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Influence of substratum heterogeneity and settled barnacle density on the settlement of cypris larvae*

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Abstract

On the Atlantic coast of Canada, *Semibalanus balanoides* (L.) is widely distributed in the mid-intertidal zone, whereas in the Gulf of St. Lawrence, this species is mostly limited to crevices. We tested the hypothesis of regional differences in microhabitat selection by barnacle larvae at settlement in 1984 and 1985 at St. Andrews, New Brunswick, Canada. Since larvae settle in microhabitats already colonized by adults, the relative influence of settled barnacle density and of different scales of substratum heterogeneity on settlement were evaluated experimentally at Capucins, Québec, (Gulf of St. Lawrence) and at St. Andrews, New Brunswick (Atlantic coast). On a large scale (> 10 cm deep crevices) of heterogeneity, results show that, in the Gulf, cypris larvae settled nearly exclusively (93%) in natural crevices rather than on adjacent horizontal surfaces. On the Atlantic coast, settlement was more important outside than inside of crevices, when the substrata were either natural or artificial. This result is unique and contrasts sharply with all known reports on barnacle settlement in relation to surface contour. The influence of barnacle density on settlement was greater than that of large scale heterogeneity. On a small scale (< 1.5 cm deep cracks), the presence of conspecifics had a stronger effect on settlement than heterogeneity in both regions. Field observations showed a relationship between larval settlement density and percentage of adult cover. Settlement increased up to 22 or 30% (Gulf and Atlantic coast) of adult cover and decreased afterwards. The results confirm the hypothesis of larval selection for cryptic habitats in the Gulf and the opposite behaviour (preferences for horizontal surfaces) on the Atlantic coast. This microhabitat selection is apparent at large scales of heterogeneity, whereas at small scales, the presence of conspecifics is the predominant factor.

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Introduction

Observations carried out at different sites on the Atlantic coast of Canada and of the New England region show that *Semibalanus balanoides* occurs on exposed as well as on protected (crevices) rocky surfaces of the midlittoral zone. This distribution is apparently common despite varying local or regional environmental conditions (rock structure, water currents, etc.). However, quantitative measurements of distribution of individuals in relation to shore topography, carried out at numerous sites in the Gulf of St. Lawrence, show that adult *S. balanoides* are found mainly in cracks and crevices (Bourget *et al.*, 1985; Bergeron and Bourget, 1986). This pattern of distribution was attributed by Bergeron and Bourget (1986) to greater settlement inside than outside crevices (97% vs 3%) rather than to differential mortality between the two microhabitats. However, since cypris larvae tend to settle near adult conspecifics (Crisp, 1974), the observed settlement in crevices could simply be due to gregariousness rather than to a strong cryptic behaviour of the larvae at settlement. Thus, the question arises as to what extent microhabitat selection by the larvae at settlement, independently of the presence of adults, might account for the geographic differences in local distribution observed. As larvae settle in microhabitats already colonized by adult conspecifics, in order to examine the cryptic response of the larvae at settlement in the two regions, the influence of adult barnacle density on settlement must be determined in addition to measuring the influence of substratum heterogeneity.

Experiments have shown that surface texture or roughness (Pomerat and Weiss, 1946; Barnes, 1956), surface contour (grooves and concavities; Gregg, 1948; Crisp and Barnes, 1954; Crisp, 1961) and intraspecific gregariousness, induced either by barnacle extracts or by the presence of the barnacles themselves (Knight-Jones and Stevenson, 1950; Knight-Jones, 1953, 1955; Knight-Jones and Crisp, 1953; Crisp, 1961; Crisp and Meadows, 1962, 1963;

Gabbott and Larman, 1971; Larman and Gabbott, 1975; Hudon *et al.,* 1983) influence settling cyprids in the choice of a settlement site.

However, few authors have examined simultaneously the relative influence of substratum heterogeneity and barnacle density on settlement. In a laboratory experiment, Crisp and Meadows (1963) showed that gregariousness was a more potent stimulus than small scale surface heterogeneity (pits). Indeed, more cyprids settled on slate panels treated with a barnacle extract than on pitted surfaces. Yule and Walker (1984) studied the effects of various treated surfaces on the temporary adhesion of barnacle larvae. Their results show that treatments can be ranked according to the forces required to remove cyprids from a surface: smooth < abraded < smooth soaked in barnacle extract < abraded and soaked in barnacle extract.

In this study, we examine the response of the settling barnacle larvae to the presence of conspecifics and to the influence of substratum heterogeneity in the field. More precisely, we test the hypothesis of regional differences in microhabitat selection by the larvae and further explore the gregarious response of the cyprids of *Semibalanus balanoides* at settlement. Our approach will be: (1) to offer to the settling larvae various combinations of barnacle densities and of scales of substratum heterogeneity (< 27 cm deep crevices) in the Gulf of St. Lawrence and on the Atlantic coast, and (2) to compare the settlement behaviour in artificially denuded and in intact natural crevices in the two regions. Different scales of substratum heterogeneity were examined because the larvae can change their behavioural patterns during the settlement process (Crisp, 1974). As aggregation is related to the presence of settled barnacles, scale sizes equivalent to that of barnacle populations (e.g. size of a crevice in the Gulf of St. Lawrence: roughly 30 cm deep) or individuals (< 1 mm) have been considered. In a companion paper, Le Tourneux and Bourget (1987) examined the cues used by the settling larvae from scales ranging from 1 m down to a few microns to determine the mechanism responsible for the patterns observed.

Materials and methods

Study area

The study was carried out in 1984 and 1985 at Andrews, New Brunswick, Canada (Lat. 45°04′N, Long 67°03′W), on the Atlantic coast of Canada and at Capucins, Québec (Lat. 49°03′N; Long 66°51′W), in the Gulf of St. Lawrence near the mouth of the Estuary (Fig. 1). The site at St. Andrews was located near the laboratory for convenience.

