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Field reproductive dynamics of the invasive slipper limpet, *Crepidula fornicata*

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ABSTRACT

At least part of the invasive success of the slipper limpet, *Crepidula fornicata*, in European waters must be due to reproductive characteristics, yet the events underlying the easily-observed brooding and non-brooding periods have not yet been studied in this species. The reproductive system dynamics were therefore investigated using topological histology and quantitative histological techniques. Specimens were sampled twice monthly for 18 months from Bourgneuf Bay, France, a mid-latitudinal point in the European distribution of *C. fornicata*. Both the testicles and ovaries showed active and resting phases, corresponding to the brooding and non-brooding periods, respectively. Maximum spermatozoan production corresponds to the female brooding period (female incubation of oviposited eggs, mid-March to late August), and allows males to possess full spermatozoan stocks at the height of fresh mature oocyte availability. The year-round presence of mature oocytes in the female gonad is misleading, since the histological aspect reveals that they are vestigial oocytes which slowly degenerate during the brooding period, possibly providing metabolites for the developing oocytes that become increasingly abundant during this period. A complete scheme of the *C. fornicata* reproductive cycle is presented, showing the events in the major reproductive organs.

The seminal vesicle shows high inter-month variability in sperm presence, suggesting year-round copulation and sperm storage in the seminal receptacle. The seminal receptacle shows a uniform covering of spermatozoa throughout the year, suggesting rapid renewal after fertilization, again in line with multiple copulation throughout the year. Given the limited available space on the seminal epithelium, against which all spermatozoa abut, as well as polyandrous copulation, it is postulated that sperm competition may take place.

1. Introduction

Invasions of terrestrial, freshwater and marine habitats by non-indigenous species are occurring with increasing frequency throughout the world (Davis and Thompson, 2000; Goulletquer et al., 2002; Wonham et al., 2005). The marine gastropod *Crepidula fornicata*, native to the East coast of the United States, is an involuntarily-introduced species that has progressively invaded the European coastline over the past decade, from the Mediterranean to Southern Norway (Blanchard, 1997; Davis and Thompson, 2000), notably in the shallow bays and estuaries where oyster farming is located. This recent rapid extension may be related to rising water temperatures, as has been observed for another temperate invader in these waters, *Crassostrea gigas* (Thieltges et al., 2004; Dutertre et al., 2009).

Along the Brittany coastline of France, sixty years after its introduction, *C. fornicata* populations constitute a considerable, and increasing, biomass (>50,000 tons for Mont Saint-Michel, Saint-Brieuc, Brest and Bourgneuf Bays — Hamon and Blanchard, 1994; Blanchard and Ehrhold, 1999; Richard et al., 2006; Sauriau et al., 2006). *C. fornicata* can completely cover the sediment with up to several thousand individuals per square meter (Ehrhold et al., 1998; de Montaudouin and Sauriau, 1999; Thieltges et al., 2004). Such high densities heavily impact the colonized habitat, irreversibly modifying the nature and structure of the bottom (Ehrhold et al., 1998; Grall and Hall-Spencer, 2003), creating local competition for resources and space with suspension-feeders of commercial interest (oysters and scallops — Blanchard, 1997; Beninger et al., 2007; Decottignies et al., 2007a,b) and disturbing both oyster farming and commercial fisheries relying on dredging (Blanchard, 1997).

Notwithstanding a recent call for an effort to evaluate positive as well as negative ecological impacts of biological invasions, and notably for *C. fornicata* (Thieltges et al., 2006), the negative impacts of *C. fornicata* on commercial activities and on the marine environment have spurred attempts, as yet ineffective, to control its biomass (Blanchard, 1995, 1997; Sauriau et al., 1998, 2006; Thieltges et al., 2004; Valdizan et al., 2009).

It is reasonable to suppose that the invasive success of the slipper limpet is at least partially related to its biological characteristics. Although potential predators such as crabs and starfish exist locally, predation pressure is low (Coum, 1979), possibly due to lack of familiarity with *C. fornicata* as a prey species. In addition, *C. fornicata* shows high ecological tolerance (Coum, 1979). It is an opportunistic, generalist suspension-feeder (Barillé et al., 2006; Beninger et al., 2007; Decottignies et al., 2007a,b), and thus able to thrive under many different dietary conditions.

