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Larvae-substrate relationships of *Eupolymnia nebulosa* (Montagu, 1818) (Polychaeta, Terebellidae): an experimental analysis

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ABSTRACT

The transition from the plankton to the benthos has been investigated experimentally with a terebellid annelid. Tests on artificial and natural sediments lead to the following results. 1) Larvae were able to displace particles with a greater weight than their own, but this was possible only if larvae had access to a perfectly immobile platform. Larvae required two kinds of substrate for settlement, one forming support, the other for building the tube. 2) Mechanisms of the substrate selection by larvae do not suppose a "choice" from several sediments presented at the same time and equally accessible; larvae were either able to use the particles which they contact or unable to do so, in which case they returned to the water column. These tests indicated more an ecological opportunity than a choice. 3) The reduced selectivity of larvae with age corresponded to an increasing ability to use particles. Larvae, at each stage of development, were able to manipulate only a well defined size range of particles. This range increased as development proceeded, and the result was the progressive utilisation developing in the direction of increasing grain sizes. 4) Selection of sediment by larvae was accomplished on the short term and did not assure the future of individuals. The result of the transition from the planktonic to the benthic phase was unrelated to the subsequent success or failure of recruitment. A contrast became visible between limited requirements in terms of grain size and shape defining a large potential zone for larval settlement, on the one hand, and a spatially limited adult area, on the other hand. These two features were made compatible by the existence of an egg mass in the life cycle.

RÉSUMÉ

Relations larves-substrat chez la polychète térébellide *Eupolymnia nebulosa* (Montagu, 1818): analyse expérimentale

La transition plancton/benthos a été analysée expérimentalement sur une annélide polychète térébellide. Les tests réalisés sur des substrats artificiels et naturels ont conduit aux résultats suivants. 1) Les larves sont capables de déplacer des particules d'un poids supérieur à leur propre poids; cela est rendu possible par l'accès des larves à un point d'appui parfaitement immobile. A l'échelle des larves, la fabrication du premier tube benthique nécessite deux catégories dimensionnelles de particules: les unes constituant le support et les autres les éléments du tube. 2) Les mécanismes d'établissement des larves sur un substrat ne reposent pas sur un choix entre plusieurs sédiments présentés simultanément et également accessibles. Les larves peuvent ou non, utiliser les particules qu'elles contactent et dans le cas négatif, elles retournent dans la colonne d'eau pour préparer un

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nouveau test. Cette succession d'essais indépendants traduit une opportunité écologique et non un choix. 3) L'apparente diminution de sélectivité avec l'âge représente une augmentation des capacités d'utilisation des particules par les larves. A chaque stade de développement, elles manipulent une gamme de taille bien définie qui s'élargit avec l'âge; le résultat est une utilisation progressive vers les tailles élevées d'un sédiment hétérogène. 4) Les larves sélectionnent le substrat sur un court terme en fonction de leur capacité de manipulation. Elles n'assurent pas nécessairement la réussite des stades ultérieurs. Un contraste apparaît entre d'une part les faibles exigences granulométriques des larves conduisant à définir une large zone potentiellement favorable à leur fixation et d'autre part la zone de présence des adultes particulièrement réduite. Ces deux éléments sont rendus compatibles par la présence d'une structure de rétention des larves dans le cycle de vie.

INTRODUCTION

A study was undertaken in the Bay of Banyuls in order to determine the factors influencing the composition and maintenance of benthic communities. Recruitment success has been discussed as an important process regulating communities. Larval behaviour, predation, local hydrodynamic patterns and substrate characteristics were the most frequently cited factors controlling larval settlement (ECKMAN, 1983; BUTMAN, 1986; WATZIN,1983). Several hypotheses have been presented to explain the long term structure of communities. From reaction patterns of first contact, active habitat selection has been suggested as opposed to passive deposition (BUTMAN, 1987). Habitat selection (MEADOWS & CAMPBELL, 1972) was proposed along with ecological opportunity (MOORE, 1975). In this second pair of hypotheses, the local distribution of adults was viewed as largely determined by selection of larvae. Other field observations supported the hypothesis of constrained settlement, i.e., this pattern resulted in a local distribution determined more by availability of suitable sites than by selection between equally accessible alternatives. These hypotheses, which are based on field observations, are probably not as conflicting as suggested by MOORE (1975); they must be tested in the laboratory. We approached this problem by using *Eupolymnia nebulosa,* Polychaeta. In the Bay of Banyuls, *E. nebulosa* is one of the most abundant species for which a large body of biological information is available. Its life cycle has been described (BHAUD & GREMARE, 1988, 1991) and preliminary experimental studies investigating settlement requirements have also been done (BHAUD, 1990b; CHA & BHAUD, 1991).

