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Induction of larval metamorphosis, survival and growth of early juveniles of the burrowing echinoid *Echinocardium cordatum* (Echinodermata)

Cristina De Amaral P. NUNES[1]* and Michel JANGOUX[1,2]
(1) *Service de Biologie Marine, Université Libre de Bruxelles (C.P. 160/15), 50, Av. F. D. Roosevelt, B - 1050 Bruxelles, Belgium*
**Corresponding author: Present address: INRB-IPIMAR, Instituto de Investigação das Pescas e do Mar, U-REMS, Unidade de Recursos Marinhos e Sustentabilidade, Avenida Brasília, 1449-006 Lisboa, Portugal*
Tel.: + 351 21 302 71 55, Fax: + 351 21 301 59 48. E-mail: cnunes@ipimar.pt
(2) *Laboratoire de Biologie Marine, Université de Mons-Hainaut, 20, Place du Parc, B-7000 Mons, Belgium*

Abstract: Recruitment in most marine invertebrates depends on the larval supply and the success of metamorphosis as well as on the growth and survival of the early juveniles. These two aspects of the early-life history of the burrowing echinoid *Echinocardium cordatum* have been investigated in the laboratory. Seven natural substrata were tested with competent larvae to assess their metamorphosis inducing capacity. Sediment (the natural substratum inhabited by the echinoids) - harbouring or not conspecific adults - was the most effective inductor, with nearly 100% metamorphosis. Cleaned sediment was much less inducing, indicating that associated microbes and/or organic matter might be the cues responsible for the starting of metamorphosis. Coralline algae were the second most inductive substratum (50-64% metamorphosis), while biofilm, detritic particles and green macroalgae (*Enteromorpha* sp.) were significantly less effective. A 47d survey of the survival and growth of early juvenile *E. cordatum* in sediment with various grain sizes (very coarse, coarse, medium, fine and silty) was undertaken. Survival was the highest in the medium grain sand, although the obtained differences among the five sediment types were not statistically significant. During the first month, growth was significantly faster in the finer sediments (fine and silt), but at the end of the experiment all sediment types were equivalent in terms of sizes reached by the juveniles. The results indicate that neither the competent larvae nor the juveniles appear to be very specific in terms of sediment type, suggesting that other factors (e.g., mortality or migration) would determine the distribution and abundance of adult populations.

Résumé : *Induction de la métamorphose larvaire, survie et croissance des juvéniles de l'oursin fouisseur* Echinocardium cordatum *(Echinodermata).* Le succès du recrutement chez la plupart des invertébrés marins est dépendant aussi bien du nombre de larves produites que de la réussite de leur métamorphose et de la croissance/survie des juvéniles résultants. Ces différents aspects du cycle de vie du spatangue *Echinocardium cordatum* ont été étudiés expérimentalement en laboratoire. Sept substrats naturels ont été testés afin de déterminer leur capacité inductrice de la métamorphose des larves de l'espèce. Le sédiment - conditionné ou non par des conspécifiques adultes - est le substrat le plus efficace, induisant près de 100% de métamorphoses. Le rinçage et séchage du sédiment diminuent considérablement son pouvoir inducteur, suggérant un rôle important de la matière organique associée (microorganismes et/ou détritus) dans le déclenchement de la métamor-

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phose. Des individus post-métamorphiques d'*E. cordatum* ont également été placés en présence de sédiments de granulométrie différente (sable grossier, moyen, fin, sablon et sable vaseux) et leur survie et croissance ont été suivies pendant 47 jours. Un plus grand nombre de juvéniles a survécu dans du sable fin, mais les différences obtenues selon le type de sédiment sont statistiquement non significatives. Le taux de croissance durant le premier mois a été plus élevé dans le sablon et le sable vaseux ; cependant, en fin d'expérience les juvéniles ont atteint une taille similaire dans les cinq types de sédiment. Ces résultats montrent que ni les larves compétentes ni les juvéniles n'ont un comportement spécifique eu égard au type de sédiment ; ils suggèrent que d'autres facteurs, tels que la mortalité différentielle ou les migrations, détermineraient la distribution et l'abondance des populations adultes d'*E. cordatum*.

Keywords: *Echinocardium cordatum* ● Metamorphosis ● Early growth ● Survival

Introduction

As in most species of marine benthic invertebrates, success of metamorphosis of competent echinoderm larvae depends on the occurrence in the surroundings of certain metamorphosis-inducing factors (for review, see Pawlik, 1992; Rodríguez et al., 1993; Pearce, 1997). Among echinoderms, these were identified for several species as being, notably, microorganisms of epibenthic films (Cameron & Hinegardner, 1974; Chen & Run, 1989; Pearce & Scheibling, 1991; Ito et al., 1991; Rahim et al., 2004), species of macroalgae (Pearce & Scheibling, 1990a & 1991; Kitamura et al., 1993; Gosselin & Jangoux, 1996; Swanson et al., 2004), or conspecific adults (Highsmith, 1982; Burke, 1984; Pearce & Scheibling, 1990b). After metamorphosis, early juveniles face particular environmental conditions which may lead to selective survival, differential growth rates and/or migration events of individuals (Ólafsson et al., 1994; Fraschetti et al., 2003). Considering the large spatial and temporal variability observed by many authors investigating echinoderm recruitment, success in metamorphosis and early juvenile life may play an equally important role in determining species population structure and dynamics (for review, see Ebert, 1983; Hunt & Scheibling, 1997; Balch & Scheibling, 2001).

