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External fertilization and excapsular development in *Crepidula fornicata*: evaluating the risk of invasion control by dredging, crushing, and on-site rejection

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Abstract – Strategies for biomass control are actively sought in response to proliferation of the introduced, suspension-feeding gastropod *Crepidula fornicata* along European coasts. Among these, dredging, crushing and on-site rejection may only be envisaged if the risk of further dissemination through the release of gametes and of excapsulated embryos/larvae is found to be nil or extremely low. This study evaluates such risk using three approaches: (1) determination of the periods of brood presence/absence, (2) external fertilizations of gametes obtained by gonad stripping, (3) cultures of mechanically-excapsulated embryos and larvae. A period of near- or total brood absence was observed between September 2006 and January 2007. The few zygotes (1.5% to 7.5%) obtained from external fertilizations did not proceed beyond the 4-cell stage. Excapsulated young embryos and mid-stages died in less than 4 days of culture. Despite their ability to swim, grow and feed, excapsulated veligers all died before metamorphosis. No juveniles were obtained from external fertilizations and excapsulated cultures, indicating that the release of crushed catches directly at sea could not present a dissemination risk from the standpoint of gamete or prematurely-excapsulated larval dispersal. Furthermore, by carrying out the crushing operations during the period of brood absence, the dissemination risk becomes nil. Negative ecological impact of such operations could be offset by attraction of motile predators, as yet unaccustomed to this introduced species, and their subsequent generalization to live slipper limpets and invasive feral *Crassostrea gigas*. At the very least, this biomass reduction approach would concentrate bottom habitat unavailability to restricted zones, freeing up the rest of the subtidal for other, formerly displaced species, as well as reducing the pressure on the trophic carrying capacity of affected habitats.

Key words: Invasive species / Biomass reduction / Reproduction / Brooding / Development / Egg capsule / Slipper limpet / *Crepidula fornicata*

Résumé – Fécondation externe et développement extra-capsulaire chez *Crepidula fornicata* : évaluation du risque de dissémination après dragage, broyage, et relargage sur site. La prolifération des crépidules, *Crepidula fornicata* gastéropode suspensivore, le long du littoral européen, a stimulé de nombreuses stratégies de contrôle de sa biomasse. Parmi elles, le dragage, broyage et relargage sur site des crépidules sont envisageables à condition que le risque de dissémination additionnel, lié au rejet de gamètes et/ou d'embryons/larves excapsulés, soit nul ou extrêmement faible. Trois études sont effectuées pour évaluer ce risque : (1) la détermination des périodes de présence/absence de pontes, (2) les fécondations externes de gamètes obtenus par scarification des gonades, (3) les cultures d'embryons et de larves mécaniquement excapsulés. Une période d'absence totale de ponte est observée entre novembre 2006 et janvier 2007. Les quelques zygotes obtenus (de 1,5 % à 7,5 %) à partir des fécondations externes ne survivent pas au-delà du stade 4 cellules. Les jeunes embryons et les stades intermédiaires excapsulés sont morts en moins de quatre jours de culture. Malgré leur capacité à nager, croître et se nourrir, toutes les larves véligères excapsulées sont mortes avant la métamorphose. Aucun juvénile n'a donc été obtenu à partir des fécondations externes et des cultures de propagules excapsulées, indiquant que le relargage de crépidules broyées en mer présenterait peu de risques de dissémination par les gamètes et par les larves prématurément excapsulées à partir du site de rejet. De plus, en effectuant les opérations de broyage hors des périodes de ponte, le risque de dissémination serait extrêmement faible. Les impacts écologiques négatifs générés par de telles opérations pourraient être compensés par l'attraction de prédateurs, encore non accoutumés à cette espèce introduite, sur les sites de rejet, pouvant stimuler leur prédation par l'abondance de crépidules vivantes et d'huîtres sauvages *Crassostrea gigas*. De plus, le rejet de broyats pourrait être concentré sur des zones délimitées, permettant ainsi de libérer des habitats, du reste de la zone subtidale, pour d'autres espèces autrefois déplacées et de réduire la pression trophique exercée par la crépidule sur les habitats infestés.

