

# Recruitment of marine invertebrates onto arborescent epibenthic structures: active and passive processes acting at different spatial scales

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**ABSTRACT:** The hypothesis that passive processes control larval settlement of benthic marine invertebrates on 3-D structures was tested for several groups at both meso (3 cm) and small (mm,  $\mu$ m) scales. Flume experiments were carried out with inert polyvinylchloride microparticles and 5 different types of silicone-coated 3-D arborescent plastic structures with 5 distal branch diameters. Microparticle attachment as a function of the distal branch diameter and the proportion of particles attached to the nodal section of each branch were used to predict the initial contact site by living marine benthic invertebrates larvae in field experiments using the same 3-D structures immersed for 2 mo. The flume experiment showed that distal branch diameter had a significant effect on the density of attached particles. The proportion of particles observed in the nodal sections (8%) was much lower than the proportion of the total surface area occupied by the nodes (20%) on each branch. Field experiments examining recruit density (6 sessile species belonging to 4 classes of marine invertebrates) on the same 3-D structures showed the same significant effect of the distal branch diameter observed in the flume. However, individuals were found in the nodal sections much more frequently (35 to 80%) than would be expected based on the results of the flume experiment (8%). Comparison of results of laboratory and field experiments suggests that passive settlement processes are sufficient to explain early recruitment patterns of the species examined on scales ca 3 cm. However, at smaller scales (ca mm) the hypothesis of active larval exploration of the substratum could not be rejected. The likelihood of post-settlement mortality processes potentially explaining the patterns observed is discussed.

**KEY WORDS:** Larval settlement · Recruitment · Benthic invertebrates · Field experiment · Flume experiment · Passive and active processes

## INTRODUCTION

Four successive phases lead to the colonization of habitats by marine invertebrates with planktonic larvae: larval growth and dispersal; determination of habitat quality; settlement; and metamorphosis (Keough & Downes 1982, Bourget 1988). The settlement phase is considered passive when it is predominantly controlled by hydrodynamics or active when the larvae select mi-

crohabitats using chemical, biological or physical cues (Crisp 1974, Bourget 1988, Butman 1989, Pawlik 1992). The process may also involve both active and passive processes acting at different spatial scales (Butman 1987, Boudreau et al. 1993, Harvey et al. 1993).

A variety of approaches have been used to document these processes. In general, indirect methods compare abundance patterns of settled larvae between experimental and control situations to determine whether the larvae have selected or rejected a particular substrata. A novel approach has recently been developed (Hannan 1984), where the distribution of settled larvae is compared to inert particles which have a fall velocity similar to larvae. If the distributions differ, then a behavioural hypothesis for selection of habitat can be proposed. This approach has resulted in numerous

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studies of flow near surfaces and enhanced our understanding of the influence of flow on larval settlement at small scales (see Eckman 1983, 1987, Mullineaux & Butman 1991, Mullineaux & Garland 1993, Pawlik & Butman 1993). With few exceptions (Eckman 1983, Harvey et al. 1995a), these studies were carried out using flat or nearly flat 2-D panels on which the larva could come into contact with the substrata more than once and further explore the substrata. Larvae either passively accepted the initial contact site or actively explored the settlement site.

Few studies have examined the relationship between larval settlement and filamentous or arborescent substrata, though these structures constitute an important category of substrata for a variety of marine epifauna. An interesting feature of such substrata, which are fully exposed to the current flow, is that they offer no 'second chance' to the settling larva should the larva be eroded away or decide to explore other sites by swimming away. On such substrata site selection should primarily result from passive entrainment or benthic larval exploration after contact. Further, the filamentous or arborescent structures consist of cylindrical rod-like units, and contain a variety of physical gradients (e.g. of shear stress) from which a larva can select. Thus, such structures offer a unique opportunity to detect responses of larval which occur during the pre-attachment phase. An additional advantage of such structures is that the flow characteristics around cylindrical rod-like elements are theoretically and experimentally well documented, aiding the understanding of the physical processes involved in the settlement phase.

In a previous study, Harvey et al. (1995a), using 3-D arborescent structures 20 cm in height, showed that a passive entrainment (hydrodynamic) model could explain spatial patterns of abundance of larvae of 4 species of bivalves at a scale of 10 to 20 cm. In the present study, we used the same 3-D structures to test the hypothesis of passive settlement (controlled by hydrodynamic processes and predicted from the patterns of inert particle contact) of larvae of sessile invertebrates, for 2 spatial scales smaller than that previously tested (ca 3 cm, the scale of the branches of the 3-D structures, and ca 1 mm scale, within the branches). We feel the latter provides a stringent test for a behavioural component in the final phase of the settlement process since the arborescent structures offer steep gradients to the settling larvae (e.g. nodes at the junction of branches, shear stress gradients), and the behavioural response to these gradients is likely to be detectable quantitatively due to the imposed bi-directionality of any migratory response of the larvae after contact with a given cylindrical element of the structure.

## METHODS

### Experimental procedures. *Laboratory experiments:*

Flume experiments were carried out with inert polyvinylchloride (PVC) microparticles and 3-D plastic structures similar in shape to ramose hydroids and filamentous benthic macroalgae, organisms colonized by larvae of numerous marine benthic invertebrate species such as bivalves, encrusting bryozoan, hydroids, and sedentary polychaetes.

Assays were conducted in a recirculating, temperature-controlled (10°C), seawater flume (described by Harvey et al. 1995a) located in the Laboratoire d'Hydraulique, Département de Génie Civil, Université Laval. The flume channel was 12 m long, 60 cm wide, and 60 cm deep. Flow from the channel discharged into a sump downstream and was recirculated by a centrifugal pump through a 15 cm diameter pipe connecting the sump to the head tank of the flume. Three baffles mounted serially in the downstream part of the head tank rectified the flow at the entrance of the flume channel. The experimental structures were 3.6 m downstream of the flume channel entrance. Experiments were carried out at a flow rate of  $5 \text{ cm s}^{-1}$ , which corresponds to the average near-bottom current speed measured in the field (Fig. 1) and used in previous flume experiments by Harvey et al. (1995a, b). Since the boundary layer (3.5 cm, as calculated from Nowell & Jumars 1987) is a small portion of the channel water depth, the structures (20 cm in height) were mostly located above the boundary layer in a frictionless flow