MATERIAL AND METHODS

EXPERIMENTAL DESIGN. — Four types of experiments were conducted (Table 1) to answer the following questions: what kind of sediments separately presented are larvae able to use? If they are able to use a given sediment, how does this ability vary with larval age and increasing particle size? What is the behaviour of larvae when offered a variety of sediments including unsorted heterogeneous sediments, artificial or natural sediments ?

1) The initial experiments were conducted to determine whether or not the larvae had the ability to use a particular sediment. Sediments of various nature, size, settling velocity and organic matter content were tested. One type of sediment was placed in a separate glass petri dish 9 cm in diameter. Rates of larval settlement were determined after four days. During preliminary experiments (BHAUD, 1990a & b) we had observed that a heterogeneously distributed sediment of variable thickness resulted in more tubes being present on the margins of the dish where the sediment layer was thinner. The number of introduced larvae was approximately 300; the percentage of free larvae was calculated after counting 100 larvae with or without tubes, at five places chosen at random but avoiding the margins. Free larvae were classified as those swimming or lying on the bottom; in the latter case, no tube has been constructed .

2) To test the effects of larval age on the ability to use a given sediment, 14-day old and 18-day old larvae were introduced into an aquarium containing either a series of sediments consisting of microbeads (MB), or decanted silt (DS) (Table 2, items 7 and 8, respectively), each available as a thin (t) or thick (T) layer. Four plates were tested in an aquarium, and each paired aquaria represented a replicate. Sediments were deposited in each aquarium on plates of equal surface area. The number of tubes on each test sediment in an aquarium, counted after four days, was expressed as a percentage of the total number present on all sediments of this aquarium.

3) To test the ability to use different sized grains of one sediment, 14 day old larvae were tested in sediments consisting of fractions of the fine sand in a range of eight degrees of fineness. Oservations were made daily on each sediment for six days.

4) In an earlier study (CHA & BHAUD, 1991), and in experiment number 2 (present paper), a greater number of larvae settled in thin layers of sediment, whether it was mud or microbeads, than in thick layers of sediment. It was

suggested that access to a hard substrate, as a result of the thin layer of sediment, facilitated settlement. To complement the earlier findings on the role of a heterogeneous substrate, larvae were offered two types of sediment combined in three substrates: decantation silt (DS, Table 2: no 8) in a thick or a thin layer, and a fraction of Fontainebleau sand (160-200 µm; Table 2: no 3) with traces of DS (approximately in the ratio 1/500). Only one enclosure was provided with 3x5cm plates in alternate positions. In addition to these perfectly sorted sediments, two natural sediments were also tested: the silty sand from the *Nephtys hombergii* community and the fine sand from the *Spisula subtruncata* community.

Supply of larvae. — Larvae were obtained from egg masses (BHAUD, 1990a) which were maintained in a tank 60 cm (l) x 40 cm (w) x 16 cm (h) filled with 50 litres of filtered sea water. Filtering was achieved by passing the water through synthetic fiber-wool. The water was circulated at a rate of 0.5 l /min using a flow-through system, the surface water was drained away by an overflow. When larvae were close to hatching from the mucoid mass, they were removed to a one litre vessel and gently agitated. After 2 hours, the emerged larvae were transfered to a second vessel with filtered sea water. Larvae were used either immediately or kept for several days until the desired developmental stage was reached. Estimates of numbers were made from a subsample obtained by immersing a tube of known diameter in the vessel containing larvae and gently agitating to obtain a homogeneous suspension (RAZOULS, 1972). A range of 500 to 60,000 larvae were collected from one egg mass with an average of about 5,000 larvae. They were collected from late February to early June.

Two methods of collecting larvae of varying age were used. a) Newly produced egg masses were collected at particular intervals and maintained at the same temperature; b) Egg masses were divided and each half was incubated at a different temperature. Development was shortened by 6 days when the temperature was increased from 12 to 18 °C for eight days.

Morphology of larvae introduced into enclosures. — Morphology and age were calculated from a known time-development series determined at the beginning of the reproductive season in March (BHAUD, 1988a). Growth rate was positively correlated with temperature (BHAUD, 1988b). Larvae of five setigerous segments in March is 14 day-old but is only eight day-old in early June. This variation in rate of larval development was minimized by characterizing the larvae by morphology rather than by age. The date of each experiment was recorded in the course of the reproductive period. Experiments were conducted in March. Larvae measured 430 to 510 µm in length in experiments 1, 2, 3 and 4; they had five setigers, each with one pair of provisional club-shaped setae and the first 4 setigers with one pair of capillary setae. They were 14 days old. A second group of larvae, also used in experiment 2, had eight setigers, each with one pair of provisional club-shaped setae, and the first six setigers with one pair of capillary setae. They were 18 days old.