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Proliferation and extension are consequences of reproduction. The reproductive traits of *C. fornicata* are thus of particular interest. The gregarious habit of *C. fornicata*, in addition to protandry with direct internal fertilization and sperm storage in both sexes (Coe, 1936; Gaffney and McGee, 1992), are all characteristics which enhance reproductive success, compared with the broadcast spawning of most other suspension-feeding molluscs (Dupont et al., 2006). Although adult chains are sedentary, the pelagic larval stage enables natural dispersion and colonization of new habitats (Collin, 2003; Dupont et al., 2006).

The reproductive success of *C. fornicata* is further enhanced by multiple spawning over the extended reproductive season (deduced from periods of brood presence), and the protection of early embryos through encapsulation and incubation (Pechenik, 1979; Richard et al., 2006; Valdizan et al., 2009). However, understanding of *C. fornicata* reproductive biology is hampered by the lack of data on the underlying dynamics of reproduction. Neither the periods of gamete production, eventual resting phases, nor of gamete transfer to male and female storage organs, have been identified to date, such that it has not yet been possible to construct a realistic sequence of the pre-brooding events in *C. fornicata*. Indeed, although abundant histological and anatomical observations of the reproductive system have previously been performed in this species (Gould, 1917a,b, 1952; Coe, 1936), we are unaware of any data on the gametogenic cycle anywhere in the world. Such information is obviously of prime importance in order to understand the invasion process of this introduced species.

In the present study, we elucidate the field reproductive cycle of *C. fornicata* in 2006–2007, by examining the dynamics of tissue and gamete types in the various reproductive organs, as well as the occurrence of broods. We chose Bourgneuf Bay, France, as a study site, as it is a mid-latitudinal point in the European distribution of this species.

2. Materials and methods

2.1. Specimen sampling and histological processing

Bourgneuf Bay (French Atlantic coast, 46–47°N, 1–2°W) is a slipper limpet-invaded ecosystem, located south of the Loire estuary (Corlay and Robert, 1986; Barillé-Boyer et al., 1997). Chains of *Crepidula fornicata* (L.) were sampled twice monthly from the intertidal, from March 2006 to September 2007. Five individual chains were collected, with a minimum of 50 slipper limpets for each monthly sample.

For each specimen, the visceral sac containing the entire gonad was removed and fixed in aqueous Bouin's fixative (Martoja and Martoja-Pierson, 1967) for at least 1 month prior to dissection, thus avoiding gamete leakage when dissecting fresh slipper limpets. In order to control for eventual gametogenetic topological variability in the gonad, it was necessary to choose a readily-identifiable, topologically-invariable external anatomical marker for section locations (Morales-Alamo and Mann, 1989). The anterior branch of the gonad was therefore sectioned in dorso-ventral planes passing through the heart (Fig. 1A, B: 10 females and 5 males per sample). The sperm storage organs (i.e. the seminal receptacle for the females and the seminal vesicle for the males) were sectioned without respect to plane, as they were spherical and well-visible from the exterior (Fig. 1A: 5 females and 5 males per sample). Samples were then dehydrated, embedded in paraffin, and 7 µm sections were cut and stained with a modified Masson's trichrome protocol (Martoja and Martoja-Pierson, 1967; Gabe, 1968). Observations, microphotographs, and analyses of the histological slides were performed using an Olympus light microscope, and image capture and processing software (LUCIA GF 4. 80).

2.2. Reproductive dynamics analysis

Stereological methods were used to determine volume fractions of different tissue types in the histological sections (Weibel et al., 1966; Briarty, 1975; Morvan and Ansell, 1988; Pazos et al., 1996; Mayhew, 2000; Beninger et al., 2001). For each section of the ovary, stereological counts were performed on 3 randomly-chosen areas using a 10×10 point matrix on a microscope projector at 100×, as described in Beninger et al. (2001) for the archaeogastropod *Megathura crenulata*. Six tissue categories were counted: developing oocytes, mature oocytes, oocytes undergoing atresia, lysed oocytes, unoccupied tubule space, and inter-tubular space. Since male gonad tissue is scarce, irregular and small in the large visceral mass (Gould, 1917a), counts were performed using a 20×20 point matrix at 200× on 3 randomly-chosen areas containing visible tubules. Six categories of male gonad tissue were counted: developing gametes, immature spermatozoa forming packets with their flagellae projecting toward the center of the tubule (heads and flagellae visible), mature spermatozoa in the tubule lumen, residual spermatozoa, unoccupied lumen, and inter-tubular space. Three counts were performed for each of the 3 randomly-chosen areas, and the means were calculated (with 95% confidence intervals). For each sample, the means for all males were plotted, as were the means for all females.