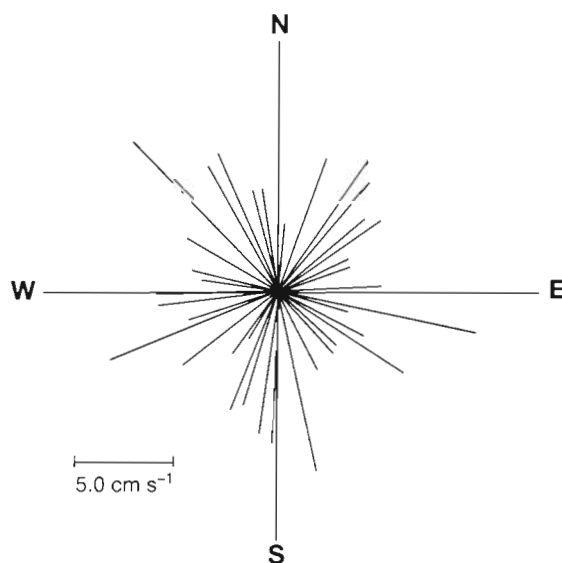


Fig. 1. Mean velocity as a function of direction measured at 30 cm above the bottom. Each line represents the average velocity for each  $10^\circ$  sector during the sampling period. Current velocity and direction were collected over a 1 min period every 10 min

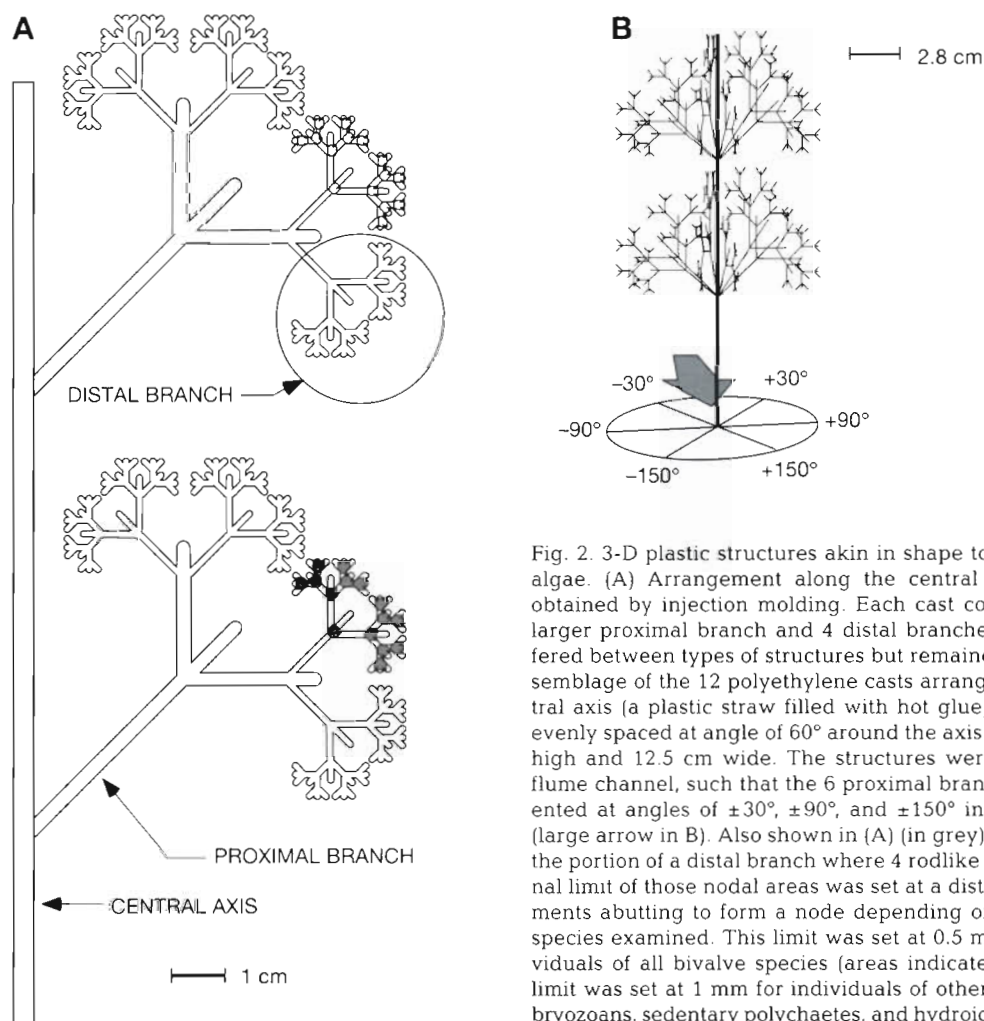


Fig. 2. 3-D plastic structures akin in shape to hydroids and benthic macroalgae. (A) Arrangement along the central axis of 2 polyethylene casts obtained by injection molding. Each cast consisted of 2 parts including 1 larger proximal branch and 4 distal branches whose branch diameter differed between types of structures but remained uniform within type. (B) Assembly of the 12 polyethylene casts arranged together such that the central axis (a plastic straw filled with hot glue) supported 2 levels of 6 casts evenly spaced at angle of  $60^\circ$  around the axis. Each 3-D structure was 27 cm high and 12.5 cm wide. The structures were placed, in the centre of the flume channel, such that the 6 proximal branches from each level were oriented at angles of  $\pm 30^\circ$ ,  $\pm 90^\circ$ , and  $\pm 150^\circ$  in relation to the flow direction (large arrow in B). Also shown in (A) (in grey) are the node areas, defined as the portion of a distal branch where 4 rodlike segments intercept. The external limit of those nodal areas was set at a distance of 0.5 or 1 mm along segments abutting to form a node depending of the size of individuals of the species examined. This limit was set at 0.5 mm for microparticles and individuals of all bivalve species (areas indicated in light grey), whereas this limit was set at 1 mm for individuals of other species including: encrusting bryozoans, sedentary polychaetes, and hydroids (areas indicated in dark grey)

regime. The bottom of the channel was hydraulically smooth for the flow rate used in our experiments ( $5 \text{ cm s}^{-1}$ , roughness Reynolds' number,  $Re < 3$ ). The flow in the working section was not fully developed (i.e. the boundary layer had not grown through entire water depth), and so conditions in the flume did not exactly replicate those in the field, where the experimental structures were assumed to be within the boundary layer. This difference in prevailing conditions in the flume and the field may limit comparisons. However, at the scale of the experimental structures, motion is essentially an unsheared, frictionless flow in both cases.