Criteria for settlement. — Settlement success was measured by the appearance of the primary tubes of juvenile benthic stages. This method ensured that the number of tubes counted represented the number of animals present in the sediment. Counts were verified by inspecting all tubes under a binocular microscope for the presence of living, dead or abandoned tubes. All larvae built tubes and never abandoned them in these short experiments. Mortality was negligible. The change from planktonic to benthic life was not a cause of mortality (BHAUD & CHA, 1992). In order to confirm that we would be able to find dead larvae, 10 larvae with six setigerous segments, were sacrificed (repeated three times). These dead larvae were still recognizable six days later. This confirmed the absence of dead larvae at the time of observations made four days after the introduction of larvae.

Feeding. — It was not necessary to feed these larvae during these experiments since they still had yolk reserves present in their digestive tract after eight days (CHA, 1990, BHAUD, 1991).

Duration of experiments. — Our study was designed to explore the behaviour of larvae at the time of settlement and the interaction between settling larvae and natural and artificial sediments. Settlement involved behaviour indicative of the benthic life stage (BUTMAN, 1987) and was defined as the first lasting contact with the bottom (KEOUGH & DOWNES, 1982). Tube building was used as an indication that settlement had occurred. The distribution of larvae was determined among several substrates four days after the introduction of larvae (Experiments 1, 2 and 4). However, this did not exclude daily observations, allowing us to register intermediate steps and in particular provisionally settled larvae as in experiment 3.

Sediment type. — The following sediments were used in one or more of the experiments. Mud obtained by sedimentation from the laboratory running sea-water supply (decantation silt: DS) then sieved through 40 and 60 µm mesh. Fontainebleau sand (FS) (Prolabo, France) was composed of grains between 100 and 315 µm in diameter. This sand was separated according to the following diameters: 100 to 160 µm, from 160 to 200 µm, from 200 to 250 µm, and from 250 to 315 µm. An artificial sediment was used (Ferro Prod., Cataphote division, USA) with microbead sizes measuring 45 to 60 µm and 100 to 150 µm. A second artificial sediment of equal sized

Pyrex particles (OSI, France) was used. Two natural sediments from Banyuls Bay were used: a) silty sand from the *Nephtys hombergii* community which was composed of 5 to 20 % clay (< 40 µm), 50 to 80 % fine sand (> 40 µm and < 200 µm) and less than 5 % of medium-sized sand (> 200 µm); b) fine sand from the *Spisula subtruncata* community which was composed of 5-10 % clay, 80-90 % fine sand and 5-10 % medium-sized sand. A fraction > 40 and < 63 µm of these natural sediments (NH and SS for *Nephtys hombergii* and *Spisula subtruncata*) was isolated and the settling rate compared with that of the other sediments. All sediments used are reported in Table 2 with the following characteristics: size, shape, sinking velocity and ability of larvae to use sediments.

TABLE 1. — Main features of the experimental design. Number of sediments used in one experiment is related to the nature of particles; number of substrates used in the same experiment is deduced from the pattern of presentation (thick or thin layer, sorted or not sorted...); replicates are given by the number of aquaria, even if several equivalent supports are present in one aquarium; S= sediment.

Exp. Nb	1: Table1.	2: Fig.1.	3: Fig.2.	4A: Table 3	4B: BHAUD & CHA, 1992
Questions asked	capability to manipulate	influence of age on the ability to manipulate particles		importance of heterogeneous particles	relation with field communities
	grains of a defined size	2 age classes face to several S	1 age classe face the size range of 1 S		
Nb of sediments (S)	14	2	1	2	2
thick (T) or thin (t) layer	T	T t	T	Tt	T
Nb of substrates to which larvae have access	1	4	8	3	2 (with large range size)
homogeneous (hm) or hete-rogeneous (ht) grain size	hm	hm	hm	hm+ht	ht
choice given	no	yes: in a space larger than the perceptive volume of the larvae			yes: inside the perceptive volume of the larvae
Nb of replicates	2	10	1	1	10
Nb of supports for substrate in each aquarium	1	2	1	5	5
incubation (days)	4	4	1 to 6	4	4
observation at the end of incubation period (E) or each day	E	E	ED	E	E
age (days) of introduced larvae	14	14+18	14	14	14
higher distance between centre of two adjoining supports (cm)	-	12	16	12	-

Among well-sorted sediments, only decanted silt (items 8 and 9 in Table 2) has some organic content (1.13 mg. g^{-1} dry weight of organic material) and probably also a large amount of microbial biomass; these elements are known to be strongly correlated with the surface area of the particles (DEFLAUN & MAYER, 1983). Previous experiments (BHAUD, 1990a, b) have shown that the suitability of a substrate was not correlated with the relative or absolute quantity of organic matter, which suggested the importance of physical characteristics of sediment for larval settlement.