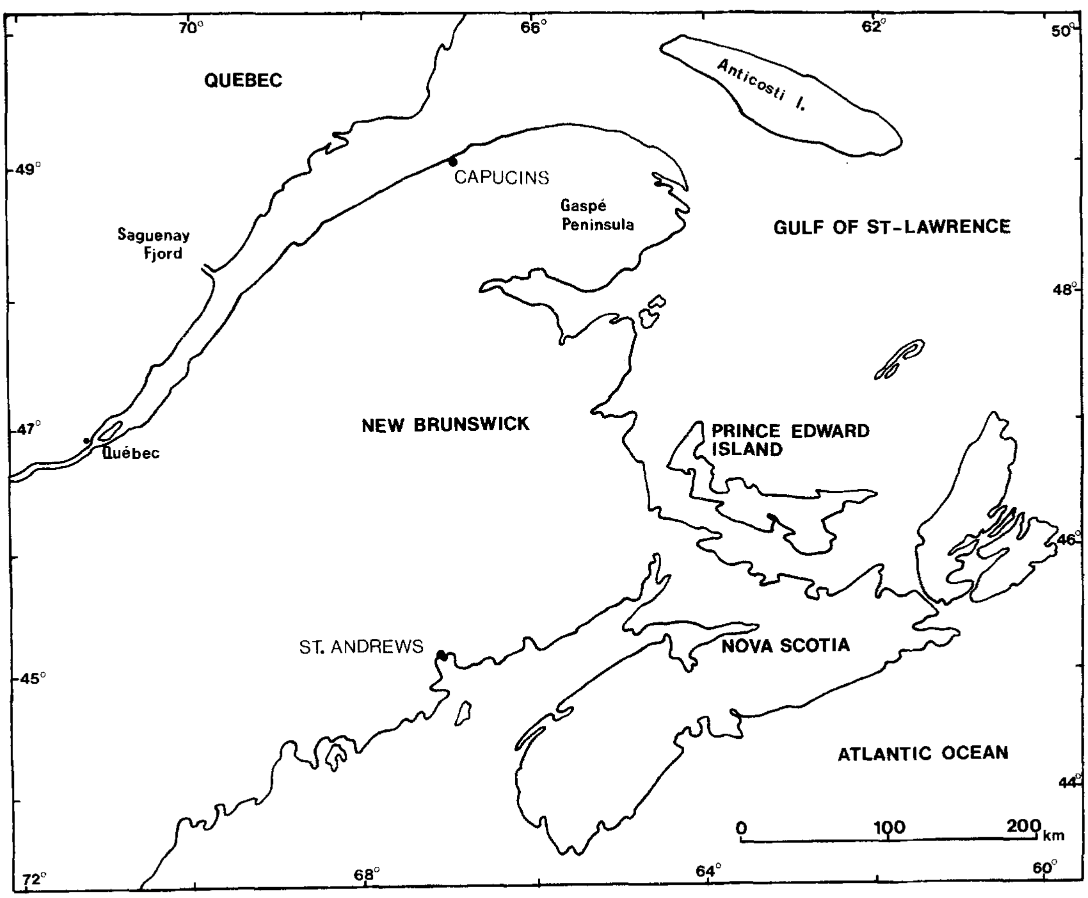

Fig. 1. Map showing the location of the study sites in the Gulf of St. Lawrence and on the Atlantic coast

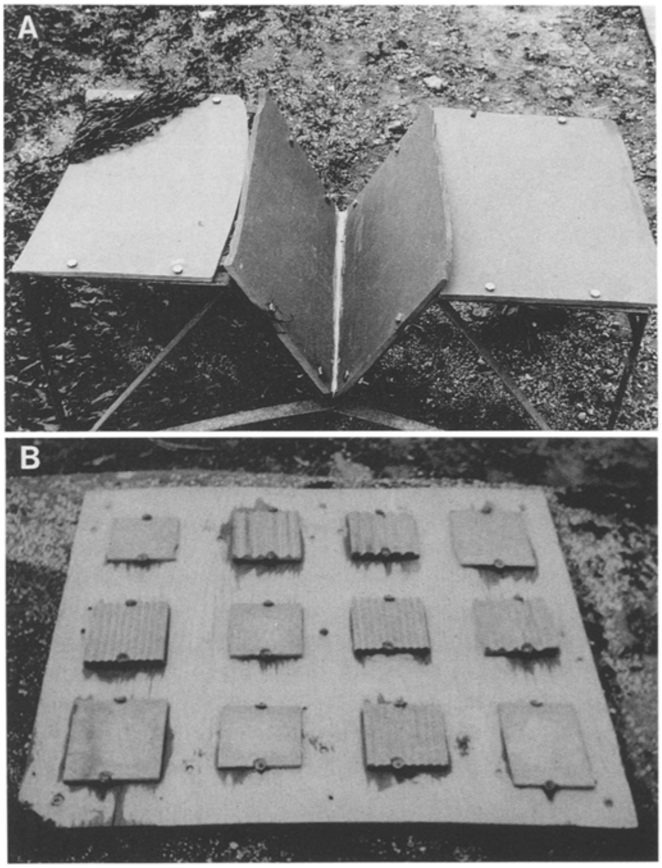

Fig. 2. Apparatus used in the experiments. (A) Artificial crevices used to examine the influence of large-scale heterogeneity and barnacle density on settlement. (B) Combination of smooth and grooved panels (colonized and uncolonized) used to examine the relative influence of small-scale (< 15 mm) heterogeneity and barnacle density on settlement

There, the rock structure ranges from sandstone to siltstone and barnacles are widely distributed in the mid-littoral zone. Other sites (Greens Point at the mouth of Passamaquoddy Bay) with different rock structures showed, nonetheless, the same overall pattern of barnacle colonization when compared quantitatively (E. Bourget, unpublished data). The settlement period of *Semibalanus balanoides* (L.) larvae occurred in May in 1984 and from mid-April to late May in 1985 (Bousfield, 1953). The site at Capucins consists of hard ordovician slate, forming smooth horizontal surfaces irregularly broken up by crevices. Barnacles are restricted to the walls of those crevices (Bergeron and Bourget, 1986). Similar patterns of colonization were observed on the north shore of the St. Lawrence where the substratum is granite. The settlement period occurred from early to mid-June in 1984 and 1985. Both sites were chosen for convenience and accessibility. The mean tidal amplitude is 5.6 m at St. Andrews and 2.5 m at Capucins and the mean water level is 4.07 m above lowest water of spring tide (LWST) at St. Andrews and 1.61 m above LWST at Capucins (Canadian Hydrographic Service, 1985).

Large-scale heterogeneity

Experiments using artificial crevices. To quantify the relative influence of substratum heterogeneity at a large scale (27 cm deep) and barnacle density on the settlement of *Semibalanus balanoides* cyprids, a two-treatment randomized factorial design (Kirk, 1982) was used. A total of eight artificial crevices (angle of 60° at the base, 26 cm deep) were built using slate panels (45 × 30 cm). Each crevice consisted of four contiguous panels (two placed horizontally on either side of the two forming the crevice itself) fastened to a metal frame with stainless steel bolts (Fig. 2A). The bottom of the crevices was filled with silicone caulking. The crevices were randomly assigned to sites previously chosen for their even surface, and comparable height in the mid-intertidal zone (eight sites from 4.97 to 4.16 m above LWST at St. Andrews and eight sites ca 2 m above LWST at Capucins). They were attached to the shore using stainless steel screws fastened into rawlplugs in the rock. Each crevice bore a total of 12 quadrats (10 × 10 cm), three on each of the two horizontal slate panels and three on each of the two slate panels forming the crevice walls. The quadrats were either bare or colonized by 50 barnacles prior to experimentation. The design contained all the possible combinations of conditions (Fig. 3): (1) pre-colonized quadrats within crevices and bare horizontal surfaces (two crevices); (2) pre-colonized quadrats on horizontal surfaces and bare crevice walls (two crevices); (3) pre-colonized quadrats inside and outside crevices (two crevices); and (4) uncolonized control quadrats (two crevices).

