	9	0.76 (0.09)	0.48 (0.04)

Table 4. *Diplosoma listerianum*. Nearest neighbour analysis for settled juveniles (numbers given in brackets). Twentyfive to 30 larvae were introduced per Petri dish. Values are the mean observed (obs.) and expected (exp.) nearest neighbour distances (mm), calculated assuming a Poisson (random) distribution of attached and scored individuals. A second batch of larvae was introduced (Experiment 2) after all nearest neighbour measurements of the first settlers were completed. Three replicates were made (A, B, C). Only one value is significant at the 5 % level; it is marked with an asterisk

Experiment	Replicates A obs.	A exp.	B obs.	B exp.	C obs.	C exp.
1	6.74	7.76	8.18	7.16	5.35*	7.30
	(23)		(27)		(26)	
2	4.83	5.16	5.58	5.55	5.88	5.89
	(52)		(45)		(40)	

DISCUSSION

The results confirm predicted settlement patterns for colonial and solitary sessile invertebrates (Jackson, 1977; Woodin, 1979). In the field, both *Botryllus schlosseri* and *Diplosoma listerianum* showed random settlement. Laboratory experiments with *D. listerianum* substantiated these results and, moreover, demonstrated that the presence of already attached juveniles did not affect the random settlement of subsequent larvae. However, the present study did not assess whether attached juveniles influenced the rate of settlement of conspecifics, as has recently been demonstrated for the colonial ascidian *Trididemnum solidum* (van Duyl et al., 1981).

The numbers of juvenile *Botryllus schlosseri* per panel were high considering that an area of 0.16 × 0.24 m may be covered by a few or even one single large colony (Brunetti, 1974). This also applies to *Diplosoma listerianum* (Schmidt, unpubl.). The advantage of such intense settlement to colonial species probably lies in its potential of rapidly pre-emting unoccupied substratum (cf. Karlson and Jackson, 1981). It would be particulary important for *B. schlosseri* which is sensitive to the presence of other species, unless these are few and small, and reacts to interspecific encounters mostly by inhibition or redirection of growth, and fragmentation rather than overgrowth (Brunetti, 1974; Schmidt, own obs.).

The aggregative settlement patterns of *Molgula complanata* and *Elminius modestus* are most likely brought about by some form of species recognition and the subsequent exercise of choice. Such a gregarious response is well known for *E. modestus* and some other solitary marine invertebrates (Knight-Jones, 1950; Scheltema, 1974 for review; Young and Braithwaite, 1980; Scheltema et al., 1981). At similar densities *E. modestus* was found to be more strongly aggregated than *M. complanata*. This may be attributed to the different requirements of nearest conspecific distance which the different modes of cross-fertilisation in these species demand (cf. Kaestner, 1967; Berrill, 1975).

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