Characteristics of the sediments may have changed during experiments if their introduction into sea water was made at the beginning of the experiment, just before adding the larvae. Sediments were prepared in filtered sea water one week before use in enclosures with the same water to avoid the possibility of a sudden development of microfauna.

TABLE 2. — Sinking velocity and preference for several sediments types by larvae of *E. nebulosa.* The ability of these larvae to use the sediment and to construct or attempt to construct tubes is assigned a value from 0 (grains not displaced) to ++++ (well constructed tubes). F S: Fontainebleau sand; MB: microbeads; DS: decantation silt; PY: Pyrex; SS: fine sand of *Spisula subtruncata* community; NH: silty sand of *Nephtys hombergii* community. Observations four days after introduction of larvae. Origin is either synthetic product (S) or a natural product (N). Sediments were sorted in sea water (sw) with a silk plankton net or sorted in dried conditions (dc) with a metallic sieve or the size range directly furnished (df). In the column "shape", is given the ratio of longitudinal to transverse axis. The different levels of the use of sediments (observed in two replicates) have been photographed and presented elsewhere (CHA & BHAUD, 1992). Free larvae are live larvae, in the water column or on the sediment but without attached particles. S: sediment.

sediment		origin	sorting methods	fall velocity		shape	ability to use sediment	observations
item	D µm			$cm.s^{-1}$	SD(N=6)			
1 F S	250-315	N	d c	3.034	0.039		0	no marks of bioturbation;100 % living and free larvae.
2 F S	200-250	N	d c	2.752	0.035	approximately spherical or weakly	0	no marks of bioturbation; 100 % living and free larvae.
3 F S	160-200	N	d c	2.340	0.038	elongated (1/2) smooth angles	+	clusters of some grains appearing; 100 % living and free larvae.
4 F S	100-160	N	d c	1.270	0.003		++	clear arrangement of grains in linesbut structure of tube inconspicuous; 70 and 76 % of free larvae.
5 F S	40-60	N	d c	0.358	0.002		+++	structure of tubes evident; 25 and 35 % of free larvae.
6 M B	105-150	S	d f	0.855	0.003	perfectly spherical	+++	structure of tubes evident, 20 and 28 % of free larvae
7 M B	45-60	S	d f	0.390	0.001	and smooth	++++	very fine tubes of good appearance, perfectly built, very long and in relief; 6 and 10 % of free larvae
8 D S	40-60	N	s w	0.462	0.003	mineral part +	++++	perfectly built tubes, appearing in prominent position relatively to the surface of the sediment; no free larvae
9 D S	< 40	N	s w	0.199	0.000	organic part	++++	same observations.
10 PY	40-60	S	d c	0.240	0.000	variable shape, never spherical, sharp angles and sharp edges	+	structure of tubes inconspicuous; arrangement of particles only in short lines (1mm); numerous tracks without larvae; 92 % and 45 % of larvae inside sediment but not in built structures.
11 PY	< 40	S	d c	0.180	0.000	same as above	+++	structure of tubes evident (3cm); 60 % and 35 % of larvae in tubes; other larvae are free; no tubes built then deserted
12 NH	< 63	N	s w	0.428	0.002	mineral + organic part	++++	perfectly built tubes.
13 SS	40-63	N	d c	0.210	0.003	approximately spherical or weakly elongated (1/2) sharp angles	++++	same observations.
14 PC	< 10	N	d f	0.178	0.008	microplates	0	no tube; 100 % living and free larvae
15	Larvae from same eggmass			0.110 0.115 0.105	0.012 0.028 0.017	12 day-old living specimens 14 day-old living specimens 16 day-old living specimens		values obtained using 30 individuals for each of the 10 fall observations in each age group.

Preparation of sediments. — Supports of sediments were set out on the bottom of aquaria. These were movable plates allowing for easy manipulation (CHA, 1990). Sediments were deposited with a pipette onto these plates in thick or thin layers. Thick layers were 1 and 2 µm thick, which prevented the larvae from reaching the bottom of the movable plates, and thin layers were one to two layers of particles (approximately 120-300 µm), which allowed the larvae to reach the surface plates and thus could be considered as a hard substratum.