Pre-colonization of quadrats was achieved by drilling 50 regularly distributed pits (< 1 mm deep). A drop of barnacle extract was placed in each pit. Extracts were prepared with ground barnacles and sea water. All barnacles not settled at preselected sites were removed daily. During the experimental period, barnacles settling outside the pits were counted and removed daily using an entomological needle. Only one individual was left in each pit in order to maintain a constant number of settled barnacles. On each diurnal low tide, pre-colonized quadrats were covered by macroalgae to prevent barnacles from desiccation. This was necessary as the slate panels constituted a very poor heat sink. The experiments at St. Andrews lasted from 5 to 26 May in 1984 and from 22 April to 19 May in 1985 and at Capucins from 1 to 10 June during both years.

Experiments using natural crevices. In 1986, the influence of large scale heterogeneity and barnacle density was measured using natural rocky crevices at Capucins and St. Andrews. Cyprid settlement was compared in crevices colonized by adult barnacles and in artificially denuded crevices. Ten and nine pairs of crevices were selected in the mid-intertidal zone at Capucins and at St. Andrews, respectively. All crevices had angles of 60° to 90° at the base and their depth was comparable (Capucins: 8.69 ± 1.10 cm; St. Andrews: 8.37 ± 0.39 cm). At each site, half of the crev-

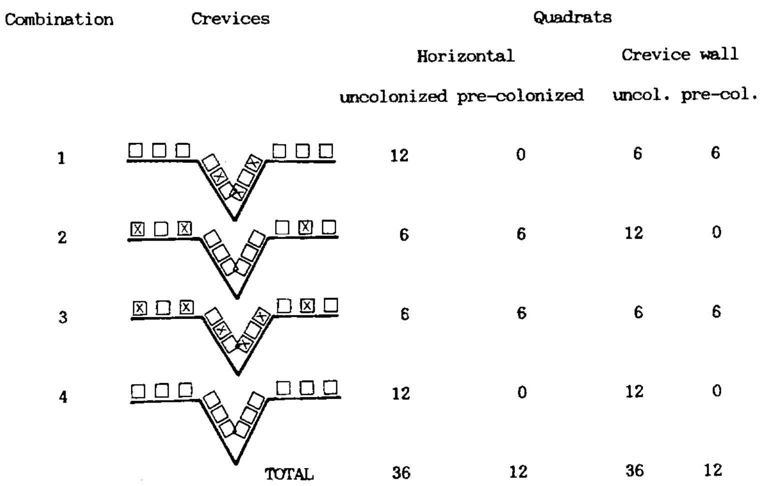

Fig. 3. Experimental design used to study the influence of large-scale substratum heterogeneity and barnacle density on the settlement of cyprids in artificial crevices. Each combination involves two crevices. —— horizontal panels, V crevice wall panels, □ uncolonized quadrats, ⊠ pre-colonized quadrats

ices and adjacent horizontal surfaces outside of crevices were completely denuded. The denuded crevices and adjacent horizontal surfaces were further cleaned using quicklime (Capucins), scraping and burning with a blow torch (St. Andrews), to remove any residual arthropodin cue. Quickline was not used at St. Andrews since the delay between denudation and the usual settlement season was too short. Both treatments are drastic and effective. Each crevice contained ten quadrats (10 × 10 cm) paired to ten others on adjacent horizontal surfaces (outside of crevices). Thus, beside being individually paired (denuded and intact), crevices were also paired with adjacent horizontal surfaces. The density of settlement was measured by counting the newly settled spat in the quadrats 10 d after the beginning of the settlement season, i.e. in June 1986. Each crevice had been photographed prior to denudation in order to estimate their initial adult density, thus ensuring that both denuded and control crevices offered comparable probability of settlement prior to treatment. The percentage of adult cover was evaluated by a random dot technique (Connell, 1961). One hundred computer-generated random points were placed on a (17.5 × 11.5 cm) plastic sheet. Three of these dotted sheets were placed successively on the (17.5 × 11.5 cm) photographs and points falling on a barnacle were counted and translated in % cover. An analogous technique has been used by Brault and Bourget (1985).

Small-scale heterogeneity

The influence of small scale heterogeneity (< 15 mm deep cracks) and barnacle density on settlement was quantified using a completely randomized factorial design (Kirk, 1982) in which slate panels (15 × 15 cm) with four different surface characteristics, namely ungrooved panels (two types) or panels bearing grooves 5- and 10-mm deep (90° angle at the base) were used (Fig. 2B). Each quadrat (10 × 10 cm), placed centrally on the panels to remove a border effect, had the same cm² area (e.g. panels with ten grooves, 5 mm-deep = panels with five grooves, 10-mm-deep). In 1984, 20 × 15 cm ungrooved panels (quadrats 13.5 × 10 cm) were also used to test the effect of increased total area due to grooves on settlement. These were discarded in 1985 and replaced with panels with 15-mm-deep grooves due to the insignificant effect of increased area observed in 1984. This experimental design also included three levels of barnacle density: (1) bare; (2) pre-colonized by 50; and (3) by 100 barnacles per quadrat of 10 × 10 cm. Thus, a total of 12 combinations were used in each experiment. At each location (Capucins and St. Andrews), plywood boards (two in 1984 and four in 1985) bore 12 equidistant panels randomly assigned to their positions on the board and secured to it with stainless steel screws and rubber washers. The boards were fastened into rawl-plugs onto the bedrock with stainless steel screws. They were set in the mid-intertidal zone at both stations (from 5.12 to 4.38 m above LWST at St. Andrews and at 2.25 m above LWST at Capucins). Pre-colonization was achieved by drilling 50 and 100 regularly distributed pits (< 1 mm deep) and by placing a drop of barnacle extract in each pit. Sampling procedures were the same as for the large scale heterogeneity experiment.