2.3. Gamete status within the seminal receptacle and vesicle

The presence of histologically-intact spermatozoa in the ampullae of the sperm storage organs was monitored. Furthermore, for each male, the percentage occupation of 3 seminal vesicle ampullae was quantified using image analysis as described above; three counts were performed for each ampulla. For each ampulla the mean was calculated (with 95% confidence intervals), and the mean for all the ampullae of the same male were calculated (with 95% confidence intervals). Subsequently, for each sampling date, the grand seminal vesicle mean for all of the males were plotted (with 95% confidence intervals). This procedure was not possible in the seminal receptacle

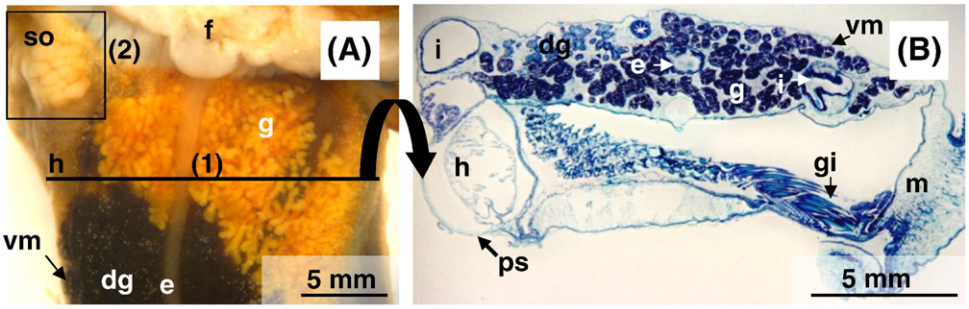

Fig. 1. Anatomy of the anterior visceral mass (vm) of *Crepidula fornicata* (ventral view). (A) Stereomicrograph showing the localisation of the sections performed (1) at the anterior branch of the gonad (g), (external marker = heart (h)), and (2) at the sperm storage organ (so, seminal vesicle in male, seminal receptacle in female). (B) Histological section of the anterior branch of the gonad, showing anatomical relationship to surrounding structures. dg: digestive gland, e: esophagus, f: foot, gi: gill, i: intestine, m: mantle, p: pericardial sac.

(=spermatheca) ampullae of females, since when present, spermatozoa abut and completely cover the ampulla epithelium, forming a single layer (Conklin, 1897; Silberzahn, 1978; Voltzow, 1994). In females, therefore, only the presence or absence of a spermatozoan layer in the seminal receptacle ampullae was recorded.

2.4. Brood presence

The incubation periods for *Crepidula fornicata*, identified by brood presence in 2006 and 2007 (Valdizan et al., 2009), were also used to assist in the interpretation of reproductive events.

2.5. Data analysis

Due to the exploratory nature of this work, no *a priori* hypotheses could be formulated. *A posteriori* tests (Kruskal–Wallis one-way non-parametric ANOVAs and Student–Newman–Keuls multiple range tests to detect significant differences in means over the course of the sampling period) were performed, but the results were uninterpretable, yielding many statistically-significant differences for which no corresponding chronological or physiological significance could be construed. We decided therefore to present the data graphically and construct our reasoning from the most obvious trends observed.

3. Results

3.1. Males

3.1.1. Histological aspect

The testicular tubules showed two phases: active and resting. During the active phase, all stages of spermatogenesis were visible, and gamete maturation proceeded from the basal syncytium toward the tubule lumen. Latent oogonia could be observed along the basal syncytium, anticipating the subsequent sex reversal (Fig. 2A, B).