Speed of sediment deposition. — Sinking sediment was timed in a test chamber 42 cm high and 6.5 cm in diameter. The chamber was filled with seawater having a salinity of 37.58 ‰, and the temperature maintained at 18 °C. The settling velocity was calculated from Stokes' Law:

$$V = r^2C, \quad \text{with } C = \text{constant}, \quad \text{or} \quad V = (D/2)^2 \times 2(d_1 - d_2)g / 9\, z\, d_2$$

with D: diameter of grains, d_1: density of the particle, d_2: density of water, g: gravitational acceleration, z: kinematic viscosity of the fluid (BUCHANAN, 1971). The vessel was placed in a controlled temperature room and surrounded by plastic plates disposed on four sides and on the top. For measuring rate of descent of the particles, the lower part of one side was open in order to observe the particles reaching the bottom. A drop of dye was added and observed to establish that temperature gradients did not exist.

Hydrodynamic conditions. — All experiments were conducted in aquaria measuring 60 x 40 x 20 cm containing still sea water 15cm deep. In this set up, the distribution of tubes could only arise from the larvae's ability to move. Larvae made multiple passes over the plates; visits of these plates were followed by a return to the water column, after leaving a mark on the sediment. Light and temperature were similar for all experiments.

Observation of larvae. — Larvae were observed at the surface of the sediment using two compound microscopes: one with a horizontal line of sight, the other with a vertical line of sight. These two microscopes were set on supports equipped with rollers which enabled them to travel on two long horizontal guides (CHA, 1990).

Quality of the biological materials. — Spawned egg masses were available in large numbers which permitted the use of large volumes in contrast to "finger bowl" ecology (SCHELTEMA, 1986). The synchrony of development within one egg mass provided individuals at the same developmental stage. The egg masses were laid in the field. The success of settlement was recorded by counting the number of tubes present. Tube building did not stop once it had commenced. All larvae appeared healthy and the number of live individuals did not change significantly during the experiments in spite of a change from pelagic to benthic ecosystems.

RESULTS

Manipulation of particles by larvae with no choice of sediment. — (exp. nb. 1 in Table 1). Table 2 shows that the maximum particle size for transport and agglomeration was about 160 µm. The optimal particle size for construction of a clearly defined individual tube was 60 µm. The use of sediment seemed to be related to the ease with which the particles could be manipulated. There was an inverse correlation between the sedimentation rate of particles and the ability of the larvae to manipulate particles and construct a tube. Sedimentation rates of live larvae were also measured as for the sediments. For 12 to 16 day old larvae, the mean, of 30 trials, (Table 2: n° 15) was one-half that for the finest silt particles (n° 9) and one-fourth that of the larger silt particles (n° 7 and 8). Items 7, 8 and 9 were the most used particles.

Comparison of the net weight of larvae and particles may explain these results. Net weight is given by $6\, z\, (d_1 - d_2)\, V\, D/2$. Larvae are viewed as spheres of 120 µm D, and are compared to sediment 7 (Table 2) which contained the larger particles (D= 60 µm) used in tube construction. All parameters giving net weight were known except for d_1; however, the density of organic matter of larvae was certainly lower than that of the sediments and $(d_1 - d_2)$ is thus lower for larvae. If Dlarvae is twice Dsediment, and Vlarvae is four times lower than Vsediment, the net weight of larvae is one-half that of the sediment. The problem of manipulating particles arises from the fact that the net weight of the larvae is lower than the particles. Under these conditions how may larvae grasp particles? We observed that for sediments 1-5, and for 6-7 (each group with same d_1), strength of use grew when D and V decreased, or when net weight of particles decreased. That suggested that use of particles by larvae was correlated with the weight of particles.

Effect of larval age.— (exp. nb. 2 in Table 1). Two stocks of larvae (14 and 18 day old, respectively) were presented (Fig. 1) with two types of sediments (microbeads, MB and decanted silt DS, items 7 and 8 in Table 1) with each sediment presented as a thick and a thin layer. Each experiment was run in two enclosures, each enclosure receiving one age class and four substrates. Ten replicates were carried out. Only a summary is given with the quantitative data reduced as the percentage of the total number of tubes in each of the four sediments. Younger larvae preferred the thin layer of sediment. Presence (in DS) or absence (in MB) of organic matter was not a source of variance. The greatest difference which occurred between the highest and lowest number of tubes was 41.8 and the coefficient of variation was 88.2. The nature and the thickness of the sediment were of lesser importance for the old larvae than for younger. The greatest difference in the number of settled larvae was only 22.5 % with a coefficient of variation of 47.1. A comparison test (χ^2) of the two series gave a significant difference at a confidence level of 0.05. An age difference of four days resulted in highly modified settlement. Older larvae were less susceptible to differences between substrates; they had a settlement capability greater than younger larvae, and they were distributed more evenly between different sediments.