Studies regarding the above mentioned aspects in spatangoid echinoids are non existent and most published data on spatangoid populations have dealt with late juveniles and adults of the cosmopolitan species, *Echinocardium cordatum* (Pennant, 1777). *E. cordatum* is a major component of the soft substratum associated macrofauna and forms patchy and dense intertidal and subtidal populations in the North Atlantic (Mortensen, 1951; Ursin, 1960). Past observations indicated that, although its larvae are present every year in the plankton (they are often the most abundant larval representatives; Rees, 1954; Ursin, 1960; Hecq, 1975; Kirby & Lindley, 2005), recruitment intensity in the species is extremely variable (from no recruitment at all to a huge number of recruits; Ursin, 1960; Buchanan, 1966 & 1967; Ziegelmeier, 1978; Beukema, 1985; Guillou, 1985). Furthermore the growth and survival of the species, and its population structure significantly differ between intertidal and subtidal areas as well as according to sediment grain size. For instance, higher growth rates are observed in clean fine sands compared to silty sediments, and in shallower (intertidal) waters compared to deeper (subtidal) waters (Buchanan, 1966; Duineveld & Jenness, 1984; De Ridder et al., 1991; Nakamura 2001).

The present work was performed in laboratory conditions. It aimed to score metamorphosis of competent larvae in response to various natural substrata, and to determine which type of sediment is the most efficient in terms of early juvenile survival and growth.

Material and Methods

Adults *Echinocardium cordatum* (average body length of ca. 50 mm) were hand collected intertidally at Wimereux (Pas-de-Calais, France: 50º46'N, 01º35'E) during spawning season (April to June; De Amaral P. Nunes & Jangoux, 2004) and maintained in running seawater aquaria (aquarium bottoms were covered by a 15-cm thick layer of sediment originating from the collection site). Rearing of larvae was performed according to the method of De Amaral P. Nunes & Jangoux (2007).

Induction of metamorphosis

Experiments were run during the echinoids natural spawning season, at 21ºC and under natural light. Each experimental series was done with larvae of the same parental origin (one single male and one single female contributed to progeny). Before experiments larval competence was properly assessed (see De Amaral P. Nunes & Jangoux, 2007). Experiments were done in cylindrical polystyrene beakers (55 mm diameter, 70 mm high) containing 90 ml of natural seawater (previously allowed to settle in large tanks for 48 h). Seven different putative inductors were tested: (1)

epibenthic biofilms (i.e., biofilms which develop on the beakers' walls after immersion for 7 d in running seawater; see Gosselin & Jangoux, 1996), (2) detritic materials (i.e., bottom detritus accumulated in running seawater aquaria), (3) untreated intertidal sediment from the collection site of *E. cordatum*, (4) untreated intertidal sediment from a site without *E. cordatum* (Luc-sur-Mer, Normandy, France), (5) cleaned intertidal sediment from the collection site (sediment washed in distilled water and oven-dried at 100°C for 24h), (6) coralline algae (*Corallina elongata* hand-collected at cap Lévy, Normandy, France), and (7) other macroalgae (*Enteromorpha* sp. hand-collected at Luc-sur-Mer). Putative inducing substrata were transferred to the experimental beakers with either a plastic pipette (inert polyethylene) or a pair of forceps (stainless steel), the bottom of the beakers being filled up of each substratum up to ca. 5 mm. Seawater in the beakers was allowed to rest for a few minutes before competent larvae were gently added with a plastic pipette. The metamorphosis inducing action of the various substrata was assessed by counting, under a binocular microscope, the number of metamorphosed individuals (i.e. postlarvae; for more details on their morphology, see De Amaral P. Nunes & Jangoux, 2007) in each experimental beaker after 24 h. Control treatments were established in cleaned beakers containing seawater only. Two experiments were performed with 5 replicates (30 larvae per replicate) for each tested condition and for the control. The obtained data were treated by means of a Kruskal-Wallis rank, followed by pairwise comparisons using the Mann-Whitney procedure (Zar, 1999).

Post-metamorphic survival

Experimental sediment was prepared in order to obtain different granulometric modes and median size particles while reproducing the shape of the grain-size frequency distribution curve of the natural sediment from the adult collection site. This was done by oven-drying at 65ºC and for 24 h sediment from the collection site before sieving it through 23 sieves ranging from 0.04 to 6.3 mm mesh-size [AFNOR series]. This showed that the sediment of the collection site consists in fine sand with 91% of the sediment particles in the size range of 125 to 250 µm (Fig. 1B). The different sediment to be tested (silty sand, fine sand, medium sand, coarse sand, and very coarse sand) were thus obtained by mixing determined amounts of particles from various granulometric fractions (Fig. 1A, Table 1).