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1 Introduction

The introduced slipper limpet *Crepidula fornicata* (Linnaeus 1758) has proliferated along European coasts over the past fifty years (Blanchard 1997), considerably impacting the shallow, soft and mixed-bottom habitats in which it is found (Le Pape et al. 2004; Arbach Leloup et al. 2008). The abundance of *C. fornicata* creates additional competition for resources and space with other suspension-feeders (Blanchard 1997; Beninger et al. 2007; Decottignies et al. 2007a, 2007b), and modification of the nature and the structure of the habitat (through biodeposition production and shell accumulation) to the advantage of this species (Ehrhold et al. 1998). It is now considered an invasive species *sensu stricto* (Davis and Thompson 2000).

Several factors have been evoked to explain the successful invasion of *Crepidula fornicata*: wide ecological tolerance, the absence of habituated predators, and in particular its reproductive strategy (Blanchard 1995, 1997). The aggregation of individuals in chains, combined with protandric sexuality, internal fertilization and gamete storage promote reproductive success (Coe 1936; Dupont et al. 2006). In addition, the protection of early developmental stages through encapsulation and incubation minimizes the loss and the predation of propagules in the plankton (Pechenik 1979).

The economic and ecological impacts of this invasive species have spurred attempts at localizing, estimating and controlling its biomass (Blanchard 1995, 1997; Sauriau et al. 1998, 2006; Thieltges et al. 2004). Mechanical removal has been proposed and implemented (Blanchard and Thomas 1998; Soulas et al. 2000; Bonnet 2006). However, on-shore transport, storage, and disposal of dredged slipper limpets is cost-prohibitive and very problematic in terms of pollution and public hygiene (Soulas et al. 2000; CREOCEAN 2006). Despite numerous attempts, including as a food species, no economically successful use for this product has been found (Soulas 1996; Fitzgerald 2007).

An alternative solution to landing dredged slipper limpets is the release of shipboard-crushed catches directly at sea. Obviously, such a procedure can only be envisaged if the crushing operation efficiently kills the dredged adult slipper limpets, which is the starting assumption for the present study. The legal framework for such a procedure is the 1972 London Convention and its 1996 Protocol, and the specific modalities adopted by each signatory country. Beyond this legal context, however, is the eminently biological question of the possibility of further dissemination of slipper shells through the release of intact capsules, gametes or excapsulated embryos/larvae which survive the treatment. Propagules within intact capsules removed from incubation do not survive when the capsules are exposed to natural seawater (Hoagland 1986). However, since capsules are incubated on a hard substrate beneath the females, surrounded by the concave shell, crushing involves appressing the shell to the substrate, potentially excapsulating the propagules contained therein.

The present study thus evaluates the risk of dissemination by following (1) the periods of brood presence, in order to avoid crushing operations during these periods, (2) the development of eggs fertilized externally, (3) the development of excapsulated embryos and larvae.

2 Materials and methods

2.1 *Crepidula fornicata* sampling

Stacks of 5 adult *Crepidula fornicata* were collected bimonthly from the intertidal at Noirmoutier Island, Bourgneuf Bay (French Atlantic coast, 46–47°N, 1–2°W) from March 2006 to November 2007. For fertilization and culture experiments, slipper limpets sampled from April 2007 to August 2007 were used. They were transferred to the laboratory, placed in flowing sea-water (15 °C–35‰) and fed with *Dunaliella salina* and *Isochrysis galbana* for 3 days prior to the experiments.

2.2 Periods of brood presence

For each female sampled, ($n \geqslant 30$ per sample), the presence or absence of incubated egg capsules was noted. This method allows the determination of brooding periods and the percentage of brooding females. For each brooding female, the egg capsule colour was assessed to evaluate the frequency of each brood type following the Chipperfield scale (1951), modified to standardize the colours using the Pantone™ 2001 universal color guide (Beninger et al. 1993): young broods containing developing embryos are yellow (100c) to orange (136c) while older broods containing veligers are light brown (4675c) to dark brown (411c).