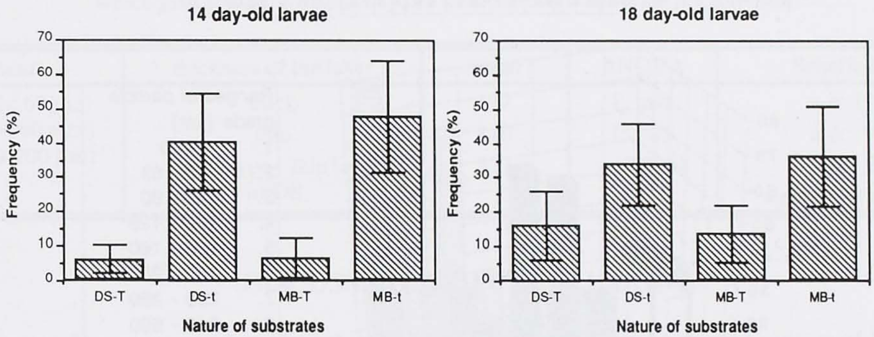

FIG. 1. — Effect of larval age on selectivity with four sediments, as shown by comparing settlement on various sediments by larvae 14 (left) and 18 (right) days old. Blocks represent the percentage of the number of tubes which appeared after 4 days for each age group in each series of four sediments. Mean from 10 replicates. Vertical bars represent standard deviations. DS: decanted silt < 60 µm; MB: microbeads 60-45 µm; T: thick layer; t: thin layer.

Effect of duration of incubation. — (exp. nb. 3 in Table 1). Approximately 2,000 larvae were introduced into an enclosure with eight size fractions of fine sand from the *S. subtruncata* community. The numbers of tubes constructed were observed daily, and these data are summarized in figure 2. Twenty-four hours after the introduction of larvae, the rates of settlement were highest on the finer sediments (1, 2 and 3), lower on middle-grade ones (4 and 5) with no settlement on the coarser ones (6, 7 and 8). Sediments of grades 1 and 2 were the preferred substrate on day 6. Sediments of grade 4 had a similar number of tubes as grade-3 sediment after six days, but they appeared later. Very few tubes were made in sediment grades 6, 7 and 8 and they were not formed until day 6.

Addition of tubes formed on each sediment grade was normalized to 100 (Fig. 2), but the number of tubes built on each sediment was not the same. Comparison of the real number of settled larvae on substrates just after disappearing from the water column (horizontal boxes "hrs2"), with the number of tubes counted on day 6 (horizontal boxes "day 6"), allows one to conclude that a numeric rearrangement occurs. Larvae settling on sediment 6-7-8 cannot use it but can still return to the water column. This increased the probability of reaching easy to use sediments. A small fraction of larvae visiting 6-7-8 and registered in boxes "hrs2" do not leave these sediments. They can wait for several days during which morphological development proceeds. Accordingly, unused sediment on the first day may be used four or six days later. On sediments 4 and 5, tubes appeared only on the third day. Sediments most easily manipulated at the start of the experiment had the highest density of tubes at the conclusion. Larvae which sedimented on sediment 1-2-3 had the opportunity to settle and did not leave.

Use of heterogeneous sediments. — (exp. nb. 4 in Table 1). The preceding experiments 1 to 3) were not directly relevant to the Bay of Banyuls where sediments are more or less heterogeneous. The most homogeneous sediment (the Well Sorted Fine Sand) is formed of 70 % of 100 to 200 µm particles plus a small fraction of larger (200 to 500 µm) and smaller (< 40 µm) particles (GUILLE, 1970). We used natural calibrated sediments, isolated or mixed together, and two natural, unsorted sediments in reconstructing natural sediments for laboratory experiments.

a) *Natural sediment, artificially sorted.* — The larvae were offered three sediments with five replicates each and were placed in one aquarium. The number of tubes were counted at day four. Treatments were randomly distributed. The three sediments were: (a) silt in thick layer giving a very fluid boundary layer, (b) silt in thin layer, (c) sand in thick layer with a thin layer of silt on the surface. Statistical comparison (Table 3) showed that a & b, and a and c, were significantly different but treatments b and c were not.