Table 1. *Echinocardium cordatum*. Median and mode values of grain size for each reconstituted sediment.

Tableau 1. *Echinocardium cordatum*. Médiane et mode de la granulométrie de chaque sédiment expérimental.

Experimental Sediment	Grain size (mm) Median	Mode
Very coarse sand	1.250	1.125
Coarse sand	0.565	0.500
Medium sand	0.250	0.225
Fine sand	0.142	0.125
Silty sand	0.138	0.125

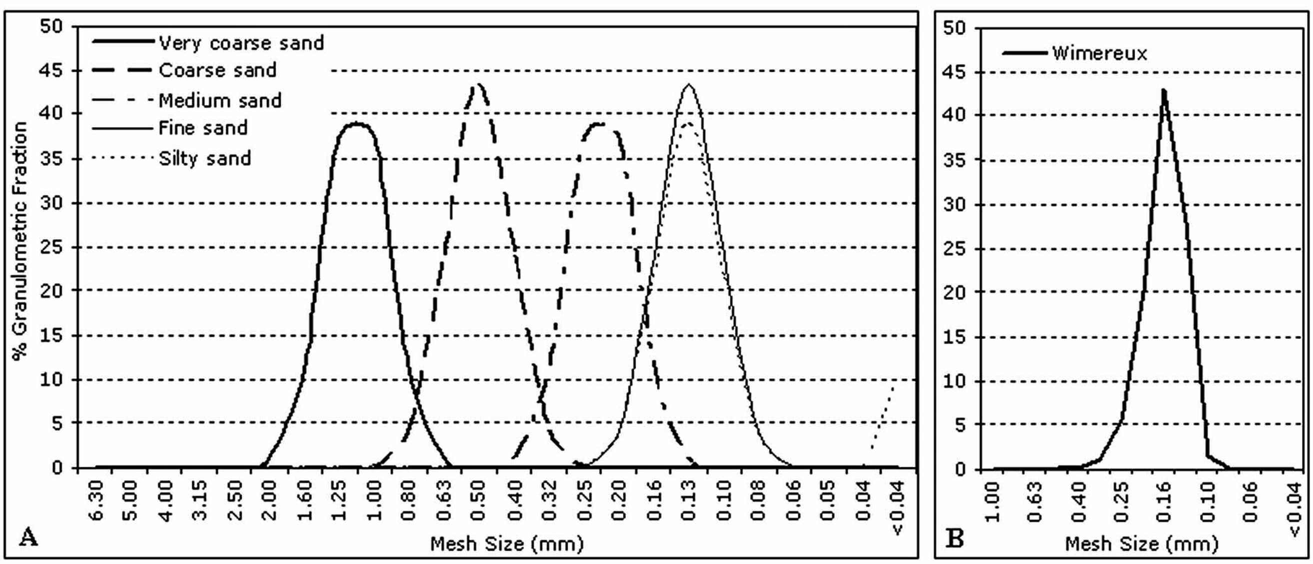

Figure 1. *Echinocardium cordatum*. Granulometry. **A.** Reconstituted sediment. **B.** Natural sediment.
Figure 1. *Echinocardium cordatum*. Granulométrie. **A.** Sédiment expérimental. **B.** Sédiment naturel.

Post-metamorphic survival was assessed over time by counting juvenile individuals placed on different sediment. Used juveniles resulted from mass metamorphosis of coralline-induced competent larvae. Five experimental and one control series were performed. Individuals to be tested were collected using a plastic pipette when being 1 d-old (after metamorphosis) and placed in especially designed PVC (Polyvinyl chloride) tubes with a sieved bottom. These are cylindrical PVC tubes (40 mm diameter, 50 mm high) placed vertically, and closed at their bottom end by a 25-µm nylon mesh. A small plastic tap allowed water to fall continuously and gently (drop by drop) through each tube in order to maintain a constant water circulation (Fig. 2). Each tube had 30 individuals, and each series consisted in 20 tubes, the bottom of which is either covered by a 5 mm thick layer of sediment (silty sand, fine sand, medium sand, coarse sand, and very coarse sand, depending on the experimental series) or has no sediment (control series). [Experimental sediment was previously placed for 3 d in slightly sloping, running seawater aquaria (see Grosjean et al., 1998) to form a natural biofilm on constituting particles.] Tubes are grouped in experimental tanks, being placed ca. 1 cm above the tanks bottom (Fig. 2). The whole system was kept at 20 ± 1ºC and with a photoperiod of 12L:12D. Experiments lasted for 23 days. At days 3, 6, 13 and 23, three tubes of each series were sampled and the number of live individuals per tube was counted. The obtained data were treated by means of a Kruskal-Wallis rank analysis, followed by nonparametric multiple comparisons adapted to unequal sample sizes (Zar, 1999).