2.3 External fertilizations

Gametes were obtained by stripping the gonad of 5 males and 10 females. Mature eggs (diameter approx. 170 μm, Collin 2003), were separated from immature eggs using a 150 μm sieve. Males were chosen on the basis of spermatozoan mobility under light microscopy. Mature, immature oocytes and spermatozoa were then rinsed with 0.45 μm filtered seawater. Experimental fertilizations were performed using mature eggs, with immature egg fertilizations as controls. Mature or immature oocytes were suspended in beakers containing 100 ml seawater at 20 °C, and oocyte concentration was determined. Spermatozoan suspensions were adjusted to obtain different spermatozoa-to-egg ratios from 1 : 1 to 50 : 1 (5 treatments). Each treatment had five replicates. First cleavage division in *Crepidula* appears in egg capsules 4 to 24 h after fertilization and is followed by the second cleavage after 18 h (Conklin 1897). Consequently, beaker contents were observed with a binocular microscope every two hours during the first day of experiment and then regularly during the following 72 h under light microscope. For each beaker, fertilization efficiency was assessed as the percentage of fertilized eggs, distinguishable from non-fertilized eggs at the first cleavage division.

2.4 Determination of intracapsular developmental stages

In order to assess the viability and extracapsular development of homogeneous stages, embryos and larvae were excapsulated from 50 broods and observed using both inverted light

microscopy and scanning electron microscopy (SEM). Samples for SEM were relaxed in 7.5% $MgCl_2$, fixed directly in slightly hyperosmotic 2.5% glutaraldehyde in 0.2M sodium cacodylate buffer (pH 7.2), dehydrated in a graded series of ethanol, and dried using hexamethyldisalizine (HMDS, protocol for mollusks, Cannuel and Beninger 2006). The specimens were mounted on a stub, sputter-coated with gold and palladium, and viewed with a JEOL JSM 6400F SEM.

2.5 Culture of excapsulated embryos and larvae

Culture method

Culture of excapsulated embryos and larvae were performed in favourable conditions for the development of *Crepidula fornicata* (Pechenik 1984; Pechenik and Lima 1984). Capsules were carefully opened, and propagules of homogeneous stage were transferred to polypropylene tanks containing 10 L of 0.45 μm filtered seawater at initial concentrations of 6.5 to 21 propagules ml^{-1}. Gentle bubbling was used to maintain the propagules in suspension. Salinity was 35‰, and water temperature was maintained at 18 °C, the mean temperature found in Bourgneuf Bay from April to August, the months of sampling and experimental culture (IFREMER REPHY data from 1995 to 2003, Noirmoutier: "Bois de la Chaise" sampling station) and within the temperature range allowing the survival, growth and spontaneous metamorphosis of naturally hatched veligers in the laboratory (Pechenik 1984; Pechenik and Lima 1984). Although several types of inducer accelerate metamorphosis in *Crepidula fornicata*, normal metamorphosis also occurs without such inducers (Pechenik 1980, 1984). No inducers were used in the present study. The rearing tanks were emptied, washed and refilled every 2–3 days, at which times propagules were provided with *Isochrysis galbana* in log phase growth at approximately 1×10^5 cell ml^{-1} or at comparable cell volume with a mixed diet of three species of *Isochrysis galbana*, *Dunaliella salina*, *Thalassiosira pseudonana* (1 : 1 : 1). The algal densities used were above the critical cell density for larvae of *Crepidula fornicata* (Pechenik 1980). For each intracapsular developmental stage, 4 (veliger stage) or 5 cultures (young stage and mid-stage) were performed. Excapsulated veliger cultures were divided into two duplicates: (1) veligers fed with a unialgal diet (U1 and U2), and (2) veligers fed with a trialgal diet (T1 and T2).

Algal consumption

Algal concentrations in the tanks were calculated just after feeding the embryos and larvae at T0 and then 2, 4, 6 and 8 hours after feeding. For each container, the water was mixed before samplings were performed. Algal concentration, based on three samples, was then determined using a glass hemacytometer (Neubauer). Cell consumptions were calculated from the decline of phytoplankton concentration over time and expressed as cells eaten propagule $^{-1}$ $hour^{-1}$.