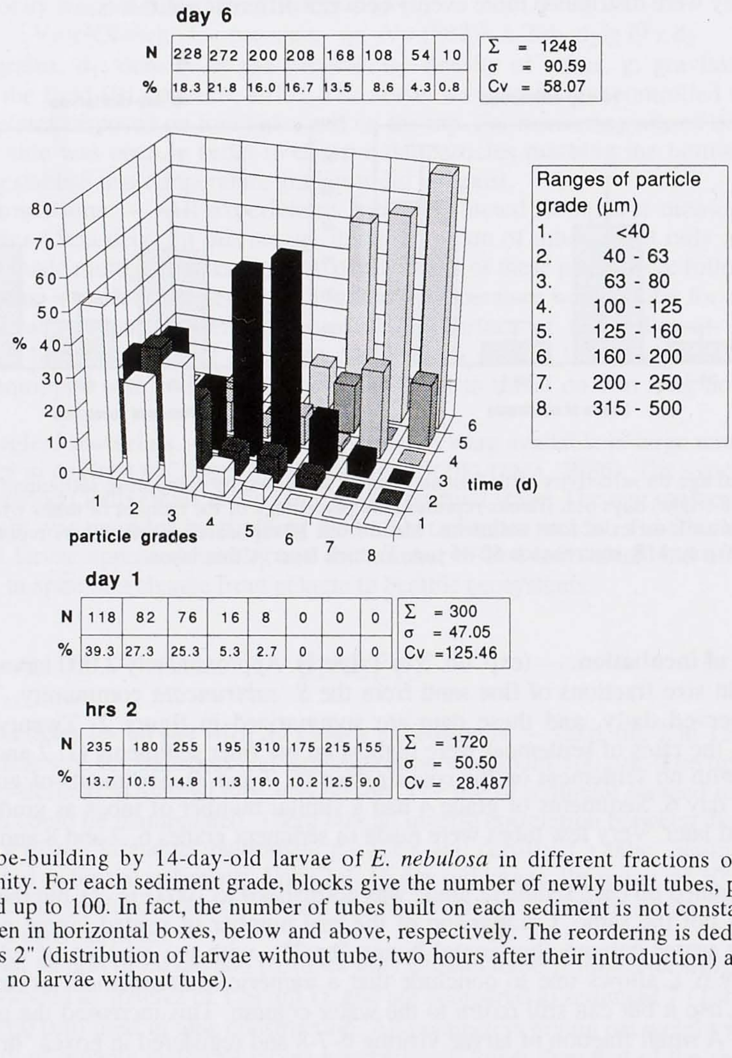

FIG. 2. — Rates of tube-building by 14-day-old larvae of *E. nebulosa* in different fractions of sand from the *Spisula subtruncata* community. For each sediment grade, blocks give the number of newly built tubes, per day. The block values in each sediment add up to 100. In fact, the number of tubes built on each sediment is not constant; the real situations for days 1 and 6 are given in horizontal boxes, below and above, respectively. The reordering is deduced from comparison of horizontal boxes "hrs 2" (distribution of larvae without tube, two hours after their introduction) and horizontal boxes "day 6" (juveniles in tube, no larvae without tube).

A grain of sand 160-200 µm in diameter was too large for tube building (Table 1). The tubes in series c were made from silt but not from the sand. The difference in colour between silt and sand made the grey silt tubes conspicuous against the white sand (CHA & BHAUD, 1992). These results suggest that conditions for settlement were similar in b and c: the material used to build tubes in both cases was silt and the large grains of sand, in c, were not used. To explain these observations, we examined the larvae in detail under a compound microscope.

This experiment suggested that a solid base is necessary for aggregation of particles with mucus by larvae and for positioning larvae as the tube is built. Construction seemed to be impossible on a fluid substrate which continually gives way and contained no solid components. This explained why the results from treatments b and c were similar as large sand grains in c acted as stable bases for settlement, similar to the bottom of dishes in treatments b.

b) *Natural sediments.* — The larval requirements for settlement, as identified by the preceding experimental observations, allow one to predict which natural sediments are suitable for juveniles of *E. nebulosa*. Juveniles required a minimum of clay and medium-sized sand grains. The silty sand of the *N. hombergii* community seems suitable, as well as the fine sand of the *S. subtruncata* community. Experiments confirmed that these sediments were potential substrates for *E. nebulosa* larval settlement. In each of 10 replicates, 50 larvae introduced into enclosures with these two natural substrates produced 50 formed tubes and inhabited by well-developed juveniles. The important result was demonstrating the ability of the larvae to use a fraction of a heterogeneous sediment. Photographs and additional results have been presented elsewhere (CHA & BHAUD, 1992).