Larvae were simulated using fluorescent inert PVC microparticles (described in Harvey et al. 1995a) with a diameter (150 to 250  $\mu\text{m}$ ) similar to that of marine benthic invertebrate larvae. Particles were introduced uniformly across the channel section of the flume through an array of 16 injection points (see methods in Harvey et al. 1995a). The width-to-depth ratio of our flume experiment (60 cm wide channel/40 cm deep water column = 1.5) violates the recommended mini-

mum width-to-depth ratio of 5 or 6 for 1-dimensional open-channel flow (e.g. see Nowell & Jumars 1987) creating a possible cross-stream circulation effect on particle distribution. This effect was previously investigated by Harvey et al. (1995a) using 5 glass rods (60 cm long) coated with a thin layer (ca 0.25 mm) of silicone grease, and installed vertically every 5.0 cm from 17.5 to 42.5 cm across the open section of the flume channel. The total number of particles attached to the glass rods over a 10 min period did not differ significantly across the section of the channel (Harvey et al. 1995a). A 100  $\mu\text{m}$  plankton net was placed in the downstream part of the channel to prevent particle recirculation into the flume.

The 3-D structures used in these experiments consisted of 3 parts. The first 2 parts were identical in form for all structures, consisting of a central axis and proximal part of ramifications (proximal branches), and the third, the distal part of ramifications (distal branches), was composed of a series of rodlike segments arranged together as opposite branches (Fig. 2A). Thus, a com-

plete structure was composed of 1 central axis, 12 proximal branches, and 48 distal branches (4 distal branches per proximal branch) arranged together such that the central axis supported 2 levels of 6 proximal branches evenly spaced at angles of  $60^\circ$  around the axis (Fig. 2B). A more detailed description of these structures is available in Harvey et al. (1995a). Five different types of structures were used in the present study. They were obtained by combining 1 pattern of distal branch ramifications (the more elaborate branching system of Harvey et al. 1995a) and 5 distal branch diameters. The total area available for particle attachment increased as a function of the distal branch diameter.

Distal branches of the structures were coated with a thin layer (ca 0.25 mm) of silicone grease (Dow Corning high vacuum grease) before installation in the flume to ensure adhesion of the microparticles. The five 3-D structures were placed, one at a time, in the centre of the flume channel 5 m downstream from the entrance of the channel. The structures were placed with the 6 proximal branches from each level oriented at angles of  $\pm 30^\circ$ ,  $\pm 90^\circ$ , and  $\pm 150^\circ$  in relation to the flow direction (see Fig. 2B). Each trial lasted 10 min. The number of particles being captured during this period was far below the number of particles necessary to saturate the surface of the structure but high enough to yield meaningful statistics. Microparticles adhering to the 4 distal branches belonging to each proximal branch were counted under a black light (Philips TLD 18 W/08 fluorescent tube). Particles attached to the straight parts of the rodlike segments and in the region of each node were counted separately. A nodal region is defined as the portion of a distal branch where 4 rodlike segments intercept. The external limit of this section was arbitrarily set at a distance of 0.5 mm along each of the 4 segments forming a node (see Fig. 2A). This distance was twice the diameter of the largest particles used in our experiments (250  $\mu\text{m}$ ). This experiment was replicated on 2 different days.

**Field experiments:** Field experiments were carried out near Port-Daniel, Baie des Chaleurs, Québec, Canada ( $48^\circ 10' 56'' \text{N}$ ,  $64^\circ 54' 88'' \text{W}$ ) in 1992. We examined small-scale spatial variability in recruitment of various species of marine benthic invertebrates settling onto filamentous epibenthic structures and compared recruitment patterns to patterns of passive particle collection in the flume. All experiments were carried out with the same 3-D plastic structures (1 pattern of distal branch ramifications and 5 distal branch diameters). In the field, 5 structures (1 of each distal branch diameter) were mounted onto PVC tubing (2.5 m long  $\times$  3.8 cm diameter) such that the distance between structures was 50 cm on each side. This distance was 4 times greater than the total width of each structure and structures were oriented in the

same direction on the tubes. Two replicate tubes were anchored to the bottom with 4 steel rods. Tubes were installed ca 5 m from one another and were similarly oriented on the bottom. Water depth at the study site was 22 m. The experiment began on 24 September and ended on 15 November 1992. At the end of the experiment, the 3-D structures were individually collected by divers, who gently covered each structure with a plastic bag before cutting the main stem and closing the bag. The bags were transported to the laboratory where the 12 proximal branches (6 per level) of each structure were identified according to position of its proximal branch (upper or lower level;  $\pm 30^\circ$ ,  $\pm 90^\circ$ , or  $\pm 150^\circ$ ) on a 3-D structure. Branches were cut and kept individually in plastic jars filled with 70% ethyl alcohol.

In the laboratory, the total number of spat of various invertebrate species present on the 4 distal branches of each proximal branch were determined using a stereomicroscope ( $60\times$ ). Spat attached to the straight parts of the rodlike segments and to the nodes were recorded separately. The external limit of a node section was set at a distance of 0.5 or 1 mm along segments forming a node depending of the size of individuals of the species. This limit was set at 0.5 mm for all bivalve species. The limit was set at 1 mm for individuals of other groups such as encrusting bryozoans, sedentary polychaetes, and hydroids (see Fig. 2B). For both groups, the distance used to delimit a node was ca 2 times greater than the mean size of individuals observed.

**Data analysis. Flume experiments:** Microparticle attachment response as a function of the diameter of the distal branches, and of the level and orientation of the proximal branches, was of primary interest in these experiments. The total number of inert particles (TNIP) attached on the 4 distal branches belonging to each proximal branch was analysed as in Harvey et al. (1995a). The effect of the total area (TA) of the 4 distal branches included within each proximal branch was eliminated by calculating density (number of particles  $\text{cm}^{-2}$ ) as  $\text{TNIP}/\text{TA}$ . A  $\log(x+1)$  transformation was carried out on this ratio to normalize data and homogenize variances. The second dependent variable used in this study was the proportion of particles attached to the nodal sections of each proximal branch (referred to as proportion;  $p$ ) calculated by dividing the total number of particles attached to the 15 nodal sections by the total number of particles attached to the 4 distal branches of a given proximal branch. An arcsine  $\sqrt{p}$  transformation was carried out on this proportion to normalize data and homogenize variances. Any distal branches where no particles or all particles were attached to the nodal sections were given a value of  $\frac{1}{4}n$  and  $1 - \frac{1}{4}n$  ( $n$  = number of nodal sections), respectively, to improve the arcsine transformation (Bartlett 1937).