Mortality and growth

After each water change, 1 or 2 ml sample was pipetted at random in all the tanks to evaluate mortality of the propagules and to determine growth and development of the excapsulated veligers. Thirty individual measurements of the longest shell axis were carried out using a compound microscope and LUCIA GF 4. 80 image analysis software, and growth rates were calculated as μm shell growth day^{-1}. The presence of competent veligers (*sensu* Pechenik 1980, 1984; Pechenik and Heyman 1987: "brimmed veligers" 700–800 μm shell length) was monitored.

2.6 Statistical analysis

All statistical tests were run using SigmaStat 2.0 (Jandel Scientific). Normality (Kolmogorov-Smirnov normality tests) and heteroscedasticity were verified for each test. One-way ANOVA was performed when the fertilization rate, the algal consumption or the veliger growth datas were normal and variances homogeneous, or Kruskall-Wallis test was performed when data were non-normally distributed and characterized by heterogeneous variances. These tests were followed by Student-Newman-Keuls multiple range tests to detect significant differences in means ($p < 0.05$).

3 Results

3.1 Periods of brood presence

Brood capsules were found over most of the sampling period, from the first sample in March 2006 to September 2006, and from February 2007 to October 2007 (Fig. 1). Maximum presence of capsules was observed from April to July 2006 and from March to August 2007, with a mean brood frequency of 69% (95% CI ± 13) and 68% (95% CI ± 11) of total females, respectively. The presence of older and young broods was concomitent and continuous during the brooding season, indicating that there was no brood synchronisation among *Crepidula fornicata* females. Broods were rare or absent in September and October for both years (brood frequency: 0% to 10.9%), and were not observed in November, December and January.

3.2 Intracapsular development baseline

Development was synchronous for all the propagules of the same brood. Based on SEM observations, three major stages of intracapsular development were subsequently easily distinguishable under light microscopy (Fig. 2): (1) early embryos from the 2-cell stage to the gastrula stage (Figs. 2A, B, C); (2) mid-stages, from trochophores to the veliger transition (Figs. 2 D, E, F); and (3) intracapsular veliger-stage larvae (Figs. 2, G, H, I).

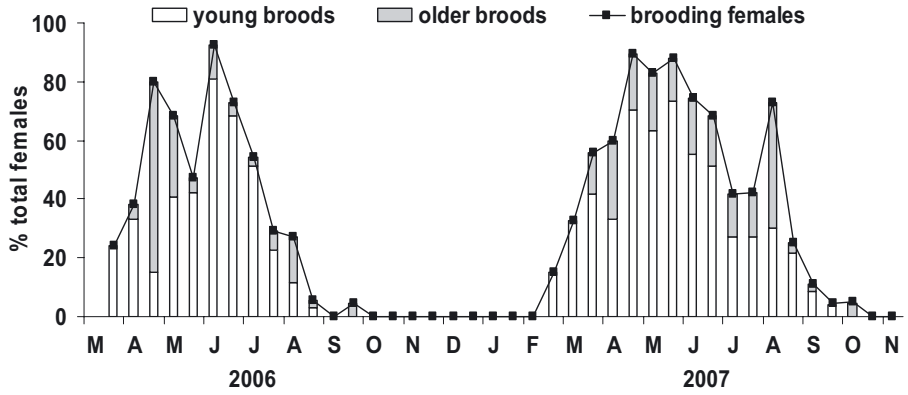

Fig. 1. Evolution of the percentage of brooding females (curve) within the female population of *Crepidula fornicata*, and frequency of young and older broods (cumulative histograms), between mid-March 2006 and November 2007.