Post-metamorphic growth

Post-metamorphic growth was assessed by measuring individuals placed on different sediment over time. A sample of sixty-two 1 d-old postmetamorphic individuals was fixed in 70% ethanol (in seawater) for initial body measurements (individual length and width). Other batches of 1 d-old metamorphosed individuals were placed in sieved tubes (30 individuals per tube; 120 tubes in the total) whose bottom was covered by a 5 mm thick layer of sediment (5 sediment types were considered plus a control series [i.e., with no sediment]). Experiment lasted for 47 days. At days 13, 23, 30 and 47, three tubes in each series and in the control were removed, and the individuals they contained were fixed and measured using a Leitz Diavert inverted microscope equipped with a Reichert PK ocular micrometer. Measurements were the longer (Length) and shorter (Width) diameters of the body test (spines and appendages were not included) obtained from the 2-D projection of the individuals lying on their oral side. Data

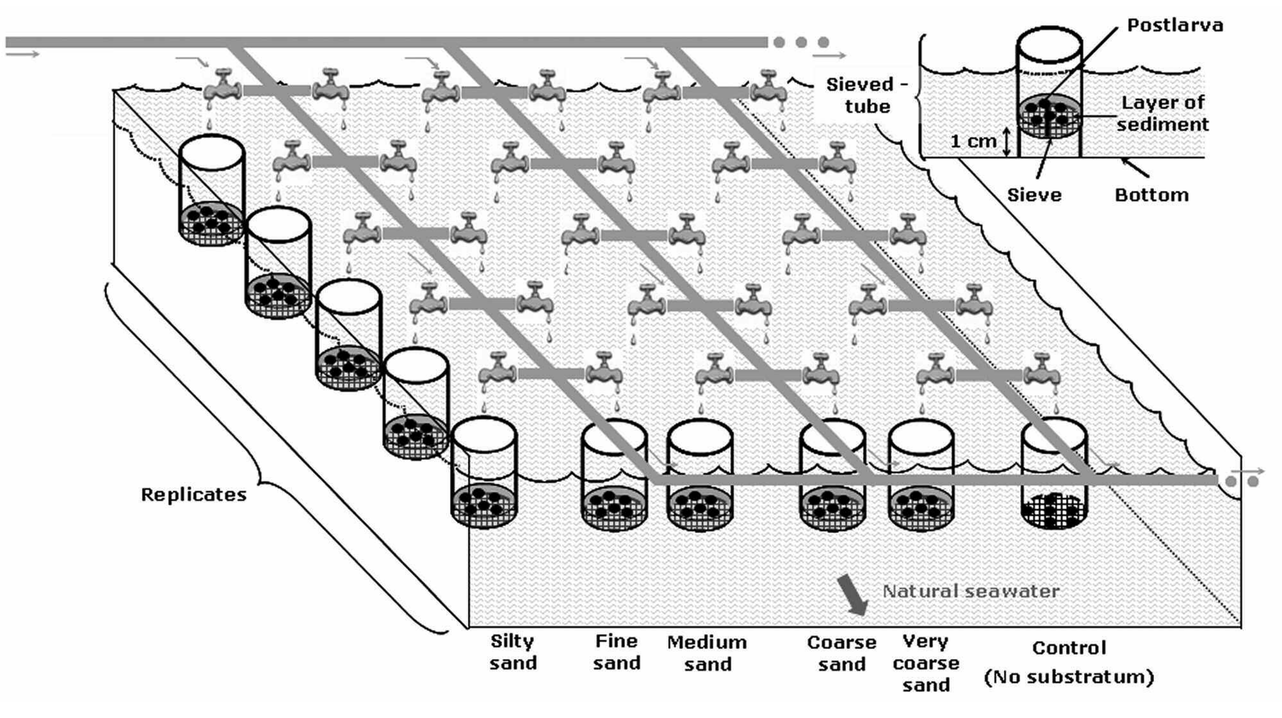

Figure 2. *Echinocardium cordatum*. Device used to experiment juvenile survival and growth.
Figure 2. *Echinocardium cordatum*. Dispositif expérimental pour le suivi de la survie et de la croissance des juvéniles.