Observations on the influence of adult density on settlement

The relationship between larval settlement, percentage of adult cover and substratum heterogeneity was also investigated using 200 quadrats (10 × 10 cm), 100 at Capucins and 100 at St. Andrews. The quadrats were located at 2-m intervals along a tape placed parallel to the shoreline in the mid-intertidal zone. The quadrats were marked with plastic paint for their exact recognition and they were photographed at the end of the settlement period in 1985 (17 May at St. Andrews and 9 June at Capucins). In each quadrat, substratum heterogeneity was subsequently measured using a curvimeter (wheel diameter = 1 cm) rolled along ten lines (10 cm of straight linear distance), five

Table 1. *Semibalanus balanoides.* Analysis of variance for the effect of barnacle density and heterogeneity at a large scale (30-cm-deep crevices) on the total number of settled barnacle larvae in artificial crevices at St. Andrews. Data were log transformed. Normality was tested using the Kolmogorov-Smirnov test for goodness of fit ($D=0.0842$, $P>0.05$; Sokal and Rohlf, 1981). Variation due to error term is homogenous (F_{max}-test, $F=6.539$, $P>0.05$; Kirk, 1982)

Source of variation	DF	MS	F
Barnacle density	1	11.354	90.275**
Heterogeneity	1	8.823	70.127**
Interaction term	1	9×10^{-5}	0.001 ns
Error	92	0.126	–

** $P<0.01$; ns = $P>0.05$

Table 2. *Semibalanus balanoides.* Settlement of larvae on four types of quadrats in or near large artificial crevices at St. Andrews. $\bar{x}$ = mean number of barnacles (log transformed), SE = standard error. A Tukey-Kramer multiple comparison of means test for unequal samples (Kirk, 1982) was calculated. Only the comparison between uncolonized horizontal surfaces and pre-colonized crevices walls was not significantly different at $P>0.05$. All other pairwise comparison were significantly different at $P<0.01$

	Uncolonized $n=36$ $\bar{x}\pm$(SE)	Pre-colonized $n=12$ $\bar{x}\pm$(SE)
Horizontal surfaces	1.229 (0.054)	2.015 (0.109)
Crevice walls	0.619 (0.059)	1.421 (0.123)

Table 3. *Semibalanus balanoides.* Parameters of the multiple regression equations of the relation between the total number of settled larvae and the effects of barnacle density and large scale heterogeneity. 96 quadrats analysed; F: variance ratio; R^2: coefficient of multiple determination; Model a: $Y=b_0+b_1BAR+b_2Het$; Model b: $Y=b_0+b_1EXT+b_2Het$; Y: total number of larvae (log transformed); BAR: effect of attraction by barnacles; EXT: effect of attraction by extract; Het: effect of crevices; b_0: intercept $\pm$(SE); b_1 and b_2: partial regression coefficients $\pm$(SE)

Partial regression coefficient	Standard partial regression coefficient	R^2	F
Model a			
$b_0=$ 1.196 (0.056)	0		
$b_1=$ 0.036 (0.004)	0.608 (0.063)	0.638	82.1**
$b_2=-0.002$ (0.0003)	–0.435 (0.063)		
Model b			
$b_0=$ 1.226 (0.055)	0	0.635	81.0**
$b_1=$ 0.016 (0.002)	0.598 (0.063)		
$b_2=-0.002$ (0.0003)	–0.527 (0.063)		

** $P<0.01$

perpendicular and five parallel to the shore, on the denuded surfaces. A heterogeneity index was calculated using the mean ratio of the ten measured distances in each quadrat on the straight linear distances. The index was equal to 1 when the quadrats were flat and increased with surface relief. Analogous methods have been used by Dahl (1973) and Bergeron and Bourget (1986). The density of larval settlement was evaluated by counting the newly settled spat from the photographs. The percentage of adult cover was determined using a Complot Series 7000 digitizing tablet and the Bioquant Image Analysis System software interfaced on an Apple IIe microcomputer. For each photograph, the outline of the barnacle bases were traced with a stylus connected to the tablet so as to measure their area.

Results

Large-scale heterogeneity

Experiments using artificial crevices. The influence of gregariousness and large-scale heterogeneity on settlement of cyprids was examined using artificial crevices. The experiments were carried out at St. Andrews and at Capucins in 1984 and 1985. As settlement on the collectors was much too low at St. Andrews in 1985 and at Capucins in 1984 and 1985 for results to be meaningful, only results obtained at St. Andrews in 1984 are presented. A two-factor analysis of variance with unequal sample sizes showed significant effects of both factors on settlement at St. Andrews in 1984 (Table 1). Sample sizes were unequal because each of the 24 pre-colonized quadrats was paired to an uncolonized one and each of the 16 slate panels bearing these quadrats was also paired to an uncolonized one (Fig. 3). Thus, a total of 72 quadrats were bare and 24 were pre-colonized. An unplanned comparison among means test, the Tukey-Kramer procedure (Kirk, 1982), was used to test differences between treatments. Results are given in Table 2. Contrary to expectation, barnacles settled significantly more heavily on horizontal surfaces than on crevice walls. Moreover, precolonized surfaces, whether horizontal or in the crevices, showed greater settlement than uncolonized surfaces.

Two types of factors caused a gregarious response during this study, namely, attraction due to the barnacle extract in pits (EXT) and attraction due to mean number of barnacles settled in pits (BAR). Both effects were considered separately in the regression analyses because the expected number of barnacles in pits (50) was not always obtained and those two factors were strongly related. Thus, two multiple regression models were used to determine the relative contribution of each factor (extract or barnacle and substratum heterogeneity) to the settlement response. The results are shown in Table 3. Standard partial regression coefficients were necessary for the comparison of factors contributing to the variance of the number of settled larvae, as the variables were not

Table 4. *Semibalanus balanoides*. Comparison of larval settlement inside and outside of denuded and occupied crevices at Capucins and at St. Andrews in 1986. $\bar{x}$: mean number of larvae (of 10 quadrats) ±SE of the mean (SE). Adult cover are mean percentage ±SE. Differences between density of settlement inside and outside of crevices as between denuded and occupied quadrats were tested with the Wilcoxon signed-rank test. $N=398$ (Capucins); $N=344$ (St. Andrews). (Difference between inside and outside of crevices: $T_s=0$, $P<0.01$, Capucins; $T_s=6$, $P<0.01$, St. Andrews; difference between denuded and occupied quadrats: $T_s=23$, $P<0.01$, Capucins; $T_s=18$, $P<0.01$, St. Andrews; Sokal and Rohlf, 1981)

A Capucins

Crevice no.	Denuded: Adult cover prior to denudation % ± SE		Inside crevices $\bar{x}$ ± SE		Adult cover prior to denudation % ± SE		Outside crevices $\bar{x}$ ± SE		Occupied: Initial adult cover % ± SE		Inside crevices $\bar{x}$ ± SE		Initial adult cover % ± SE		Outside crevices $\bar{x}$ ± SE	
1	–	–	2.10	0.46	0.00	0.00	0.00	0.00	4.00	1.14	6.30	1.14	0.00	0.00	0.00	0.00
2	6.70	1.10	2.30	0.47	0.00	0.00	0.00	0.00	4.33	0.88	5.90	1.39	0.00	0.00	0.10	0.10
3	3.00	0.86	4.60	0.97	0.00	0.00	0.40	0.22	7.50	1.28	8.10	1.98	0.00	0.00	1.00	10.42
4	8.00	1.53	6.40	1.40	0.00	0.00	0.30	0.15	7.50	1.23	11.00	1.26	0.00	0.00	1.20	0.71
5	–	–	9.00	1.52	0.00	0.00	1.30	0.97	10.00	1.62	20.30	2.45	0.00	0.00	1.00	0.39
6	3.67	0.62	1.40	0.52	0.00	0.00	0.00	0.00	6.50	0.85	2.60	0.56	0.00	0.00	0.00	0.00
7	6.00	0.15	1.50	0.48	0.00	0.00	0.10	0.10	3.33	0.33	3.60	0.72	0.00	0.00	0.00	0.00
8	5.67	0.56	2.90	0.43	0.00	0.00	0.20	0.20	6.67	2.08	6.30	1.25	0.00	0.00	0.00	0.00
9	4.50	1.06	0.10	0.10	0.00	0.00	0.00	0.00	3.33	0.33	1.00	0.44	0.00	0.00	0.67	0.55
10	5.00	1.63	3.40	0.48	0.00	0.00	0.80	0.33	4.50	0.85	2.00	0.52	0.00	0.00	0.00	0.00
			Mean $\bar{x}$ ± SE				Mean $\bar{x}$ ± SE				Mean $\bar{x}$ ± SE				Mean $\bar{x}$ ± SE	
			3.42	0.35			0.31	0.11			6.78	0.67			0.39	0.11