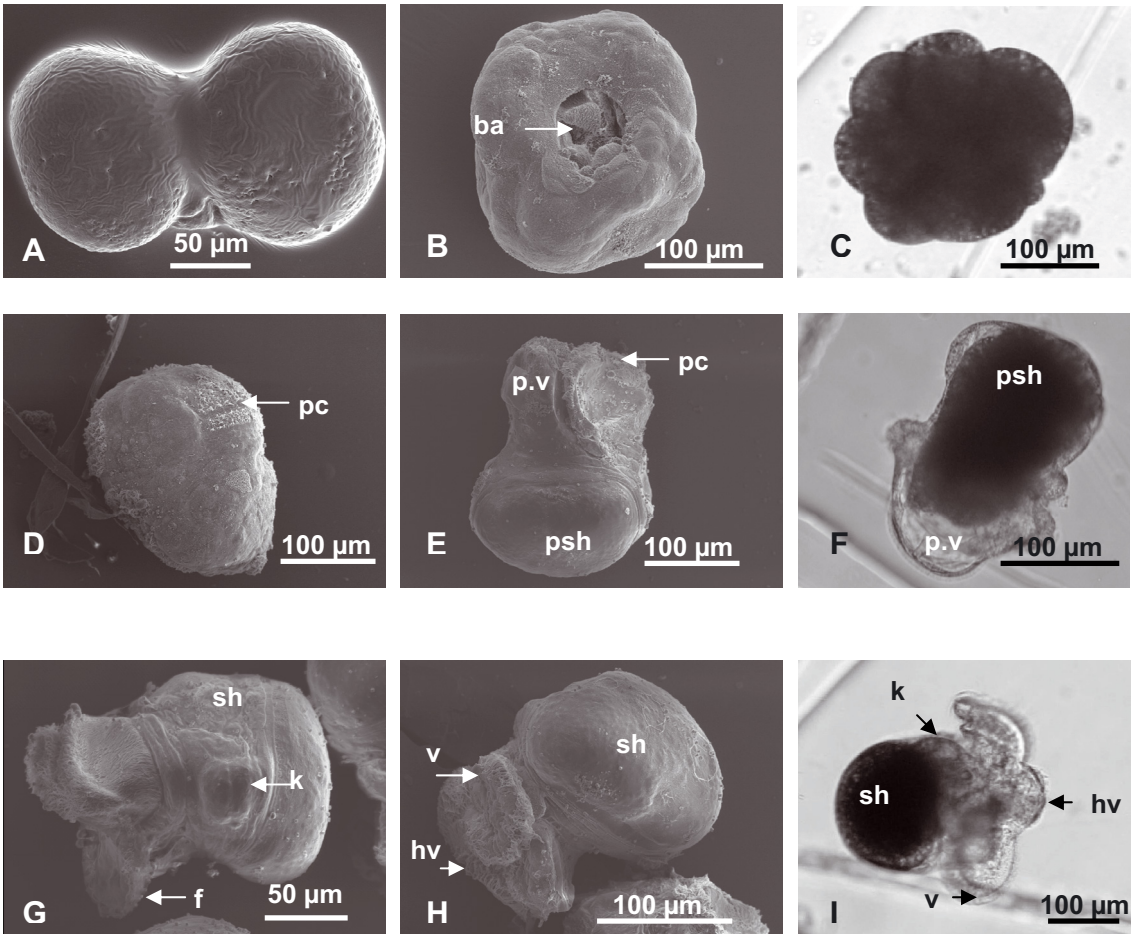

Fig. 2. Major stages of the intracapsular development of *Crepidula fornicata*. A, B, C: early embryos. SEM of (A) a 2-cell embryo, (B) a gastrula, ba: blastopore, (C) light micrograph of a multicell embryo. D, E, F: mid–stages. SEM of (D) a ciliated trochophore, (E) a trochophore in veliger-stage transition showing the beginning of the lateral extension of the velum, *pc*: patches of cilia, *psh*: presumptive shell, *pv*: presumptive velum, (F) light micrograph of a trochophore in veliger-stage transition. G, H, I: intracapsular veliger-stage larva. SEM of (G), (H) an intracapsular veliger, (I) light micrograph of an intracapsular veliger, *f*: foot, *hv*: head vesicle, *k*: embryonic kidney, *sh*: shell, *v*: velum.

Table 1. *Crepidula fornicata* external fertilizations: fertilization efficiency, embryo survival and early development.