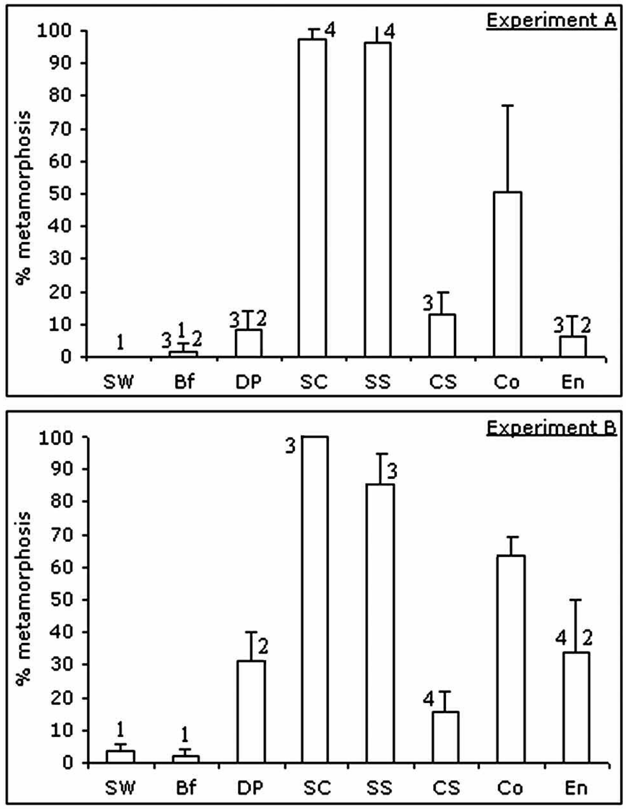

Figure 3. *Echinocardium cordatum*. Induction of larval metamorphosis (values are given in %; mean ± standard deviation, n = 5). Experiments were done with competent larvae of 18 d-old (experiment A) and 20 d-old (experiment B), respectively. SW: natural seawater (control); Bf: 7 d-old biofilm; DP: detritic particles; SC: sediment from the sea urchin collection site; SS: sediment from a site without sea urchin; CS: cleaned sediment; Co: coralline algae; En: *Enteromorpha sp*. Same numbers above columns mean not significantly different (Mann-Whitney, p > 0.05).

Figure 3. *Echinocardium cordatum*. Induction de la métamorphose (valeurs en % ; moyenne ± écart-type, n = 5). Les expériences ont été réalisées, respectivement, avec des larves compétentes de 18 jours (expérience A) et de 20 jours (expérience B). SW : eau de mer naturelle (contrôle) ; Bf : biofilm âgé de 7 jours ; DP : particules détritiques ; SC : sédiment provenant de la localité où les oursins adultes ont été récoltés ; SS : sédiment provenant d'une localité dépourvue d'adultes de *E. cordatum* ; CS : sédiment traité (rincé et séché) ; Co : algues corallines ; En : *Enteromorpha sp*. Des chiffres identiques en haut des barres de l'histogramme signifient que les échantillons correspondants ne sont pas significativement différents (Mann-Whitney, p > 0,05).

were treated by means of a Kruskal-Wallis rank analysis, followed by nonparametric multiple comparisons adapted to unequal sample sizes (Zar, 1999).

Results

Induction of metamorphosis

Figure 3 shows the results of the experiments done with competent larvae of 18 d-old (experiment A) and 20 d-old (experiment B), respectively. They indicate that natural sediment, either from the collection site or from the site without sea urchins, is the most inductive with nearly 100% metamorphosis. On the contrary, cleaned sediment and detritic particles alone induced rather few larvae to metamorphose: from 12.9 ± 7.0% to 15.4 ± 6.4% for cleaned sediment; from 8.2 ± 5.7% to 31.5 ± 8.6% for detritic material. As for the beakers covered with biofilm, it appeared to be almost non-inductive (results were not statistically different from the control one, p = 0.42). Response of larvae to macroalgae was less intense than to the sediment. Yet, induction in presence of coralline algae

Table 2. *Echinocardium cordatum*. Survival of post-metamorphic individuals (%) according to the type of sediment (mean ± standard deviation, n = 3) (VCS: very coarse sand; CS: coarse sand; MS: medium sand; FS: fine sand; SS: silty sand). Numbers in brackets correspond to the total number of live individuals at each time.

Tableau 2. *Echinocardium cordatum.* Pourcentages de survie des individus post-métamorphiques en fonction du type de sédiment (moyenne ± écart-type, n = 3) (VCS : sable grossier ; CS : sable moyen ; MS : sable fin ; FS : sablon ; SS : sable vaseux). Les chiffres entre parenthèses correspondent au nombre total d'individus vivants à chaque moment.

Days	% survival Sediment type					
	Control (C)	VCS	CS	MS	FS	SS
0	100 (150)	100 (150)	100 (150)	100 (150)	100 (150)	100 (150)
3	94.5 ± 4.0 (141)	100 (150)	84.1 ± 10.5 (127)	85.0 ± 9.2 (128)	92.1 ± 5.5 (137)	86.5 ± 8.8 (130)
6	88.6 ± 11.8 (132)	98.7 ± 1.8 (148)	88.2 ± 9.5 (131)	86.5 ± 8.0 (130)	94.7 ± 4.6 (141)	89.9 ± 8.1 (135)
13	13.3 ± 7.4 (20)	84.4 ± 12.7 (127)	83.5 ± 10.7 (125)	97.0 ± 3.0 (145)	96.1 ± 3.0 (144)	93.9 ± 5.0 (140)
23	10.0 ± 10.4 (15)	47.3 ± 6.8 (71)	68.7 ± 6.1 (103)	86.0 ± 13.2 (129)	48.7 ± 44.5 (73)	55.5 ± 42.9 (84)

Table 3. *Echinocardium cordatum*. Post-metamorphic growth (mm of body length, mean ± standard deviation) at various time intervals and according to sediment types (VCS: very coarse sand; CS: coarse sand; MS: medium sand; FS: fine sand; SS: silty sand). Figures in brackets correspond to the number of individuals analysed.