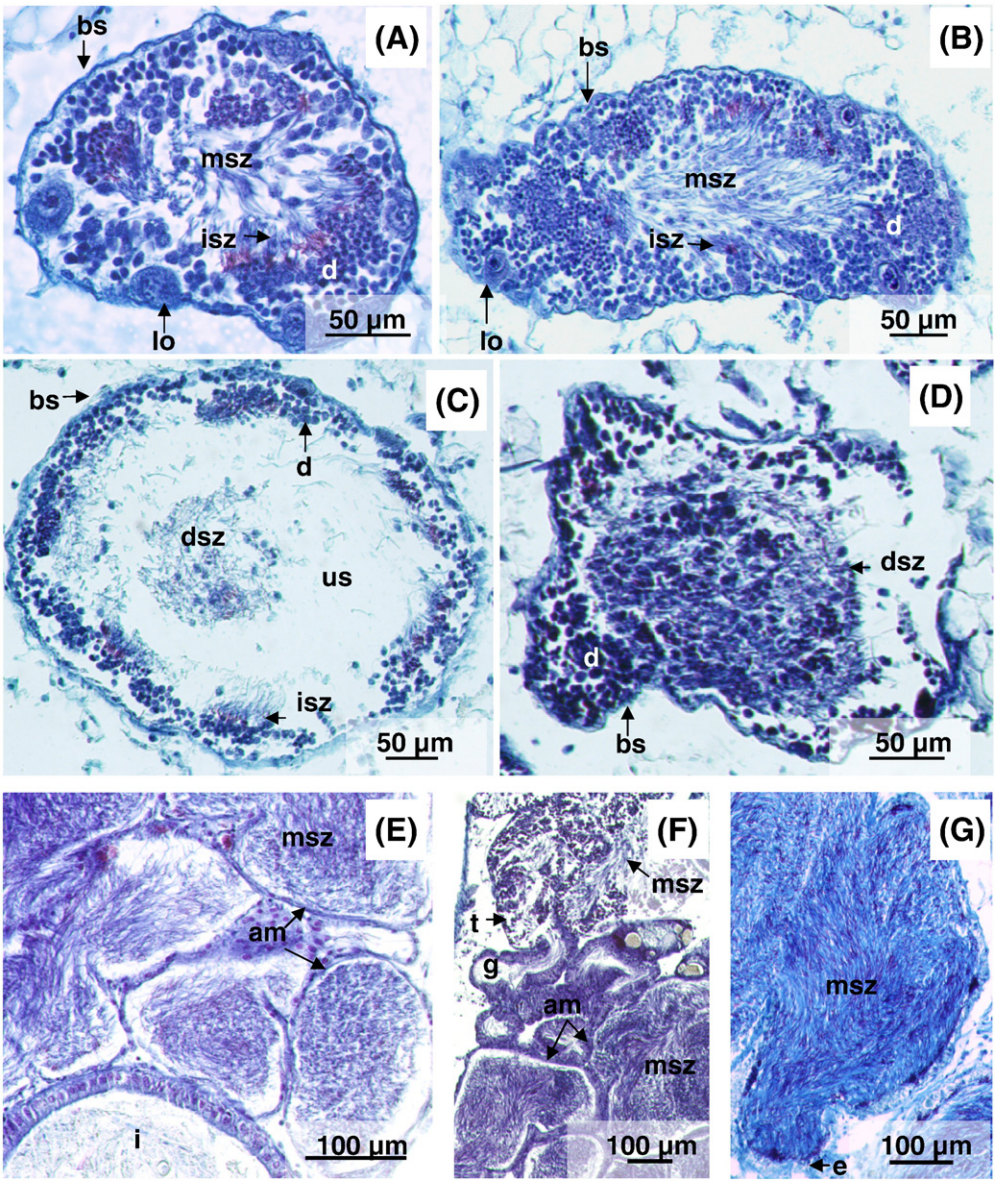

Fig. 2. Histological sections of the testis and of the seminal vesicle of *Crepidula fornicata*. (A), (B), (C), (D) Tubule of the testis. (A), (B) Tubule during the sexual activity period showing all stages of spermatogenesis from the basal syncytium (bs) to the center: developing gametes (d) forming a germinal layer, immature spermatozoa (isz) and free mature spermatozoa (msz) filling the lumen. Latent ovogonia (lo) are observed along the tubule walls. (C), (D) Tubule in degeneration, during the sexual rest period, showing: degenerating residual spermatozoa (dsz), unoccupied space (us). Developing gametes, still present, are scattered. (E), (F), (G): Seminal vesicle. (E) Ampullae (am) of the seminal vesicle, near the intestine (i), filled with mature spermatozoa. (F) Detail of ampullae connected to the testis (t) by the gonoduct (g). (G) Detail of an ampulla showing the epithelial wall (e) and free mature spermatozoa in the center.

During the resting phase, the tubule lumen is dominated by the presence of degenerating male gametes and unoccupied space, yet developing gametes are still present along the tubule walls (Fig. 2C, D).

The seminal vesicles were replete with mature spermatozoa in all histological sections (Fig. 2E, F, G). The spermatozoa were randomly oriented, in contrast to the situation in the seminal receptacle (see below).