Spermatozoa: egg ratio	Fertilization efficiency % (± SD) $n = 5$	Mean % survival to developmental stage		
		2 cells	4 cells	8 cells
1 : 1	0	0	0	0
5 : 1	1.4 (± 2)	46	0	0
10 : 1	7.5 (± 6)	20	0	0
25 : 1	1.7 (± 1)	19	5	0
50 : 1	3.6 (± 2)	100	7	0

3.3 External fertilizations

No fertilizations were obtained for the control treatments (immature oocytes) and for the experimental treatment (mature oocytes) with a spermatozoa-to-oocyte ratio of 1 : 1 (Table 1). Low fertilization success was observed when the spermatozoa-to-oocyte ratios ranged between 5 : 1 to 50 : 1 (Table 1), with a mean percentage of fertilization efficiency between 1.4 to 7.5. No significant differences were found between the fertilization efficiencies obtained for the different spermatozoa-to-oocyte ratios ($p = 0.053$), but a tendancy was observed, revealing higher values of fertilization for the spermatozoa-to-oocyte ratio of 10 : 1 compared to the other treatments.

Subsequent to the few successful fertilizations, small proportions of the fertilized eggs survived during the embryonic development (Table 1): 19% to 100% of the zygotes reached 2-cell stage, and only 5% to 7% reached 4-cell stage. Embryonic development did not proceed beyond these stages.

3.4 Cultures of excapsulated embryos and larvae

Culture of excapsulated early embryos and mid-stages

No feeding was observed in these cultures (no significant decline in algal concentrations over time, $0.056 \leqslant p \leqslant 0.778$, for the different cultures). From day 1 to day 4, mortality in all cultures reached 100%. Early embryos disintegrated in less than 24 h, and mid-stages which were actively moving at the beginning of the cultures, were observed expelling yolk and disintegrating by day 1 or 2. Propagule densities decreased rapidly, the totality of the cultures were lost on day 4. No veligers were obtained.

Culture of excapsulated veligers

Algal consumption

Following addition to the culture flasks, algal concentrations decreased with time in all treatments ($p = 0.04$, $p = 0.001$, $p = 0.016$ and $p = 0.029$ respectively for U1, U2, T1 and T2), indicating consumption by the veligers. Mean algal consumptions from day 1 to day 3 ranged from 0.01 to 0.13 cells veliger^{-1} hour^{-1} for the unialgal diet cultures and from 0.1 to 0.21 cells veliger^{-1} hour^{-1} for the trialgal diet cultures. These low consumptions, which never exceeded 0.3 cells veliger^{-1} hour^{-1}, ceased completely after day 4.

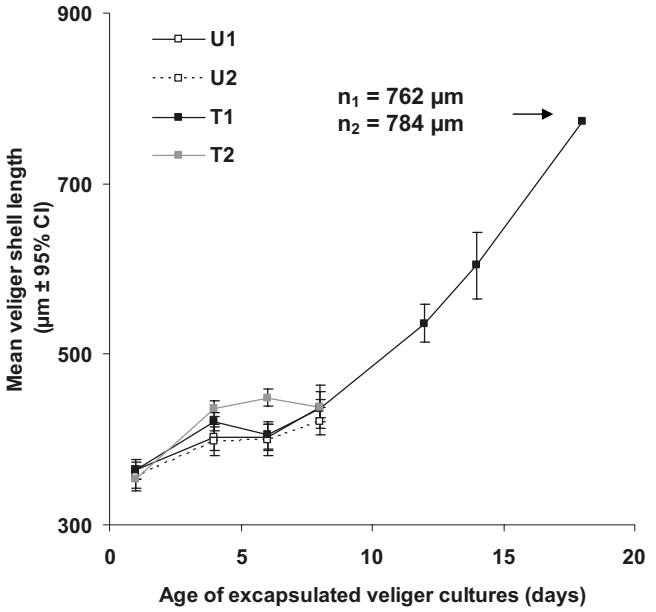

Fig. 3. Mean shell growth of excapsulated veligers fed with a unialgal (U1, U2 – *Isochrysis galbana*) or trialgal (T1, T2 – *Isochrysis galbana*, *Dunaliella salina*, *Thalassiosira pseudonana*) diet. Vertical bars are ± 95% confidence intervals (CI), not present when measurements were inferior to 5.