B St. Andrews

Crevice no.	Denuded: Adult cover prior to denudation % ± SE		Inside crevices $\bar{x}$ ± SE		Adult cover prior to denudation % ± SE		Outside crevices $\bar{x}$ ± SE		Occupied: Initial adult cover % ± SE		Inside crevices $\bar{x}$ ± SE		Initial adult cover % ± SE		Outside crevices $\bar{x}$ ± SE	
1	11.67	1.56	0.30	0.21	–	–	1.30	0.52	18.33	0.92	14.70	1.81	–	–	24.90	4.68
2	16.83	2.10	0.40	0.22	19.17	3.11	2.20	0.57	11.83	1.20	4.90	1.35	13.33	1.12	6.90	1.19
3	13.17	1.35	32.90	4.09	18.83	1.51	64.10	6.89	19.50	2.41	78.30	6.89	16.17	1.11	233.60	24.54
4	2.17	0.83	0.00	0.00	0.00	0.00	0.00	0.00	11.50	0.96	0.50	0.22	0.00	0.00	4.10	1.37
5	20.83	1.58	17.50	4.77	16.83	1.74	55.50	9.68	14.67	2.12	22.10	2.38	14.17	0.87	45.80	4.39
6	–	–	33.40	6.78	–	–	48.30	6.34	25.33	3.27	74.10	7.65	–	–	147.40	14.55
7	19.33	1.20	141.17	18.52	29.00	1.15	206.83	20.93	24.67	0.33	195.83	7.01	27.00	1.53	303.67	17.29
8	6.17	1.14	0.00	0.00	6.00	0.97	0.00	0.00	5.88	0.55	0.20	0.20	5.50	0.56	1.10	0.50
9	21.17	1.87	42.20	10.17	15.17	1.78	83.00	13.24	19.00	1.39	77.20	7.23	10.67	4.49	67.10	21.52
			Mean $\bar{x}$ ± SE				Mean $\bar{x}$ ± SE				Mean $\bar{x}$ ± SE				Mean $\bar{x}$ ± SE	
			24.58	4.34			44.01	6.40			45.29	6.00			82.92	11.25

measured in the same units (Sokal and Rohlf, 1981, p 621). The effect was significantly greater (difference between standard partial regression coefficient was tested with a Student's *t*-test; $P<0.05$) than that of heterogeneity, but the presence of EXT did not have a greater influence on settlement than heterogeneity (Student's *t*-test, $P>0.05$). These models explained nearly 65% of the variance of larval settlement.

Thus, larval settlement on the Atlantic coast was greater outside than inside artificial crevices. Furthermore, the influence of presettled barnacle density was greater than that of heterogeneity at St. Andrews.

Experiments using natural crevices. Since the large-scale experiment with artificial crevices did not permit us to compare the settlement patterns at this scale in the Gulf of St. Lawrence and on the Atlantic coast, the influence of large scale heterogeneity on the settlement of cyprids on natural substratum was examined in 1986 in both regions. Results of the settlement response in denuded and occupied crevices are shown in Table 4. At both sites, the effect of adult barnacle density was significant as settlement was significantly greater in occupied crevices than in denuded ones. At Capucins, barnacle larvae settled nearly exclusively (93%) in crevices whether they were colonized

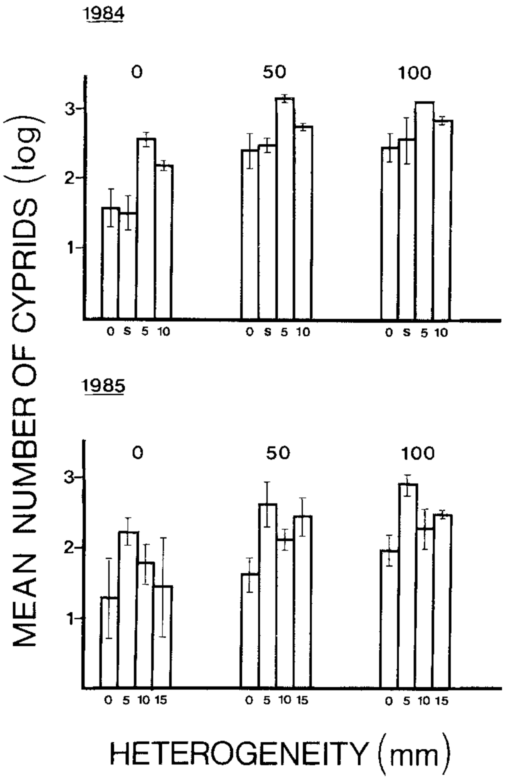

Fig. 4. *Semibalanus balanoides.* Cyprid settlement response (mean number of individuals) on quadrats (10 × 10 cm) of different levels of heterogeneity (0, 5, 10, 15 mm deep grooves) and of pre-colonization (0, 50, 100 ind dm^{-2}). Data were log transformed. S indicates the result of settlement on the control panels (heterogeneity = 0) for increased total area due to grooved surface (13.5 × 10 cm). 24 quadrats in 1984, 51 quadrats in 1985. Vertical lines represent standard errors of the mean

or not by adults. At St. Andrews, however, the microhabitat selection was opposite. Cyprids settled significantly more on horizontal surfaces than in crevices. The overall finding confirms our results obtained with the artificial crevice experiment at St. Andrews.