3.1.2. Reproductive cycle

Male tubules were scarce in the large visceral mass, as evidenced by the high percentages (41%–69%) of inter-tubular tissue found throughout the sampling period; the lowest percentages corresponded to the periods of gametogenic activity, observed from April to early September 2006, and from March to late August 2007. During these periods, developing gametes, immature spermatozoa, and mature spermatozoa in the lumen occupied almost the entire gonad, with mean percentages (±95% CI) of 19.2% (±2.7), 10.5% (±1.7) and 13.9% (±1.6) respectively (Fig. 3A). During the active periods, the percentage of mature spermatozoa was maximal with respect to the annual cycle, and no major decrease in these percentages was observed (Fig. 3A). Unoccupied space (not shown) and degenerating spermatozoa (Fig. 4A) were rare, with mean percentages (±95% CI) of 1.9% (±0.4) and 2.1% (±1.1) respectively.

A steady increase in degenerating gametes was observed beginning in July in both 2006 and 2007, with a maximum in October 2006 (data unavailable for 2007) (Fig. 4A). The percentages decreased from October 2006 to April 2007, and the tail-end of such a decrease was also observed in March and April 2006 (Fig. 4A). Free mature spermatozoa occupied a low proportion of the tubule lumen during this gametogenically inactive phase (Fig. 4A). Although developing gametes were present in relatively high proportions along the tubule walls, they did not proceed to increase the proportions of mature gametes in succeeding months, indicating arrested development during this phase (Fig. 3A).

3.1.3. Activity of the seminal vesicle

Decreases in the mean volume fraction of spermatozoa stored in the seminal vesicle ampullae indicate occurrences of copulation. Such decreases occurred frequently, with no discernable pattern over the course of the sampling period (Fig. 5). The longest period of decrease was from mid-October to mid-December 2006, corresponding to the period of maximum spermatozoan atresia in the testicle (Fig. 5).

3.2. Females

3.2.1. Histological aspect

During brooding periods, tubules were dominated by the presence of mature oocytes undergoing atresia, characterized by irregular shapes, deformed appearances and sometimes cell membrane ruptures