Table 3. *Echinocardium cordatum*. Croissance en taille (longueur du corps en mm, moynne ± écart-type) des individus post-métamorphiques au cours du temps et en fonction du type de sédiment (VCS : sable grossier ; CS : sable moyen ; MS : sable fin ; FS : sablon ; SS : sable vaseux). Les chiffres entre parenthèses indiquent le nombre d'individus mesurés.

Days	Length (mm)					
		Sediment type				
	Control (C) (x)	VCS (■)	CS (▲)	MS(◆)	FS (△)	SS (◇)
0	0.48 ± 0.04 (62)	0.48 ± 0.04 (62)	0.48 ± 0.04 (62)	0.48 ± 0.04 (62)	0.48 ± 0.04 (62)	0.48 ± 0.04 (62)
13	0.65 ± 0.15 (18)	0.87 ± 0.15 (77)	0.97 ± 0.22 (91)	0.96 ± 0.25 (94)	1.38 ± 0.33 (68)	1.41 ± 0.31 (61)
23	0.55 ± 0.15 (31)	1.14 ± 0.28 (47)	1.25 ± 0.23 (72)	1.03 ± 0.25 (92)	1.55 ± 0.28 (52)	1.60 ± 0.35 (50)
30	-	1.35 ± 0.42 (33)	1.36 ± 0.32 (38)	1.62 ± 0.40 (42)	1.59 ± 0.22 (45)	-
47	-	1.37 ± 0.25 (33)	1.41 ± 0.28 (36)	1.54 ± 0.31 (18)	1.62 ± 0.24 (49)	1.73 ± 0.36 (19)

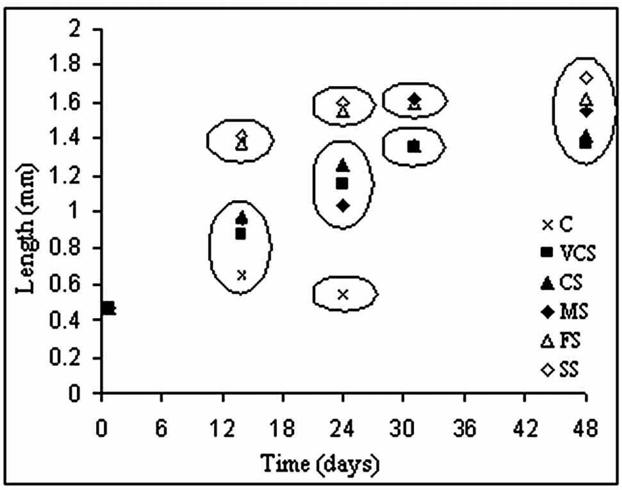

Figure 4. *Echinocardium cordatum*. Post-metamorphic growth (mm of body length; only mean values are indicated) according to sediment types (for abbreviations see Table 3). Results of statistical analysis (nonparametric multiple comparisons tests adapted to unequal sample sizes): ovals group samples which are not significantly different ($p > 0.05$).

Figure 4. *Echinocardium cordatum*. Croissance en taille (longueur du corps en mm; seules les valeurs moyennes sont présentées) des individus post-métamorphiques en fonction du type de sédiment (pour les abréviations voir Table 3). Résultats de l'analyse statistique (tests non-paramétriques de comparaisons multiples adaptées à des tailles d'échantillons différentes) : les ovales groupent les échantillons qui ne sont pas significativement différents (p > 0,05).

was significantly more efficient than with *Enteromorpha sp*. ($p = 0.008$): 50.3 ± 26.5% against 6.1 ± 6.5% (experiment A); 63.7 ± 5.5% against 33.6 ± 16.5% (experiment B).

Post-metamorphic survival

Survival rates at day 3 and day 6 were high in all series including the control (Table 2). By day 13, a huge mortality occurred in the control series while survival remained high in every other (survival in silty, fine and medium sands was higher than in coarse and very coarse sands (Table 2), although differences were not statistically significant [nonparametric multiple comparisons, $p > 0.05$]). Comparing day 13 to day 23, it appeared that survival rates were higher in medium sand than in coarse and very coarse sands (differences, however, were not statistically different; nonparametric multiple comparisons, $p > 0.05$). As for silty and fine sands, survival rates at day 23 lowered considerably, though they are highly variable according to the batches.

Post-metamorphic growth

Body length and width showed to be strongly correlated (within this range of juvenile sizes), the latter representing ca. 84.5% of the former (linear regression, $r^2 = 0.98$); hence data analysis have only considered one of them (i.e., length). Growth of individuals occurred in each series except the control (no individual from the control could be measured after day 23 due to 100% mortality) (Table 3 and Fig. 4). When measured at day 47, no significant differences in size were noted among the different series. Yet, up to day 30, growth was significantly higher for individuals on silty and fine sediment than on other sediment ($p < 0.001$).