Small-scale heterogeneity

The influence of barnacle density and substratum heterogeneity along a small-scale gradient (0, 5, 10 and 15 mm deep grooves) was studied in 1984 and 1985 at St. Andrews and at Capucins. However, at the latter station, settlement was too low to allow any statistical comparison. For St. Andrews, ANOVA results shown in Table 5 indicate that both factors had a significant influence on settlement. No significant interaction could be detected. The mean values of settled barnacles for different levels of treatments are shown in Fig. 4. In 1984, all sample sizes were equal and the Student-Newman-Keuls procedure (Kirk, 1982) was used to analyse the differences between means. The comparison between larval settlement using the levels of 50 and 100 barnacles per quadrats showed no significant difference ($P > 0.05$). Similarly, no significant

Table 5. *Semibalanus balanoides.* Analysis of variance for the effect of barnacle density and heterogeneity at a small scale (< 15 mm deep cracks) on the total number of settled larvae on slate panels at St. Andrews. Data were log transformed. Normality was verified using the Kolmogorov-Smirnov test for goodness of fit (D = 0.1130, $P > 0.05$, 1984; D = 0.0749, $P > 0.05$, 1985; Sokal and Rohlf, 1981). Homogeneity of variances was tested using the Bartlett's test ($\chi^2 = 15.667$, $P > 0.05$, 1984; $\chi^2 = 4.663$, $P > 0.05$, 1985; (Sokal and Rohlf, 1981)

Source of variation	DF	MS	F
(A) 1984			
Barnacle density	2	1.582	49.60**
Heterogeneity	3	0.822	25.79**
Interaction term	6	0.034	1.08 ns
Error	12	0.032	–
(B) 1985			
Barnacle density	2	1.984	19.46**
Heterogeneity	3	2.225	21.82**
Interaction term	6	0.094	0.93 ns
Error	39	0.102	–

** $P < 0.01$; ns = $P > 0.05$

difference in settlement could be observed between the two types of ungrooved panels (large 13.5 × 10 cm and small quadrats 10 × 10 cm) ($P > 0.05$) using the SNK test. Thus, the increase in total area due to the grooves on the rough plates had no significant influence on settlement. In 1985, sample sizes were unequal, so the comparison of means was performed using Scheffe's S procedure (Kirk, 1982). No significant differences in settlement occurred between 10- and 15-mm grooved panels ($P > 0.05$). All other comparisons led to significant differences at $P < 0.05$, i.e. settlement was significantly greater on grooved (5, 10 mm) than on ungrooved panels and on precolonized (50, 100 ind dm^{-2}) than on uncolonized panels. The greatest settlement response for either years was observed on panels with 5-mm-deep grooves.

As in the previous experiment, two types of factors caused a gregarious response (BAR and EXT) and were examined separately to assess their influence on settlement. Table 6 shows the results of the multiple regression analyses. A comparison of the standard partial regression coefficients indicates that, in 1984, the mean number of barnacles had a greater influence on settlement than heterogeneity (Student's *t*-test, $P < 0.05$). This model explained 71% of the variance of the total number of settled larvae (Y). In the other model (EXT + Heterogeneity), 59% of the variance was explained.

The effect of extract was not significantly different from that of heterogeneity (Student's *t*-test, $P > 0.05$). In 1985, however, the contribution of either BAR or EXT were not as important as that of the previous year, presumably because of the lower level of precolonization reached prior to experimentation. No significant difference between the effect of gregariousness (BAR or

Table 6. *Semibalanus balanoides.* Parameters of the multiple regression equations of the relation between the total number of larvae (log transformed) and the effect of small scale heterogeneity and barnacle density. 24 quadrats analysed in 1984; 51 quadrats analysed in 1985; Model a: $Y = b_0 + b_1 BAR + b_2 Het$; Model b: $Y = b_0 + b_1 EXT + b_2 Het$; Mean number of presettled larvae: 38±3.7, in 1984; 22.2±3.6, in 1985. Difference between mean number of presettled larvae was tested with the Mann-Whitney test ($t_s = 2.97$, $P < 0.01$; Zar, 1974). Abbreviations as in Table 3

Partial regression coefficient	Standard partial regression coefficient	R^2	F
(1) 1984			
Model a		0.714	26.2**
$b_0 = 1.809$ (0.109)	0		
$b_1 = 0.018$ (0.003)	0.735 (0.117)		
$b_2 = 0.056$ (0.014)	0.459 (0.117)		
Model b		0.586	14.9**
$b_0 = 1.870$ (0.130)	0		
$b_1 = 0.008$ (0.001)	0.642 (0.140)		
$b_2 = 0.051$ (0.017)	0.418 (0.140)		
(2) 1985			
Model a		0.159	4.5*
$b_0 = 1.678$ (0.141)	0		
$b_1 = 0.007$ (0.004)	0.259 (0.154)		
$b_2 = 0.040$ (0.014)	0.408 (0.154)		
Model b		0.351	13.0**
$b_0 = 1.487$ (0.126)	0		
$b_1 = 0.007$ (0.002)	0.500 (0.116)		
$b_2 = 0.031$ (0.011)	0.317 (0.116)		

** $P < 0.01$; * $P < 0.05$

EXT) and the effect of heterogeneity (Student's *t*-test, $P > 0.05$) was observed.

Observations on the influence of adult density on settlement

Since the small-scale experiments (with artificial substrata) did not lead to valid comparisons of settlement patterns between the two regions studied, field observations were carried out to examine the relationship between larval density and percentage of adult cover and small-scale substratum heterogeneity. Settlement in 100 quadrats, partially colonized by adults, was examined at both locations. In the quadrats studied, larval density was higher at St. Andrews (6.71±0.17 larvae cm^{-2}) than at Capucins (0.24±0.02 larvae cm^{-2}). The latter density overestimates greatly the overall abundance of barnacles in the field since quadrat location had to be chosen to include adults to measure the effect of adult density. Prior to any assessment of the relative influence of barnacle density and heterogeneity on settled larval density, the relationship between settlement and adult cover was examined. Curve-fitting methods were utilized with the NLIN (Non LINear) least squares fitting procedure (Ihnen and Goodnight, 1985) to determine which model fitted best this relationship.

At St. Andrews, a quadratic equation was determined:

$$Y = -21.33X^2 + 12.68X + 5.42, \tag{1}$$

where Y is the larval density (number of larvae cm^{-2}) and X is the percentage of adult cover. This model was significant at $P < 0.01$ (F ratio = 5.41; $n = 99$). It explained 16% of the variance of Y. The model shows that after an initial increase in the settlement response with increasing adult cover (up to 30%), a decrease of settlement density occurs presumably because of an avoidance of well colonized sites.

At Capucins, the best fitting regression equation was a negative exponential model:

$$Y = 0.323\, e^{-22.39X}. \tag{2}$$

This relationship indicates that the settlement of larvae would rapidly reach a plateau, since the curve becomes asymptotic when the adult cover reaches about 15%. This model gave the least residuals ($R^2 = 0.21$), probably because settlement remains low in this region of the Gulf of St. Lawrence and values of adult cover necessary to trigger an avoidance of the sites have not been observed there. Fig. 5 A shows that at Capucins 95% of the quadrats had values of adult cover under 22%. A quadratic response would probably have been obtained had a larger number of quadrats with greater adult cover been measured. Here, the overall weight of the values below 22% most probably determines the fit obtained. It is interesting to examine the best quadratic equation fitting the data. It is:

$$Y = -5.68X^2 + 2.47X + 0.08, \tag{3}$$

This model explained 18% of the variance of larval density. The inflexion point of the two quadratic functions was sought by equalling the derivative to 0, in order to determine the maximum density of settlers. This point was reached at 29.7% of adult cover at St. Andrews and 21.7% at Capucins.