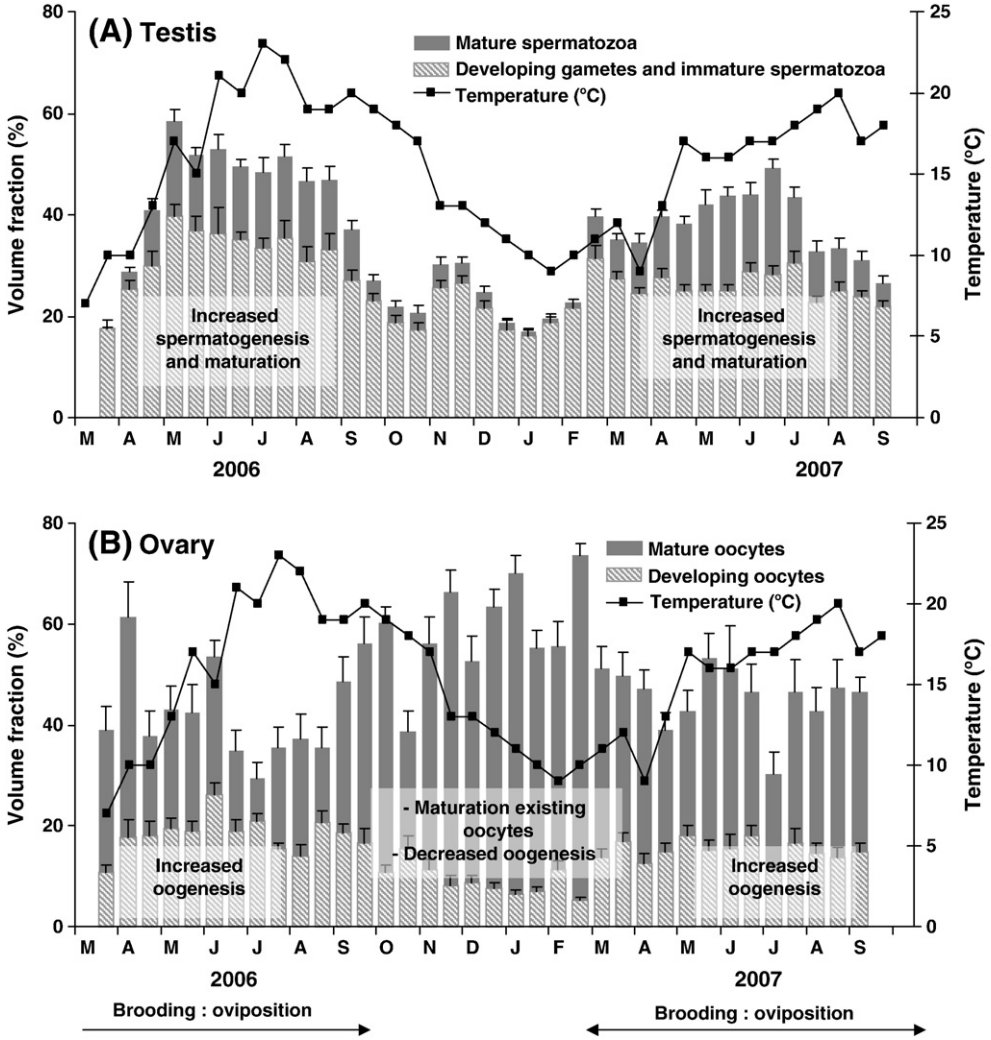

Fig. 3. Stereological results for *Crepidula fornicata* gonads and water temperature from March 2006 to September 2007. (A) Seasonal variations of mature spermatozoa and of developing gametes/immature spermatozoa percentages in the testis. (B) Seasonal variations of mature oocytes and of developing oocytes percentages in the ovary.