A split plot design was used to analyse density and proportion as a function of (1) the day of experimentation, (2) the diameter of the distal branches, which were whole plot effects, and (3) the level and (4) the orientation of the proximal branches which were subplot effects (Milliken & Johnson 1989). The ANOVA model for the analysis was:

$$Y_{ijkl} = \mu + \text{day}_i + \text{diameter}_j + \gamma_{ij} + \text{level}_k + \text{orientation}_l \\ + (\text{level} \times \text{orientation})_{kl} + \beta_{ikl} + (\text{diameter} \times \text{level})_{jk} \\ + (\text{diameter} \times \text{orientation})_{jl} + (\text{diameter} \times \text{level} \\ \times \text{orientation})_{jkl} + \epsilon_{ijkl}$$

The interaction terms containing the factor day were used as error terms ( $\gamma$ ,  $\beta$ , and  $\epsilon$ ) in both the whole plot and the subplots analyses. An assumption for the ANOVA used here is that both density and proportion are equally correlated on any pair of proximal branches within a given 3-D structure (the 2 levels and the 6 orientations). This assumption may not have been met in our experiments because the experimental units (proximal branches from the 2 levels or within each level) in the subplots analyses may not have been spatially independent. Therefore, to reduce the probability of making a type I error (i.e. the rejection of a null hypothesis when it is in fact true), the level of significance  $\alpha$  was decreased from 0.05 to 0.01 for all terms tested in the subplot analysis. Terms used in the whole plot analysis (day and diameter) were tested at  $\alpha = 0.05$ . Student-Newman-Keuls (SNK) multiple-comparisons tests were performed to determine which means differed when the main effects were statistically significant at  $p \leq 0.05$  (whole plot analysis) or  $p \leq 0.01$  (subplots analysis).

The proportion of the total number of microparticles which settled in the nodal sections of each distal branch was examined in relation to the proportion of the total surface area available for settlement represented by the nodal sections of the same distal branch. The latter variable was calculated by dividing the surface area available for settlement in the nodal sections by the total surface area available for settlement including both surface area of the straight parts of the rodlike segments and the surface area of each node. Total surface areas were calculated using the total length (mm) of the 4 distal branches belonging to a given proximal branch and the distal branch diameters (0.5 to 1.7 mm) used in this study. The nodal surface areas were calculated using distal branch diameters and the distance along segments used to set the external limit of a node section (0.5 mm for microparticles). A chi-squared goodness of fit test between observed (proportion of total number of particles settled in the nodal sections) and expected (proportion of the total surface area available for settlement being represented by the nodal sections) proportions was

carried out separately for each experimental unit (the 24 proximal branches from the two 3-D structures composed of 12 proximal branches each). The  $\chi^2$  values obtained from the 24 proximal branches belonging to each category of distal branch diameter were added together and tested with 24 degrees of freedom (Fleiss 1981). The total  $\chi^2$  was then subdivided into  $\chi^2$  homogeneity and  $\chi^2$  association as suggested by Fleiss (1981).

**Field experiment:** The total number of spat of various marine benthic invertebrate species present on the 4 distal branches belonging to each proximal branch and the proportion of individuals settled in the nodal sections were analysed following procedures described above for the flume experiment. The experimental design used to analyse densities of each benthic invertebrate species and proportions of animals settled in the nodal sections was the same as that used for the flume experiment except that the variable 'day' was replaced by the variable 'frame' which corresponded to the 2 groups of five 3-D structures anchored on the sea bottom. The ANOVA model was used to test separately both density and proportion of individuals settled in the nodal sections for 4 classes of marine benthic invertebrates (encrusting bryozoans, sedentary polychaetes, hydroids, and bivalves). Each class was represented by only one species (see 'Results') except for the bivalves which included *Mytilus edulis*, *Chlamys islandica*, *Cerastoderma pinnulatum* and *Hiatella arctica*. Pooling bivalves species was necessary because sample sizes for each individual species were too small to yield meaningful statistics. Because hydroids and bivalves were not present on all experimental units (proximal branches) tested in the ANOVA model, only proximal branches on which they were present (57 and 67% of the branches in bivalves and hydroids respectively) were considered in the analysis. This procedure was necessary since an important assumption of the ANOVA would have been violated by including a large number of zero values in the analysis. Therefore, for bivalves and hydroids, the null hypothesis must be modified and is only applicable for proximal branches on which animals (bivalves or hydroids) were present.

The proportion of the total number of recruits in the nodal sections (field experiment) was compared to the proportion of the total number of inert microparticles attached to in the nodal sections (flume experiment) using a  $\chi^2$  goodness of fit test. The microparticle proportion was chosen because it represented the expected relative number of settled individuals in the nodal sections with respect to the hydrodynamical processes in the vicinity of those areas that is passive settlement. A  $\chi^2$  goodness of fit test was carried out for each experimental unit separately following pro-

Table 1. Flume experiment. Split-plot factorial ANOVAs on inert particle density and proportion of particles attached in the node areas. Main effects were (1) the day of experimentation, (2) the diameter of the distal branches, and the (3) level and (4) orientation of the proximal branches. Significant p-values (<0.05, whole plot; <0.01, subplot) are shown in bold