The positive portion of both curves (i.e. adult cover lower than 22% at Capucins and 30% at St. Andrews) was withheld for further analysis. In this portion of the curve it is presumed that gregariousness is not influenced negatively by other factors such as a limitation of available space for settlement. Linear regressions were calculated. Results are shown in Fig. 5 A. Comparison of slopes carried out using an F-test (Sokal and Rohlf, 1981) indicates that the gregarious response at St. Andrews was significantly greater than that at Capucins (F = 5.26, $P < 0.05$).

Multiple regression analyses were carried out to examine the relative influence of adult cover and small-scale heterogeneity (< 1 cm) on the settlement response. The parameters of the regression equations at both sites are indicated in Table 7. Standard partial regression coefficients were used for determining the influence of adult cover and heterogeneity. The results show that the in-

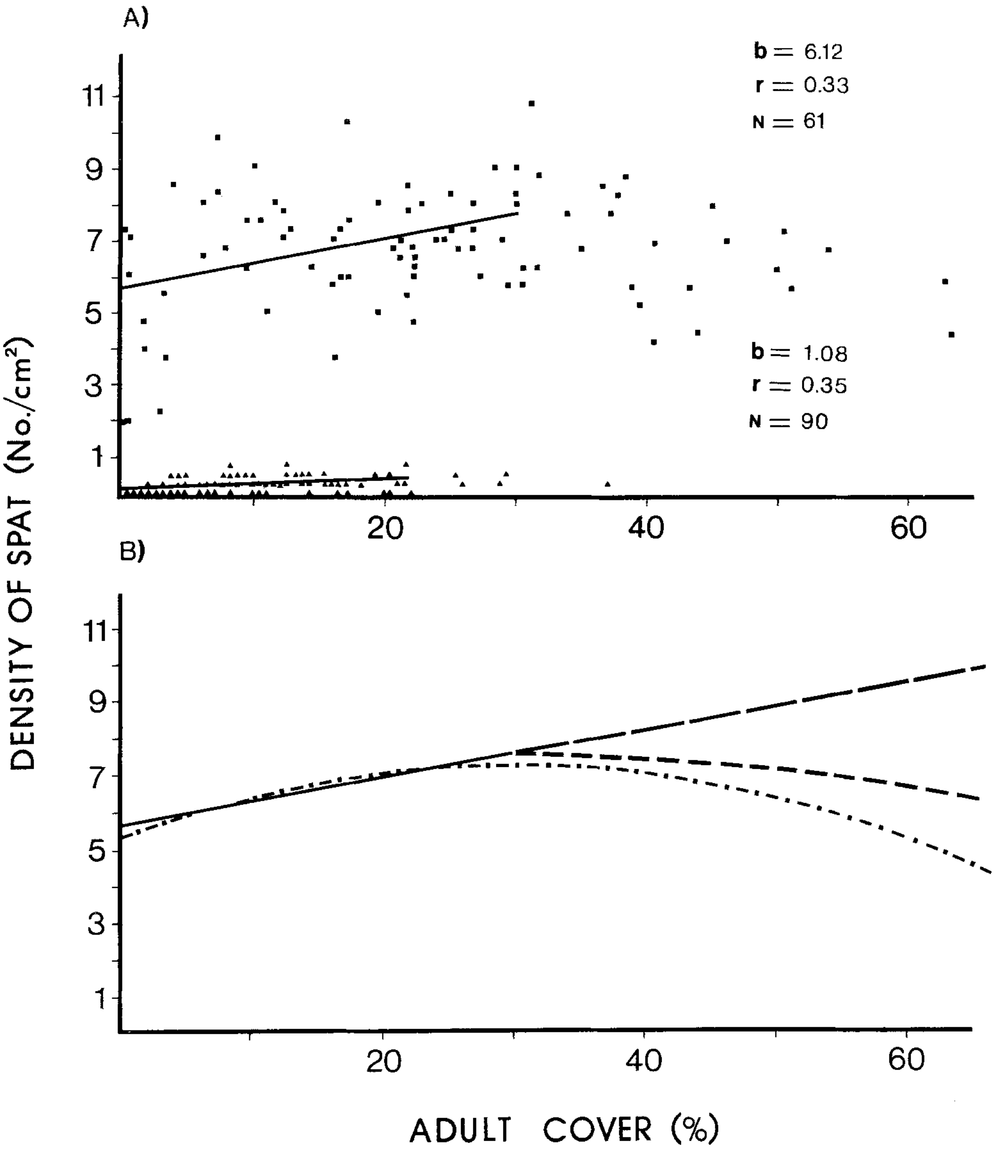

Fig. 5. *Semibalanus balanoides.* Effect of adult cover on spat density. (A) Relationship between adult cover (%) and density of spat at St. Andrews ■ and Capucins ▲. The linear regressions were calculated only on the positive portion of both curves. In this portion, it is presumed that gregariousness is not influenced negatively by other factors. b: slope of the regression lines; r: correlation coefficient; N: number of quadrats included in the analysis. (B) Expected and observed relationships between adult cover and density of spat at St. Andrews. The expected reduction of density of spat is calculated as if it was due only to the reduction of free space. Influence of adult density: ————; expected reduction of density of spat: — — — —; observed relationship: —·—·—

Table 7. *Semibalanus balanoides.* Parameters of the multiple regression equations of the relation between larval density and % of adult cover (X) and substratum heterogeneity (Het). Only quadrats with adult cover below 30% at St. Andrews and 22% at Capucins were retained for the analysis. 90 quadrats were analysed at Capucins, 61 quadrats were analysed at St. Andrews; F=6.30, $P<0.01$ (Capucins); F=3.71, $P<0.05$ (St. Andrews); $R^2=0.125$ (Capucins); $R^2=0.112$ (St. Andrews); b: partial regression coefficient ±(SE); st.b: standard partial regression coefficient ± (SE); b_X are significant ($P<0.05$), b_{Het} are not significant ($P>0.05$)

b_X	st.b_X	b_{Het}	st.b_{Het}
Capucins			
1.075 (0.323)	0.332 (0.100)	−0.023 (0.020)	−0.116 (0.100)
St. Andrews			
6.421 (2.360)	0.344 (0.126)	0.283 (0.537)	0.067 (0.126)

fluence of barnacle density is more important than that of small-scale heterogeneity at both sites, the contribution of the latter to the variance of larval settlement not being significant at $P>0.05$.

Discussion

Our objectives in carrying out this study were to determine: (1) the relative importance of two major factors influencing the settlement of *Semibalanus balanoides* larvae, the presence of conspecifics and substratum heterogeneity at scales considered by the larvae during broad exploration and close exploration, and (2) whether differences of local distribution observed between the Gulf of St. Lawrence and the Atlantic coast result from differences in microhabitat selection by barnacle larvae.