Veliger concentration and growth

All veligers swam consistently in the batches after excapsulation. Mean shell lengths increased significantly over the treatment durations ($p = 0.001$ for the four cultures, Fig. 3) accompanied by morphological changes such as velum growth, losts of the embryonic kidneys and the head vesicle, the progressive transparency of the shell and the appearance of the tentacles. Veligers of U1, U2, T1 and T2 grew uniformly during the first eight days of culture, with a mean shell size (± CI) comprised between 354 and 365 μm (±13 and 13.5) at day 0 and between 421 and 438 μm (±25 and 17.5) at day 8 (Fig. 3), corresponding to a mean growth rate of 8.2 to 10.5 μm day^{-1} (Table 2). Nevertheless, larval mortality was high in all cultures (Table 2). Indeed, most of the excapsulated veligers became progressively less active, and visibly lost a considerable amount of tissue before a mass mortality at day 8. For U1, U2 and T2, larval mortality was 70%, 47% and 48%, respectively, on day 4 and reached 100% after day 8 (Table 2). No competent veligers were obtained. For T1, from day 1 to day 7, larval mortality was below 50% (Table 2), and between day 7 and day 9 the larval concentration decreased 40 fold (8 ml^{-1} to 0.2 ml^{-1}). The remaining veligers were placed in a 1L beaker, where the larval concentration continued to decrease with respectively 0.01 ml^{-1} and 0.005 ml^{-1} at days 11 and 15. At day 18, two brimmed veligers, of shell sizes of 762 μm and 784 μm, were observed in this final culture but they rapidly died before reaching metamorphosis. The totality of the excapsulated veligers thus disappeared by day 20 (Table 2), and no viable juveniles were obtained.

Table 2. Mortality and growth of excapsulated veliger larvae fed with a unialgal (U1, U2) or trialgal (T1, T2) diet; d = day.

Cultures	Mean cumulative mortality (%)						Growth rate from d_0 to d_8 (μm day^{-1})
	d_0	d_4	d_8	d_{12}	d_{16}	d_{20}	
U1	0	69.7	98.6	100	100	100	8.2
U2	0	46.7	99.8	100	100	100	9.2
T1	0	17.6	97.0	99.9	99.9	100	9.0
T2	0	47.6	87.6	100	100	100	10.5

4 Discussion

The very low rates of external fertilization obtained (means < 8%) using mature gametes, indicate that an extra-uterine fertilization is very difficult to achieve in *C. fornicata*. Several factors provided during normal internal fertilization may be necessary, e.g. the mucus secretions which normally accompany the mature eggs in the uterus lumen, and the protected environment in which gametes develop and are transferred to the uterus, either directly from the ovaries (female) or from the male tract and then the seminal receptacles where spermatozoa are stored (Coe 1936; Martin 1985).

4.1 Viability of non-capsulated / excapsulated embryos and mid-stages

The aborted development of the few embryos obtained from the external fertilizations suggests that the "embedding" of the fertilized eggs in the uterus is essential to the survival of the subsequent embryos. The rapid disintegration of the excapsulated embryos and mid-stages is congruent with studies on *Crepidula fecunda*, which showed that excapsulated embryos were unable to capture and ingest suspended food particles, due to lack of a velum at this stage (Chaparro et al. 2002a, 2002b). Similarly, Pechenik et al. (1984) showed low survival of pre-shelled embryos of the marine prosobranch *Nucella lapillus* after excapsulation.

Encapsulation protects the embryos of marine invertebrates against microorganisms, predation, osmotic stress and desiccation (Pechenik 1979, 1982). In addition, the intracapsular fluid provides the early embryos and mid-stages of Calyptraeidae a concentrated "package" of major ions necessary for their initial development (Maeda-Martinez 2008), and it has been suggested that the organic matter contained in this fluid might provide extra-nutrients to the embryos (Ojeda and Chaparro 2004).

Given the above, it is clear that non-capsulated and excapsulated embryos and mid-stages do not present a dissemination risk after a crushing, dumping at sea, since no viable juveniles were obtained under the very favourable rearing conditions of the present study.