Discussion

Investigating metamorphosis induction in a given species of marine benthic invertebrate needs that a large number of competent larvae can be produced and that there is a complete control of the metamorphic process in that

Table 4. *Echinocardium cordatum.* Literature data on the distribution of juveniles and adults according to the type of sediment.
Tableau 4. *Echinocardium cordatum.* Informations bibliographiques sur la distribution des juvéniles et des adultes en fonction du type de sédiment.

Location	Sediment characteristics	Stage	Information available	Source
North-East coast Britain	clean fine sand, 2% silt/clay	Adults	inshore coast Britain population (5-10 ind.m^{-2})	Buchanan, 1966
	fine sand, 20-40% silt/clay		offshore population (~40 ind.m^{-2})	
	same conditions as above	Juveniles	no new recruitment during 7 years, either offshore or inshore	
Delta area (Dutch West coast)	medium sand, silt/clay content increases towards inland (estuary)	Adults	present throughout the estuary except further inland	Wolff, 1968
		Juveniles	present subtidally outside the estuary	
Bay Seine (Normandy, France)	medium to coarse sand, 5-20% silt/clay	Adults	absent from coarse sand offshore and from silty sand of the estuary	Péquignat, 1970
Provence, France	clean well sorted fine sand (bays)	Adults and Juveniles	present between 5-11 m depth	Massé, 1972
North Sea	sandy sites (Southern areas)	Adults	represent 50% benthic biomass	Duineveld & Jenness, 1984
	muddy sites (Northern areas)		represent 5% benthic biomass	
Frisian Islands (Dutch North coast)	fine sand, 0-5% mud content	Juveniles	recruitment mainly between 8-12 m depth; no clear relationship with mud content	Beukema, 1985
Bay Douarnenez (Brittany, France)	fine sand	Juveniles	regular recruitment at 5-10 m; irregular for depths > 10 m	Guillou, 1985
Bay Seine (Normandy, France)	fine sand, 5% mud (except at 5 m depth with 25% mud)	Juveniles	regular recruitment at 15-25 m; irregular or no recruitment below 10 m	De Ridder et al., 1991
Seto Inland Sea (Japan)	muddy sand (> 90% silt/clay)	Adults	present at 15-25 m depth (10 ind.m^{-2})	Nakamura, 2001
		Juveniles	recruitment in the same area, juveniles survive or collapse completely, depending on the year	

particular species. This has been achieved recently for *E. cordatum* larvae (De Amaral P. Nunes & Jangoux, 2007), and thus rendered possible the present study which investigated for the first time the response of competent larvae of *E. cordatum* to several natural substrata in terms of metamorphosis. Results showed that when placed on fresh field-collected sediment, more than 85% of competent larvae of *E. cordatum* metamorphosed. This was true whether the sediment originated from areas inhabited by adult individuals of the species or not. Competent *E. cordatum* larvae thus do not need the occurrence of adult conspecifics to settle in a given area, contrarily to what was observed in some clypeasteroid species (Caldwell, 1972; Highsmith, 1982; Burke, 1984; Pearce & Scheibling, 1990b). Yet, *E. cordatum* individuals are often distributed as large discrete patches in subtidal and infralittoral soft bottoms (Ursin, 1960; Buchanan, 1966). Considering this at a large scale, one may hypothesize that the patchy distribution of *E. cordatum* populations would more likely be the result of the settlement of larvae by physical passive deposition in areas of low hydrodynamism and/or larval retention and not of a particular gregarious behaviour of competent larvae.

Irrespective of the presence of conspecifics, occurrence of fresh sediment, however, was important for larvae to enter the metamorphic process (note that oven-drying and washing the sediment decreased substantially its inductive ability) and observations of competent larvae testing the sediment using their first podia were made repeatedly (De Amaral P. Nunes & Jangoux, 2007). Moreover, *E. cordatum* larvae did not metamorphose in large numbers in the presence of (aquarium-produced) sediment-free detritic material or biofilms, suggesting that the main metamorphic cue could be various ubiquitous sediment-associated micro-organisms.

E. cordatum competent larvae also metamorphosed in

Table 5. *Echinocardium cordatum*. Age assessment of field individuals (from literature data).
Table 5. *Echinocardium cordatum*. Estimation de l'âge des juvéniles en milieu naturel (données bibliographiques).