Variable	Source of variation	df	SS	MS	F-value	p-value
Total density of particles	Whole plot analysis					
	Day	1	0.003	0.003	0.035	0.8598
	Diameter	4	3.418	0.855	9.980	<b>0.0234</b>
	Error 1	4	0.342	0.086		
	Subplot analysis					
	Level	1	0.011	0.011	1.700	0.2189
	Orientation	5	0.709	0.142	22.105	<b>0.0001</b>
	Level × Orient.	5	0.034	0.007	1.046	0.4387
	Error 2	11	0.071	0.006		
	Diam. × Level	4	0.025	0.006	1.694	0.1685
	Diam. × Orient.	20	0.153	0.008	2.100	0.0202
	Diam. × Level × Orient.	20	0.109	0.005	1.500	0.1300
	Error 3	44	0.160	0.004		
Percent of particles settled in the node areas	Whole plot analysis					
	Day	1	0.006	0.006	0.007	0.9360
	Diameter	4	0.006	0.002	0.195	0.9290
	Error 1	4	0.032	0.008		
	Subplot analysis					
	Level	1	0.001	0.001	0.698	0.4211
	Orientation	5	0.010	0.002	2.081	0.1445
	Level × Orient.	5	0.005	0.001	0.991	0.4660
	Error 2	11	0.011	0.001		
	Diam. × Level	4	0.004	0.001	1.012	0.4118
	Diam. × Orient.	20	0.015	0.001	0.774	0.7284
	Diam. × Level × Orient.	20	0.019	0.001	0.963	0.5204
	Error 3	44	0.042	0.001		
Error 1: (Day × Diam.)						
Error 2: (Day × Level) + (Day × Orient.) + (Day × Level × Orient.)						
Error 3: (Day × Diam. × Level) + (Day × Diam. × Orient.) + (Day × Diam. × Level × Orient.)						

cedures described above for the flume experiment. Because both bivalves and hydroids were not present on all proximal branches, the degrees of freedom used to test the  $\chi^2$  values for these species were adjusted as a function of the number of proximal branches on which animals were present in the field experiment.

## RESULTS

### Flume experiments

The overall density (mean  $\pm$  1 SE) of inert particles attached on the 3-D structures varied with the diameter of the distal branches and with the orientation of the proximal branches (Table 1, upper part). Proximal

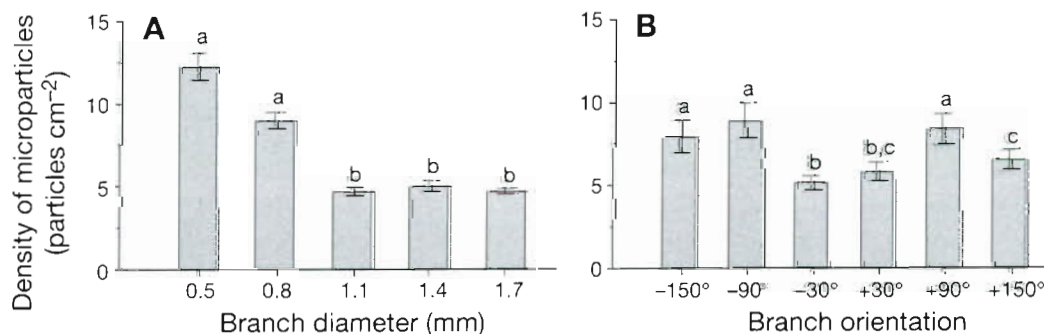


Fig. 3. Flume experiment. Total density ( $\pm$  1 SE) of inert microparticles on proximal branch as a function of (A) branch diameter and (B) branch orientation. Bars having dissimilar letters above them differ significantly from each other by SNK's procedure

branches with the smallest distal branch diameter collected 2.4 times more inert particles per unit area than those with the largest diameter (Fig. 3A). Proximal branches oriented perpendicularly ( $\pm 90^\circ$ ) to the flow direction collected 1.6 and 1.2 times more inert particles per unit area than those oriented at  $\pm 30^\circ$  and  $\pm 150^\circ$  respectively (Fig. 3B).

The mean proportion of inert particles attached to the nodal sections was not significantly affected by the diameter of the distal branches nor by the level and the orientation of the proximal branches (Table 1, lower part). The relative surface area of the node sections represented ca 20% of the total surface area available for particle attachment (Fig. 4) but on average, only 8% of the particles observed on the distal branches were attached in the nodal sections (Fig. 4). According to the results of the  $\chi^2$  goodness of fit tests, percentages of particles attached in the nodal sections were significantly lower (2.5 times) than the expected values based on the relative proportion of the surface area of the node sections to the total (see Fig. 4).

### Field experiments

The overall density of each of the 4 groups of marine benthic invertebrates observed on the 3-D structures varied between 1 and 30 ind. 10 cm<sup>-2</sup>. There were significant effects of the diameter of distal branches on the overall density of larvae, but no significant effects of level and orientation of the proximal branches on the density of any of the groups (Table 2). No significant interactions between factors was observed, except for the encrusting bryozoans (*Flustrella hispida*) which showed a significant level  $\times$  diameter interaction). Because of the significant level  $\times$  diameter interaction observed for *F. hispida*

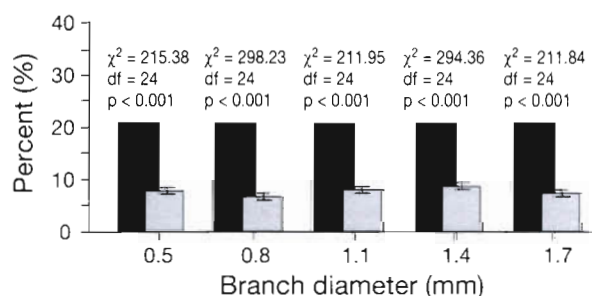


Fig. 4. Flume experiment. Mean proportion ( $\pm 1$  SE) of inert microparticles attached to the node areas (grey bars) and percentage of the total surface area represented by the node areas (black bars) for structures with each of 5 branch diameters. Also on this figure are results of the  $\chi^2$  goodness of fit tests comparing the proportion of total number of particles attached to the node areas to the proportion of the total surface area available for attachment in the same nodal areas