4.2 Viability of excapsulated veligers

Excapsulated veligers were capable of swimming and feeding, suggesting that the abilities of swimming and of removing particles from the water are acquired during the intracapsular phase, with the development of the velum, as previously observed in *Crepidula fecunda* (Chaparro et al. 2002a; 2002b). However, our observations demonstrate that despite this anatomical disposition, feeding functionality is extremely limited, since the highest algal consumption calculated was 0.21 cells veliger^{-1} hour^{-1}, compared to mean values of 3000 to 15 000 cells veliger^{-1} hour^{-1} for cultures of naturally-hatched veligers of *Crepidula fornicata* raised in the same conditions of temperature and of algal concentration and of the same range of size (400–800 μm) (Pechenik 1980; Blanchard et al. 2008). A similar observation has been made for *C. fecunda* (Chaparro et al. 2002a).

The extreme (total) mortality of excapsulated *Crepidula fornicata* veligers, observed in the present study, may be contrasted with that of naturally-hatched veligers reared under similar conditions, with only 5% mortality at metamorphosis, and even these being due to the desiccation of metamorphosed individuals crawling above the water levels of the batches (Pechenik 1980, 1984). Moreover, the growth rates for the four excapsulated veliger cultures (values comprised between 8.2 and 10.5 μm d^{-1}) were only 1/2 to 1/4 of the average growth rate observed in naturally-hatched larvae raised in similar conditions of temperature (18 °C–20 °C) and algal concentration (1 – 2 × 10^5 cells ml^{-1}) (24.5 μm d^{-1}– Pechenik 1980; 45.3 μm d^{-1}– Pechenik 1984; 40.1 μm d^{-1}– Pechenik and Lima 1984). In view of these results, prematurely-excapsulated veligers could not present a dissemination risk. Recent work on *Crepidula fecunda* suggested that intracapsular veligers were dependent on organic components, mostly proteins, in the intracapsular fluid (Ojeda and Chaparro 2004), and this might be the cause of the massive mortalities of prematurely-excapsulated *C. fornicata* veligers.

4.3 Biological feasability of dredging, crushing, and on-site rejection of *Crepidula fornicata*

It is difficult, if not impossible, to guarantee that a given event will never occur. However, the results of the present study show that the dumping at sea of crushed stacks of *Crepidula fornicata* does not present a dissemination risk from the standpoint of gamete or prematurely-excapsulated larval dispersal. Since it was necessary to ensure that the larvae studied were indeed intracapsular, it was not possible to include larvae immediately prior to hatching, hence the risk from the penultimate larval stages is not known. As brood deposition is not synchronous within a *C. fornicata* population (Richard et al. 2006, present study), it is impossible to avoid the penultimate larval stage in a dredging strategy. However, it may be surmised that the mortalities of such larvae would be high following crushing and ship-board storage of the crushed animals prior to dumping, in addition to natural mortality of *Crepidula*

fornicata larvae in the field after dumping (about 50% per day – Pechenik and Levine 2007). Furthermore, the crushing operations could be performed during the periods of absence of broods, which occur from early to mid-winter throughout the worldwide distribution of *C. fornicata* (Richard et al. 2006). In the Bay of Bourgneuf, this period extends from November to January (present study). The dissemination risk becomes nil.

Although the shipboard release of crushed *C. fornicata* might engender ecological problems such as bottom anoxia, these might be offset by the attraction of motile predators not accustomed to *C. fornicata* as a food source (e.g. crabs, rays, starfish, which have not yet produced effective predation strategies on this introduced species). This might then stimulate predation, both on slipper limpets and on the abundant stocks of feral oysters (*Crassostrea gigas*) which have also begun to pose serious proliferation problems and which may be presumed to compete with farmed oysters. Although oyster farmers could have concerns about the subsequent increase in predator numbers, it must be remembered that inaction will not alleviate their anxieties either, and that the increased number of predators (whose juveniles might enter the oyster-bags) might be offset by the advantage of increased predation on farmed oyster competitors.

The crushed biomass could also be dumped on existing concentrations of live slipper limpets, such that any ensuing bottom anoxia would not alter bottom habitat availability for other species, and even help to reduce live slipper shell biomass. Finally, the goal is to reduce the impact of slipper limpets on carrying capacity of both trophic resources and habitat; thus, concentration of bottom habitat unavailability to limited areas (dumping zones) will free up the rest of the subtidal for other species such as flatfish, displaced by the slipper limpet populations.

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