Influence of large-scale heterogeneity and barnacle density

At Capucins, cypris larvae of *Semibalanus balanoides* settled nearly exclusively (93%) in natural crevices, avoiding adjacent horizontal surfaces. This result is analogous to that of Bergeron and Bourget (1986) who observed 97% of the larvae settling in crevices. The finding reported here is that this microhabitat selection also persists strongly in denuded crevices. Therefore, our results tend to indicate that cryptic behaviour is indeed the predominant factor determining patterns of distribution in the Gulf, the larvae displaying a definite preference for crevices at settlement. In contrast to the Gulf, on the Atlantic coast (St. Andrews), barnacle larvae settled significantly more on horizontal surfaces than in crevices (on uncolonized and on colonized surfaces) either when the substrata were artificial (slate panels) or natural (rocky surface). Further, settlement on bare horizontal surfaces was comparable to that on pre-colonized surfaces in crevices. This habitat selection by cyprids differs from that reported by Wethey (1984), in which barnacle larvae settled preferentially in cracks. However, since the dimensions of cracks are not given, it is impossible to compare this result with ours. Our result from the Atlantic coast also contrasts markedly with all known reports, which show that barnacle larvae settle preferentially in depressions of the substratum (see Crisp, 1974).

Our results are also clear in showing that the presence of conspecifics had a significantly greater effect on settlement than did large-scale heterogeneity on the Atlantic coast. These results support the hypothesis of different microhabitat selection by the larvae at settlement between the Gulf of St. Lawrence and the Atlantic coast. In a companion paper, Le Tourneux and Bourget (1987) examined the mechanisms accounting for the patterns of distribution of settled larvae observed in the two regions.

On the Atlantic coast, the combined effect of larvae settling preferentially outside crevices and of enhanced settlement due to gregariousness results in widespread distribution in the mid-intertidal zone.

The small-scale heterogeneity experiment in 1984 showed that the effect of pre-colonization by juveniles had a stronger influence on settlement than heterogeneity. These results are comparable to those of Crisp and Meadows (1983) and Yule and Walker (1984) using active barnacle extracts. However, since chemical cues are short-lived in the field if not replenished (Wethey, 1984), it is not surprising that in our experiments barnacle extracts were not more effective than heterogeneity.

The multiple regression model used to examine the relative influence of heterogeneity and barnacle density on larval settlement explained less variance in 1985 than in 1984. The mean number of barnacles in pits was significantly lower in 1985, which may explain why the effect of barnacle density was not more important than heterogeneity that year. The overall result is clear: the influence of small-scale heterogeneity is never more important than the influence of barnacle density.

When considering only the effect of small-scale heterogeneity, there was definitely a preference for smaller cracks (5 mm), indicating the importance of scale size similar to that of the larvae. Further experiments on larval preferences in relation to substratum heterogeneity should consider surface contour down to a few microns to determine if other scales of heterogeneity are effective in the settlement process (Le Tourneux and Bourget, 1987).

Comparison of spatial patterns of settlement among sites

Using the number of spat at a given time during the settlement season as an indicator of the settlement intensity, it appears that the presence of conspecifics was a positive stimulus for settlement until 22 to 29% of the free space was covered by adults at both sites (Fig. 5 A). Above this percentage of adult cover, a decrease in the settlement response occurred. Such a reduction of settlement could not be detected in the natural crevices experiment. Indeed, in the occupied crevices, adult densities never exceeded 29% of surface cover above which settlement decreases. Further, the maximal surface occupied by the spat (3%) was obtained when adult reached maximal cover (27% of surface cover). In the denuded crevices, maximal spat cover was too low (2%) to have a negative influence on the settling cyprids (Table 4). An analogous reduction of settlement was observed by Crisp (1961) in a laboratory experiment at densities greater than ten individuals per cm^2, a density considerably higher than that observed in our study ($0.9\ cm^{-2}$). This reduction of settlement when % of adult cover is high can be explained either by reduction of free space or by some kind of repulsion.

It is not too difficult to estimate whether the reduction in settlement above 30% observed in the field at St. Andrews is due to reduced available surface or indeed to a genuine repulsion. If we assume that the influence of gregariousness remained the same up to 100% total adult cover and no negative effect of increased abundance of adult was observed, then the expected settlement can be extrapolated from the initial slope of settlement (up to 30%). If we now assume that no negative effect other than that due to reduced free space counteract settlement (at St. Andrews, only 4.02 ± 0.85% of the larvae settled on conspecifics), then the expected number of spat can be calculated by inferring that a given % available space reduction will induce the equivalent % reduction in larval settlement. The results of this estimation, shown in Fig. 5 B, indicate that the observed reduction is most probably caused by the reduction of free space, since both the expected and the observed curve decreased in the same manner. This result is interesting since it shows that larvae of *Semibalanus balanoides* avoid settling on conspecifics. This result, therefore, is akin to the results obtained by Moyse and Hui (1981). Presumably, reduction of available space diminishes survival probability. This behaviour is analogous to that encountered when larvae select substrata of different qualities (Doyle, 1975).

A comparison of the relationship between larval density and adult cover seems to show that the effect of the presence of conspecifics is stronger on the Atlantic coast than in the Gulf. Indeed, (1) the slopes of the regression lines indicate that larval settlement increases significantly more rapidly at St. Andrews than at Capucins and (2) the inflexion point is reached more rapidly at Capucins than at St. Andrews.

Results of the multiple regression showed that gregariousness was more important than small-scale heterogeneity (1 cm) in the Gulf and on the Atlantic coast, thus confirming the results of the small-scale heterogeneity experiments. Those results should be interpreted with some care, however, since the variables (dependent variable = larval density and, independent variables = adult cover and heterogeneity) are subject to natural variability and measurement error.

Our results show that heterogeneity is important for barnacle settlement at large scales (e.g. 20 cm), in the Gulf of St. Lawrence, i.e. presumably during the broad exploration phase (Crisp, 1974), but the presence of conspecifics is much more important than heterogeneity at the smaller scales (1 cm) corresponding to the scales sampled during close exploration of the larvae prior to definitive settlement.

Concerning geographic variations in patterns of settlement, our results confirm the hypothesis of greater larval selection for cryptic habitats in the Gulf of St. Lawrence than on the Atlantic coast. Whether these differences are genetically or environmentally mediated is still unclear. On one hand, given the known plasticity of *Semibalanus balanoides,* it is easy to imagine that under selective pressures such as those occurring outside crevices in the semi-enclosed Gulf of St. Lawrence (>95% mortality on juveniles due to ice-scouring), the evolutionary shifts could be rapid and substantial. On the other hand, such a large number of environmental factors have been reported to influence barnacle microhabitat selection (although usually very locally), that further work will need to be carried out before we can subscribe to one or the other hypothesis (but see Le Tourneux and Bourget, 1987).

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