TABLE 3. — Number of tubes distributed among three substrates, each presented on five plates in one aquarium; surface of plates 63 cm^2. ANOVA shows that total means are significantly different from each other, which justifies comparisons in pairs showing that b and c are not different.

sediment	thickness of the layer	mean	ANOVA	Scheffe F. Test
a DS (< 60 µm)	thick	197	F. test:	a-b: 13.58*
b DS (< 60 mm)	thin	410	15.726	a-c: 9.68*
c sand (160-200 µm)	thick layer + thin layer of DS	377	p: 0.0004	b-c: 0.329
				* significant at 0.05 level

DISCUSSION AND CONCLUSIONS

The four groups of experiments described provided information concerning several aspects of larval settlement: (1) the need for a heterogeneous substrate, (2) the importance of the age of larvae at the time of first contact with the sediment, and (3) the mechanism of substrate selection. Sediment preferences may change as larvae increase in size and age. This may not be applicable to other species; a behavioural variability may occur (RAIMONDI & KEOUGH, 1990).

A solid settlement base is required for coordinated movements of the animal. These coordinated movements assume that the compression of the coelomic fluid, permitting extension of the body, is possible. The manipulation and arrangement of particles is then possible. This dual constraint (solid base plus manipulable building particles) is related to the nature of the substrate. It differs from previous conclusions which suggested that only grain size was important for settlement (WILSON, 1948, 1952 for *Ophelia bicornis*; GRAY, 1967 for *Protodrilus rubropharyngeus*; DORSET, 1961 and HEMPEL, 1957 for *Polydora ciliata*). Our experiments show also the importance to settlement of the thickness of the layer of sediment. Similar observations have been made for *Polydora ciliata* (LAGADEUC, 1991) and *Thelepus setosus* (DUCHÊNE, 1983). WILSON (1952), among the 29 points of his summary, and BUTMAN (1987), in her review, did not approach this question.

Larvae at each stage of development are able to manipulate only a well-defined size range of particles. This range increases as development proceeds. The older the larvae are when they touch the substratum for the first time, the weaker their ability to return to the water column if the substrate is unsuitable. In the same way, the suitable particle size range increases with age, and the probability of successful settlement increases. These behavioural modifications are probably very frequent during development. They are linked to the morphological development by the increasing number of tentacles, setigers and oncinial plates in *E. nebulosa*. These modifications have also been observed in molluscs (AABEL, 1983, 1984; LABOURG & LASSERRE, 1980).

The mechanism of substrate selection is dependent upon whether the larvae can use a particular particle or not. In this context it is possible to refer settlement to opportunity rather than choice. A choice supposes a comparative action on a small time and space scale between equally and simultaneously accessible alternatives. Opportunity results from tests developed by larvae faced with only a single sediment size present.

Application of experimental results to the life cycle of *E. nebulosa* under natural conditions is limited. Experimental work on *E. nebulosa* settlement can only be partially transferred to the field. Larvae were able to settle on two natural sediments: the fine sand and silty sand of *S. subtruncata* and *N. hombergii* communities, respectively. However, juveniles have not been collected from these sediments during a one year study of the Bay of Banyuls (GREHAN, pers. comm.) nor in epibenthic sledge samples collected two months after the first spawning period (BHAUD & CHA, pers. obser.). Adults have not been found in soft substrates in the Bay of Banyuls (GUILLE, 1970). Adult habitat requirements of *E. nebulosa* suggest that their absence in both the *N. hombergii* and the *S. subtruncata* communities is not surprising. Adults of *E. nebulosa* are collected among rhizomes of the seagrass *Posidonia* in Banyuls (KERNEIS, 1960). LAUBIER (1966) stated that *E. nebulosa* is present in low numbers on coralline substrates on the French Mediterranean catalan coast. This species also occurs on algae of the upper part of the infralittoral level, on the "silty coastal bottoms" and in the Lacaze-Duthiers canyon (LAUBIER & PARIS, 1962). A detailed study in the English Channel (LANG, 1986) showed that individuals were protected by boulders buried in the sediment; their tubes were located on the lower side of the boulders, in close contact with the sediment. Thus, *E. nebulosa* is a species that lives at the interface of hard and soft substrate. Heterogeneity of substratum is acknowledged as a requisite for settlement of larvae and points to the way of life of adults. While larvae can settle on a variety of substrates, these may not be able to support adult populations. The presence of egg masses restricts the dispersal of larvae, and larval retention represents the coordination between two elements of the life cycle: modest granulometric requirements defining a large potential area for settlement, and a spatially limited adult distribution. The mucous structure makes these two elements compatible by limiting larval dispersal.

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