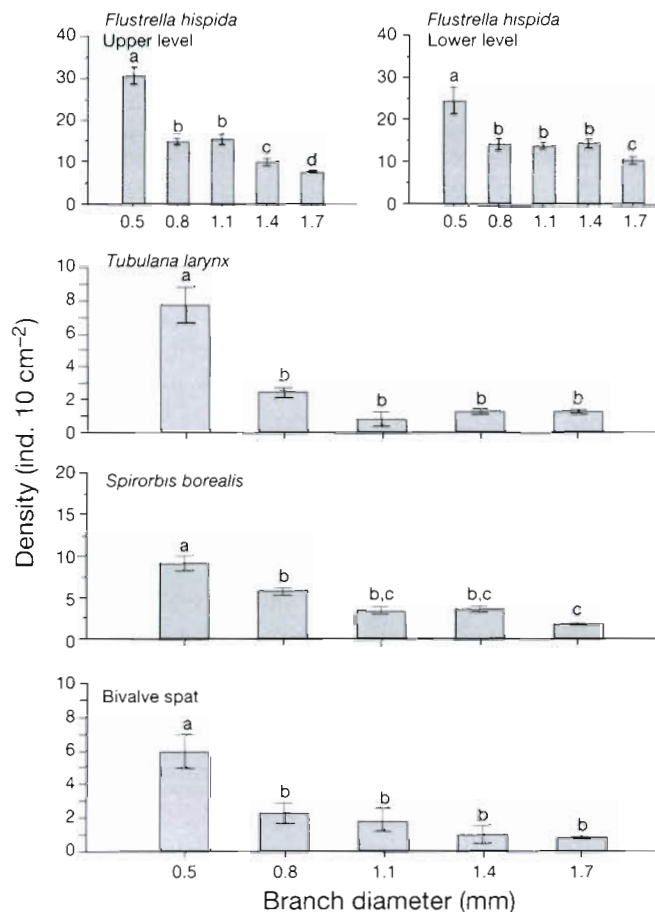


Fig. 5. Field experiment. Total density ( $\pm 1$  SE) of individuals as a function of branch diameter for each of the 4 classes of marine benthic invertebrates observed on the 3-D structures (encrusting bryozoans *Flustrella hispida*, sedentary polychaetes *Spirorbis borealis*, hydroids *Tubularia larynx*, and bivalve spat). Given the significant interaction between diameter and level obtained using ANOVA (see Table 2), results from upper and lower levels are presented separately for *F. hispida*. Bars having dissimilar letters above them differ significantly from each other by SNK's procedure

SNK's multiple-comparison tests of diameter effects were carried out separately for each level of proximal branches (Fig. 5). On average, for all 4 groups of marine benthic invertebrates, proximal branches with the smallest distal branch diameter collected 4.4 times more individuals per unit area than those with the largest distal branch diameter (Fig. 5).

In 2 of the 4 categories of marine benthic invertebrates observed on our 3-D structures (*Flustrella hispida* and *Tubularia larynx*), the proportion of recruits in the nodal sections varied significantly with the diameter of the distal branches (Table 3). There was no significant effect of level or orientation of the proximal branches on the proportion of recruits in the nodal sections for any of the categories of marine benthic inver-





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Table 4. Field experiment. Results of the  $\chi^2$  goodness of fit tests comparing proportion of animals settled in the nodal sections with the proportion of the total number of inert particles settled in the nodal sections. Those analysis were carried out in each of the 4 categories of marine benthic invertebrates observed on the 3-D structures including: encrusting bryozoans *Flustrella hispida*, sedentary polychaetes *Spirorbis borealis*, hydroids *Tubularia larynx*, and bivalve spat.  $\chi^2_{\text{ass}}$  is large compared to  $\chi^2_{\text{hom}}$ , indicating a strong degree of association of the different groups to the nodes. The significant  $\chi^2_{\text{hom}}$  indicates strong homogeneity (reduced variance) in the results. Significant p-values (<0.05) are shown in bold

Species	Branch diameter (mm)	df	$\chi^2_{\text{total}}$	p-value	$\chi^2_{\text{ass}}$	p-value	$\chi^2_{\text{hom}}$	p-value
<i>Flustrella hispida</i>	0.5	24	1288.82	<b>&lt;0.001</b>	1233.61	<b>&lt;0.001</b>	55.21	<b>&lt;0.001</b>
	0.8	24	1341.57	<b>&lt;0.001</b>	1268.17	<b>&lt;0.001</b>	73.40	<b>&lt;0.001</b>
	1.1	24	2235.88	<b>&lt;0.001</b>	2097.45	<b>&lt;0.001</b>	138.42	<b>&lt;0.001</b>
	1.4	24	3562.85	<b>&lt;0.001</b>	3351.86	<b>&lt;0.001</b>	210.99	<b>&lt;0.001</b>
	1.7	24	3261.93	<b>&lt;0.001</b>	3214.42	<b>&lt;0.001</b>	47.50	<b>0.002</b>
<i>Tubularia larynx</i>	0.5	21	90.68	<b>&lt;0.001</b>	37.37	<b>&lt;0.001</b>	53.31	<b>0.001</b>
	0.8	15	168.18	<b>&lt;0.001</b>	133.64	<b>&lt;0.001</b>	34.54	<b>0.002</b>
	1.1	12	101.59	<b>&lt;0.001</b>	71.28	<b>&lt;0.001</b>	30.31	<b>0.001</b>
	1.4	15	309.19	<b>&lt;0.001</b>	268.06	<b>&lt;0.001</b>	41.13	<b>&lt;0.001</b>
	1.7	17	292.33	<b>&lt;0.001</b>	243.16	<b>&lt;0.001</b>	49.17	<b>&lt;0.001</b>
<i>Spirorbis borealis</i>	0.5	24	415.36	<b>&lt;0.001</b>	285.38	<b>&lt;0.001</b>	129.99	<b>&lt;0.001</b>
	0.8	24	573.44	<b>&lt;0.001</b>	494.69	<b>&lt;0.001</b>	78.75	<b>&lt;0.001</b>
	1.1	24	608.38	<b>&lt;0.001</b>	500.27	<b>&lt;0.001</b>	108.10	<b>&lt;0.001</b>
	1.4	24	953.45	<b>&lt;0.001</b>	895.62	<b>&lt;0.001</b>	57.83	<b>&lt;0.001</b>
	1.7	24	454.47	<b>&lt;0.001</b>	377.51	<b>&lt;0.001</b>	76.96	<b>&lt;0.001</b>
Bivalve spat	0.5	18	226.16	<b>&lt;0.001</b>	173.61	<b>&lt;0.001</b>	52.55	<b>&lt;0.001</b>
	0.8	7	132.79	<b>&lt;0.001</b>	108.42	<b>&lt;0.001</b>	24.37	<b>&lt;0.001</b>
	1.1	15	311.41	<b>&lt;0.001</b>	273.19	<b>&lt;0.001</b>	38.22	<b>&lt;0.001</b>
	1.4	11	208.16	<b>&lt;0.001</b>	186.91	<b>&lt;0.001</b>	21.25	<b>0.019</b>
	1.7	17	277.47	<b>&lt;0.001</b>	249.16	<b>&lt;0.001</b>	27.95	<b>0.032</b>

tebrates, and no significant interactions between factors. In both *Flustrella hispida* and *Tubularia larynx*, the proportion of individuals settled in the nodal sections were 1.6 and 2.0 times higher respectively on distal branches with the largest diameter than on those with the smallest diameter. The relative proportion of recruits in the nodal sections varied between 45 and 80% and, in all categories of animals and all distal branch diameters, the percentages of individuals settled in the nodal sections were significantly higher than the expected values based on the relative proportion of inert particles which settled in the node sections in the flume experiment (Table 4, Fig. 6).