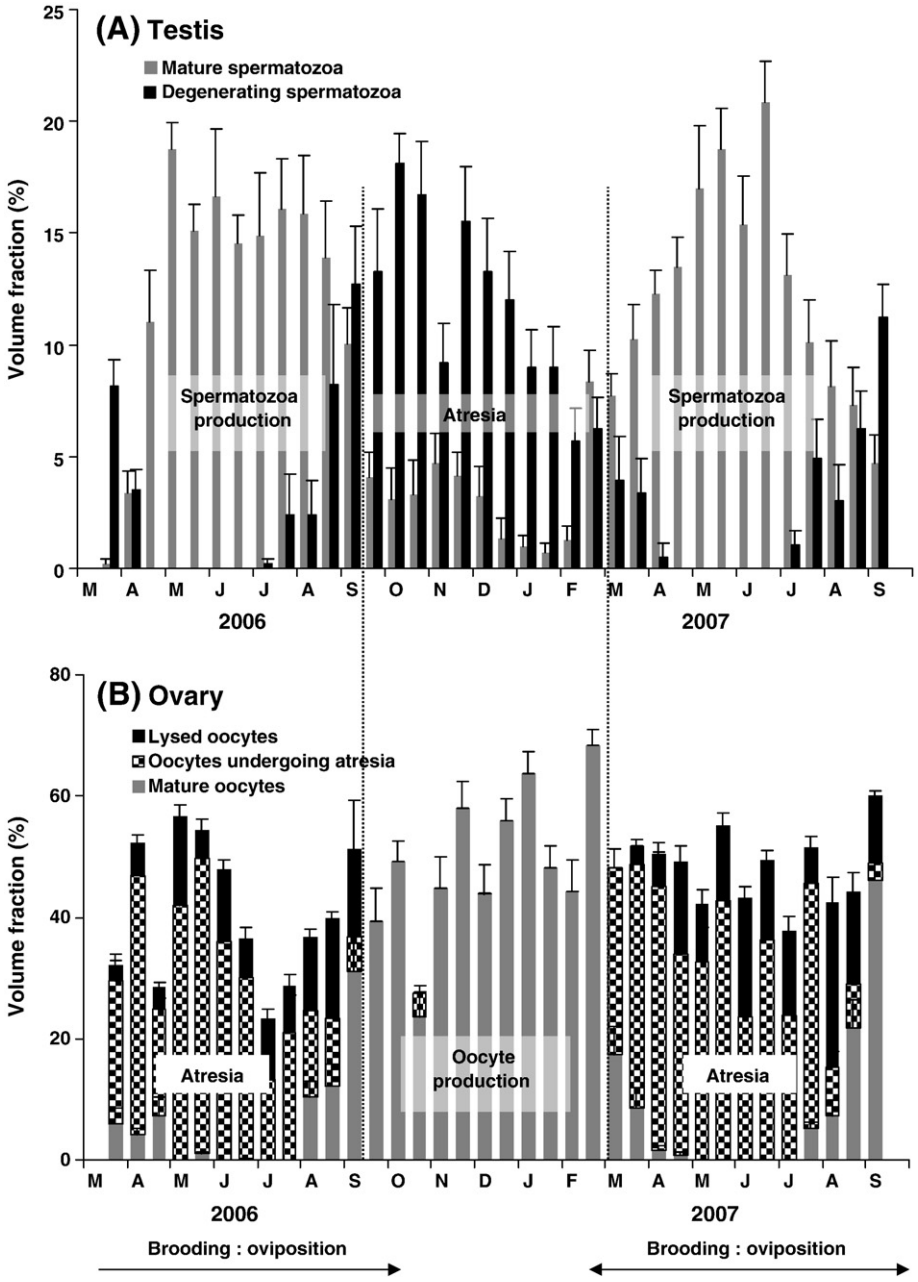

Fig. 4. Stereological results for *Crepidula fornicata* from March 2006 to September 2007. (A) Seasonal variations of mature and degenerating spermatozoa percentages in the testis. (B) Seasonal variations of percentages of mature oocytes, oocytes undergoing atresia, and lysed oocytes in the ovary. Arrows, indicating brooding periods, are from Valdizan et al. (2009).

discharging vitellin droplets inside the tubules (Fig. 6A, B, C), and by the presence in the tubule wall of high proportions of developing oocytes (Fig. 6B, C). Lytic debris, coming from totally lysed oocytes, were scarce (Fig. 6B, C), and did not seem to spread throughout the tubules.

These characteristics may be contrasted with those of the non-brooding period (autumn 2006–late winter 2007), in which the ovarian tubules were dilated and filled with voluminous mature oocytes (Fig. 6D, E, F), while developing oocytes were rare, and sometimes totally absent (Fig. 6E, F). The mature oocytes were characterized by their large size (not shown; mean ± 95% CI = 148.5 μm ± 1.4) and by the large density of very distinct vitellin droplets, often as large as 10 μm in diameter, and dispersed in the cytosol rather than condensed (Fig. 6F).

The ampullae of the seminal receptacle presented a uniform covering of mature spermatozoa conjoined to the inner epithelium (Fig. 6G, H, I). Only the basal nuclei were visible in the inner epithelium, suggesting a lack of cellular integrity at the interface with the spermatozoan heads.

3.2.2. Reproductive cycle

The histological profiles of the two ovarian periods described above indicate an active oogenic phase during the brooding periods, and a relatively inactive oogenic phase during the non-brooding period, as observed in the male gonad (Fig. 3).

The brooding phases were characterized by low percentages of intact mature oocytes (mean ± 95% CI = 1.9% ± 1.3, between March and late July 2006 and 2007), as illustrated in the histological profiles (Fig. 4B). Indeed, the majority of the mature oocytes were undergoing atresia (mean ± 95% CI = 27.7% ± 5.4), and a minor proportion of them were totally lysed (mean ± 95% CI = 10.3% ± 2.5) (Fig. 4B). The persistence of oocytes in degeneration during the brooding phases may indicate a very gradual atresia, favoring the survival and

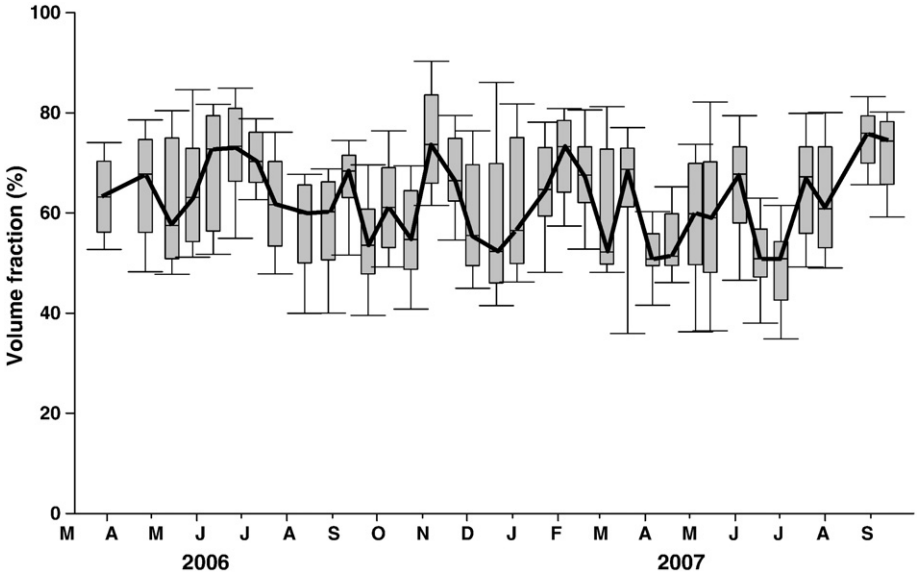

Fig. 5. Percent occupation of spermatozoa within the seminal vesicle ampullae, from March 2006 to September 2007. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicates the 90th and 10th percentiles. The curve links all the medians.

development of the developing oocytes. Indeed, the presence in high proportions of developing oocytes in the tubule wall was observed, with mean percentages (±95% CI) raising as far as 20% (±2.9) in April 2006 and 21% (±2.7) in May 2007 (Fig. 3B), indicating an active oogenesis.