Location	Length information	Field sampling date	Expected age	Author
North Sea	1-6 mm (average: 3 mm)	August	> 3 months	Ursin, 1960
Normandy, France	6 ± 1 mm	August	3 months	Péquignat, 1970
	12 ± 1 mm	November	6 months	
Brittany, France	6-10 mm	October	About 2 months	Guillou, 1985
Frisian Islands Dutch	over 10 mm	September	About 3 months	Beukema, 1985
(North Coast)	15 mm	May	Nearly 1 year	
Normandy, France	5-10 mm	October	2-6 months	De Ridder et al., 1991

rather large number in presence of coralline algae. The latter are known to enhance settlement and metamorphosis in various regular echinoids as well as in several other invertebrates such as asteroids, corals and gastropods (Burke, 1983; Pearce, 1997; Daume et al., 1999; Heyward & Negri, 1999; Roberts, 2001). That coralline algae and spatangoids occur in clearly different habitats suggests these macroalgae could develop some non-specific metamorphosis inducing factors. Coralline algae indeed contain GABA-mimetic compounds (Morse, 1985), and GABA (i.e., γ-aminobutyric acid, a neurotransmitter) is known to trigger metamorphosis in various marine invertebrates (Morse, 1985; Pearce & Scheibling, 1990a; Rahmani & Ueharai, 2001; Roberts, 2001).

Results of experiments using sorted natural sediment indicated that survival rates in both postlarvae (i.e., individuals of less than 3 d-old) and early juveniles (i.e., individuals of no more than 13 d-old) remained high whatever the grain size of the tested sediment, while in older juveniles (i.e., individuals of more than 13 d-old) it was higher in medium sand. As field observations show that although both juveniles and adults *E. cordatum* seem to be quite tolerant regarding grain size, yet they indicate some preference for medium to fine sands (Table 4). As for growth performance, our results showed that younger juveniles (i.e., less than 23 d-old) grew faster in fine to silty sands while older ones reached by the end of the experience similar sizes irrespective of the grain size of the tested sediments. These ca. 45 d-old individuals measured less than 2 mm long, a size seemingly smaller than those of English Channel and North Sea individuals for which similar age was assessed (Table 5). This could mean either that age assessment of small field individuals was under-estimated or that our rearing conditions became limited by the end of the experience (the tubes with finer sediment showed some clogging and less effective water circulation, which could have lead to less favourable rearing conditions).

Nevertheless, early juveniles showed an initial preference in growth for fine to silty sands. Recruitment of juveniles is also reported in the literature to occur mainly in areas where sand particles are finer and/or have some content in silt/clay (Table 4). These correspond to locations where bottom hydrodynamism is presumably lower: juveniles may be found either subtidally (off wave-exposed coasts; e.g., Ursin, 1960; Buchanan, 1966), or in shallower waters (in sheltered areas like bays and estuaries; e.g. off the French coast; Péquignat, 1970; Guillou, 1985; De Ridder et al., 1991). Early juveniles, due to their size (see Table 3), obviously cannot swallow particles larger than silt and clay ones (< 62 mm grain diameter; Buchanan, 1984) and as a consequence in the finer sediments they may have been able to ingest more food. Moreover, finer sediment is known to contain more organic matter and may therefore be nutritionally richer than coarser ones (Bordovskiy, 1965). The observation of the juveniles gut contents has shown that while the gut of small 1 to 2 w-old individuals only contains bacteria and fine particulate matter, the gut of 6 w-old individuals clearly has sand grains (De Amaral P. Nunes, pers. obs.). These possible more favourable conditions found in finer sediment could in part explain the initial higher growth rates observed.

The present study suggests that in *E. cordatum* pre-settlement hydrodynamic conditions may have a predominant role in determining the distribution of settling larvae and the amount of larval supply to a given area, as larvae showed not to be very specific in terms of soft substratum choice. Nevertheless, although larvae of this species are common and frequently reported in the plankton in the English Channel and the North Sea (Rees, 1954; Ursin, 1960; Hecq, 1975; Kirby & Lindley, 2005), recruitment in those areas is known to be sporadic (Ursin, 1960; Buchanan, 1966 & 1967; Ziegelmeier, 1978; Ebert, 1983; Beukema, 1985) and to occur at infrequent intervals (Beukema, 1985). It is therefore likely that post-settlement factors (related with differential growth, mortality, predation and/or strong hydrodynamism; Ólafsson et al., 1994; Hunt & Scheibling, 1997; Fraschetti et al., 2003) are mainly responsible for the recruitment success and the distribution and abundance of *E. cordatum* adult populations. Among these factors, sediment grain-size is presumably an important one, especially during the first weeks of juveniles' life. It may not only condition the physical environment and the survival of the individuals, but also the efficiency of food ingestion and subsequent growth, as these preliminary results seem to point out. Additionally, it is reported in literature that the area occupied by adults of *E. cordatum* is broader compared to the locations where young recruits are found (Table 4), suggesting that other post-settlement processes such migration events may also determine *E. cordatum* individuals distribution. The occurrence of such post-settlement migrations was suggested by various authors who reported that *E. cordatum* would settle in subtidal areas of weak hydrodynamism and migrate afterwards (as early as in 4m-old individuals; Moore, 1936) into more littoral sites (Moore, 1936; Nichols, 1962; Buchanan, 1966; Massé, 1971; Beukema, 1985; Guillou, 1985; De Ridder et al., 1991).

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