## DISCUSSION

In this study we tested the hypothesis that larval settlement of several marine benthic invertebrate species onto filamentous epibenthic substrata is entirely or partly controlled by passive processes by comparing positions of initial contact of inert microparticles on 3-D plastic filamentous structures in a flume experiment with the positions of recruits of 4 groups of marine benthic invertebrates on the same 3-D structures in a field experiment. Results from these experiments clearly suggest that for the species of marine benthic invertebrate larvae tested, settlement patterns on filamentous substrata result from both passive (hydro-

dynamic) and active (behavioural) processes acting over different spatial scales. Passive processes would operate at small spatial scales (> cm) by affecting initial contact sites—i.e. the probability of larvae coming into contact with the different parts of a filament, including upstream and downstream positions. Active processes would operate at smaller spatial scales (mm,  $\mu\text{m}$ ) and are applicable only for the later settlement phases (exploration and attachment). Larvae would actively explore the substratum, possibly to find low shear stress attachment sites before metamorphosing.

### Initial contact phase

Density of attached inert particles in the flume and density of spat in the field both decreased as a function of distal branch diameter (Figs. 3A & 5), clearly indicating that the initial contact stage of larval settlement was controlled by hydrodynamic processes. According to Harvey et al. (1995a), who used the same 3-D structures in similar flume and field experiments, the rate at which both particles and larvae come into contact with the rodlike segments (encounter rate) depends on the flow dynamics around those segments which, in turn, is a function of the segment's Reynolds number ( $Re = ud/\nu$ ; where  $u$ ,  $d$  and  $\nu$  are respectively equal to flow speed, rodlike segments diameter and sea water kinematic viscosity,  $\nu \approx 0.01 \text{ cm}^2 \text{ s}^{-1}$ ). At  $Re$  values well

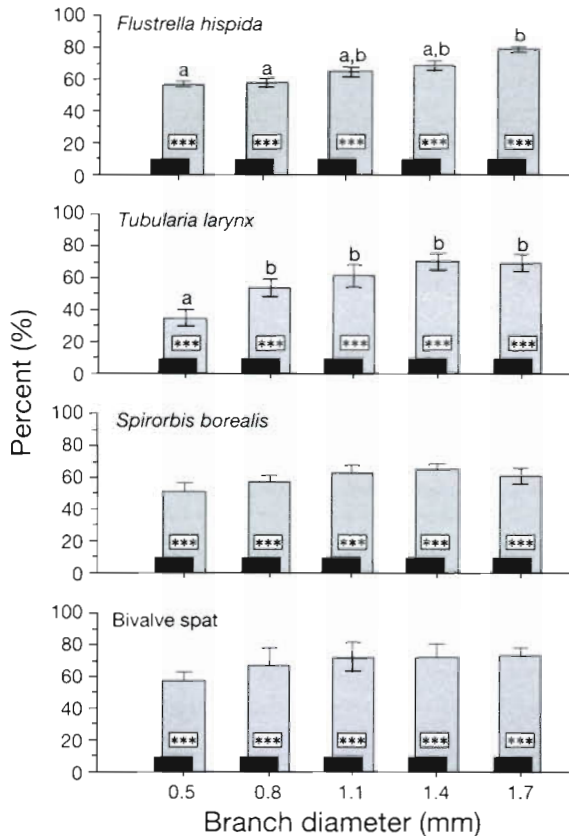


Fig. 6. Field experiment. Mean proportion ( $\pm 1$  SE) of recruits settled in the node areas (grey bars) and percentage of the total surface area represented by the nodal areas (black bars) as a function of branch diameter in each of the 4 classes of marine benthic invertebrates (encrusting bryozoans *Flustrella hispida*, sedentary polychaetes *Spirorbis borealis*, hydroids *Tubularia larynx*, and bivalve spat) collected on our 3-D structures. Bars having dissimilar letters above them differ significantly from each other by SNK's procedure. Significant differences ( $\chi^2$  goodness of fit tests) between the proportion of recruits in the node areas and the proportion of the total surface area available for settlement being represented by the node areas are indicated as: \*\*\* $p < 0.001$

below unity ( $\ll 1$ ), a smooth and vortex-free flow surrounds the rodlike segments. At Re values between about 10 and 40, flow separation occurs, forming attached, slowly rotating vortices on the downstream side of the segments. Attached vortices add 2 flow separation points and 1 additional attachment point around the segments, providing a supplementary site for particle or larvae encounter by direct interception on the downstream side. At  $Re > 40$ , lee vortices are shed periodically on each side of the wake implying that within a given time period, fewer particles and larvae encounter segments on the downstream side. Therefore, considering the diameters of the rodlike segments (0.5, 0.8, 1.1, 1.4, and 1.7 mm) and the average current velocity observed in the field and used in

our flume experiments ( $5 \text{ cm s}^{-1}$ ), the Re values of the rodlike segments used were equal to 25, 40, 55, 70 and 85 respectively. Given the theoretical considerations and the segment's Re values, 3-D structures with the smallest distal branch diameter ( $10 < Re \leq 40$ ) should encounter more particles and spat per unit area and unit time than those with the largest diameter ( $Re > 40$ ). The latter prediction agrees with results in Harvey et al. (1995a) who also showed that 3-D structures with the smallest distal branch diameter collected more particles (flume experiment) and spat (field experiment) per unit area than those with the largest distal branch diameter. The significant distal branch diameter effect observed in the present study and the concordance of results from both field (recruitment) and flume (attachment of inert particles) experiments clearly indicated that the initial contact stage of larval settlement on 3-D epibenthic filamentous structures is predominantly controlled by passive hydrodynamic processes.

Flume experiment results showed that proximal branches oriented perpendicularly ( $\pm 90^\circ$ ) to the flow direction collected more inert particles per unit area than those oriented at  $\pm 30^\circ$  and  $\pm 150^\circ$  (Fig. 3B), consistent with hydrodynamic processes. Because the projected area normal to the flow was smaller for proximal branches oriented at  $\pm 30^\circ$  and  $\pm 150^\circ$  than for those oriented at  $\pm 90^\circ$  (both type of branches had the same total length), the number of particles encountered by direct interception, within a given time, should have been lower for the  $\pm 30^\circ$  and  $\pm 150^\circ$  branches compared to  $\pm 90^\circ$  branches. This projected area effect was not observed on 3-D structures placed in the field probably because of variable currents.

Results showing that inert particles attached less frequently to the nodal sections than expected based on nodal surface area (Fig. 4) may also be explained by hydrodynamic processes occurring in the vicinity of the nodal sections. The proximity of the rodlike segments at the level of each node and the spatial arrangement of the segments within the nodal sections were similar to the spatial arrangement of 2 circular cylinders placed close together in a plane normal to the flow. According to Bearman & Wadcock (1973), distinct regimes of flow behind a pair of cylinders occur depending on both the Reynolds number and the cylinder spacing. For spacing greater than 1 diameter, the cylinders shed vortices in the form of 2 vortex streets (as discussed for the diameter effect), whereas when the cylinders are nearly touching alternate vortex shedding is detected with only 1 street forming. This destabilization of the vortex-street structure generates a pressure drop in the flow between cylinders and contributes to a decrease in the encounter rate between suspended particles and rodlike segments, thereby reducing the efficiency of particle capture per unit area within the nodal sections.

### Exploration and attachment phases

Results from the field experiment showed that spat were much more frequently attached to the nodal sections (ca 35 to 80%) than would be expected based on the proportion of inert particles observed in the nodal sections (ca 8%) in the flume experiment. In contrast, on the straight parts of the rodlike segments, recruitment was proportionately (50×) lower than the number expected from flume experiments. While this difference may have been due to passive erosion of larvae or new recruits from the straight parts of the rodlike segments under the current regime at the study site, it seems more likely that larvae used post-contact exploratory behaviours (e.g. crawling, swimming, tumbling) to find low shear stress attachment sites before metamorphosing. The latter hypothesis was supported by significant increase in the percent of recruits in the nodal sections with increasing distal branch diameter in 2 of the 4 categories of marine benthic invertebrates examined. In the flume experiments, however, there was no change in the proportion of inert particles attached to the nodal sections as a function of distal branch diameter. Higher shear stress associated with larger distal branch diameter would be responsible for the higher proportion of spat found in the nodal section of those branches. Furthermore, the significant distal branch diameter effect, as predicted by the flume experiment was observed after 2 mo of immersion in the field, and suggests that passive erosion of larvae, or new recruits, and early post-settlement mortality were low during the study period.

One could argue that passive settlement could have been important at the mm scale, but that the abundance patterns were modified by some other processes (e.g. post-settlement mortality due to predators, overgrowth, competition for food and space, or some other physical factors; see Gosselin & Qian 1996, Carroll 1996) which could influence the distribution and abundance of organisms in the field. A comparison of the results obtained in the field and those obtained in the flume experiments do not support this hypothesis. Indeed, in the flume, the passive model predicts that only 8% of the inert particles attached should be located in the nodal sections. By contrast, in the field experiments 80% of the post-larvae attached to the structures were observed in the nodal sections. Thus to achieve the proportion predicted by the passive model, up to ca 920 times more larvae than observed {No. of expected post-larvae on the straight part of the rodlike segments = [proportion of the post-larvae observed in the nodal segments in the field (80%) × proportion of particles observed on the straight parts of the rodlike segments in the laboratory (92%)] ÷ proportion of particles observed in the nodal segments (8%)} should have settled on the straight parts of the rod-like segments, this as-

suming that post-settlement mortality would have occurred only on the straight-part segments of the rodlike elements. Clearly this is unlikely, as the necessary expected densities to support this hypothesis as the sole mechanism responsible for the observed patterns are incompatible with the observed settlement densities in the region considered. Hence, the small-scale patterns observed suggested that final settlement site results from an active selection for low shear stress microhabitats or structural heterogeneity (often 2 unseparable covariables, but see Mullineaux & Garland 1993) in the species studied. Shear stress has been shown to influence settlement site in a variety of species, some preferring habitats with low shear stress (e.g. *Bugula turrita*, Mullineaux & Garland 1993; some foraminifera, Mullineaux & Butman 1990; *Balanus amphitrite*, Mullineaux & Butman 1991) and others preferring strong shear stress (*Tubularia crocea*, *Schizoporella unicornis*, Mullineaux & Garland 1993; *Phragmatopoma lapidosa californica*, Pawlik & Butman 1993).

An earlier study using the same 3-D structures in field and flume experiments showed that hydrodynamic processes dominate settlement or recruitment patterns in the bivalves *Chlamys islandica*, *Mytilus edulis*, *Cerastoderma pinnulatum*, and *Hiatella arctica* at scales of 10 to 20 cm (Harvey et al. 1995a). The present study, carried out with the same 3-D structures, has demonstrated that passive processes are sufficient to explain recruitment patterns at the scale of the proximal branches (ca 3 cm) in a variety of species (the encrusting bryozoan *Frustrella hispida*, the hydrozoan *Tubularia larynx*, the sedentary polychaete *Spirorbis borealis* and the same bivalves mentioned above). However, at a smaller scale (ca mm), all species examined in this study as well as those examined in similar studies carried out in our laboratory including 10 species from 6 classes of sessile invertebrates (Ectoprocta, Entoprocta, Annelida, Coelenterata, Crustacea, Mollusca), we have been unable to reject the hypothesis of active larval exploration of the substratum during the final attachment phase (see also Hudon et al. 1983, Chabot & Bourget 1988, Le Tourneux & Bourget 1988, Harvey et al. 1995a, b, Lemire & Bourget 1996, Miron et al. 1996).

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