During the non-brooding winter phase, the percentages (±95% CI) of developing oocytes progressively decreased (from 13.8%±1.6 in September 2006 to 6.9%±0.8 in late January 2007) confirming the histological profile which indicated a reduction of the gametogenic activity, to the benefit of the maturation of these young oocytes. Indeed, the greatest proportions of mature oocytes were observed during the non-brooding winter phase with a mean percentage of (±95% CI) of 53.4% (±6.6), tapering off on either end of this phase (Fig. 4B). The multiple minor decreases in the mature oocyte percentages during this period (Fig. 4B) indicates that the bulk of the mature oocytes were transferred to the uterus during the non-brooding period. Degenerating gametes (Fig. 4B) and unoccupied areas (not shown) were small and rare, with mean percentages of 0.1% and 0.18% (±0.1 and ±0.2, ±95% CI), respectively.

3.2.3. Activity of the seminal receptacle

The uniform spermatozoan cover of the seminal receptacle ampullae was observed throughout the sampling period, indicating continuous sperm storage by the female (Fig. 6G, H, I).

4. Discussion

4.1. Reproductive events underlying brooding and non-brooding phases

To our knowledge, the results of the present study constitute the first complete report on the reproductive cycle in *Crepidula fornicata*. As was observed in the present study, a distinct brooding period has been observed for *C. fornicata* at all of the sites at which broods have been monitored. A latitudinal effect appears to exist, with the later onsets of the brooding period in more northern latitudes (Richard et al., 2006). The present study documents the reproductive events underlying the brooding–non-brooding periods.

Taking together the quantitative histological observations of both male and female *C. fornicata*, it is possible to construct an annual sequence of reproductive events (Fig. 7). Whereas the seminal vesicle and seminal receptacle do not show marked seasonal phases of activity, both the testicle and the ovary show active and resting stages. A clear example of this is the arrested development of male gametes from late September 2006 to early February 2007. Although Coe (1936) showed a schematic of an active phase, no resting phase was mentioned. Here we present the histological aspect of both the active and the resting phase in *Crepidula fornicata* for the first time.

The alternating phases of the gonads largely conform to the alternating brooding and non-brooding periods of the females (Fig. 7), and show a close population synchrony in the timing of reproductive events (Fig. 7), notwithstanding a lack of such synchrony when considering only brooding events within a single chain (Richard et al., 2006). Female *C. fornicata* are here shown to invest heavily in oocyte production and vitellogenesis during the active oogenic phase in advance of the following brooding season. This strategy permits the rapid production of broods as soon as the minimal water temperature (10 °C) is attained to initiate oviposition (Werner 1948; Chipperfield 1951; Thieltges et al., 2004; Richard et al., 2006).

The period of most copulation occurs when the testis is in resting phase; this disposition favors renewal of the vesicle with freshly-generated sperm in the winter, although the amount of renewal is limited to about 25%.

4.2. Importance of the sperm storage organs

The exact reciprocal timing of the active and resting phases in males and females is enabled through the sperm storage organs, which allow the males to copulate throughout the year (as evidenced by the multiple and sequential decreases in spermatozoan volume of the seminal vesicle), and which also allow the females access to a sperm reservoir throughout the year (the seminal receptacle). Although the possibility of long-term sperm storage in the genus *Crepidula* was suspected by Coe (1936, 1942) and Hoagland (1975, 1978), this study constitutes the first histological demonstration that spermatozoa can viably exist in the seminal receptacle and vesicle of *C. fornicata* during winter. Metabolites for spermatozoan survival in the seminal vesicle may be provided by secretions of the epithelial cells (Martin 1985). It is also generally agreed that they are inactive at this stage, so that their energetic requirements are probably minimal (Runham, 1988).

Storage of exogeneous spermatozoa in the seminal receptacle, with much the same histological profile as that of *C. fornicata*, has been

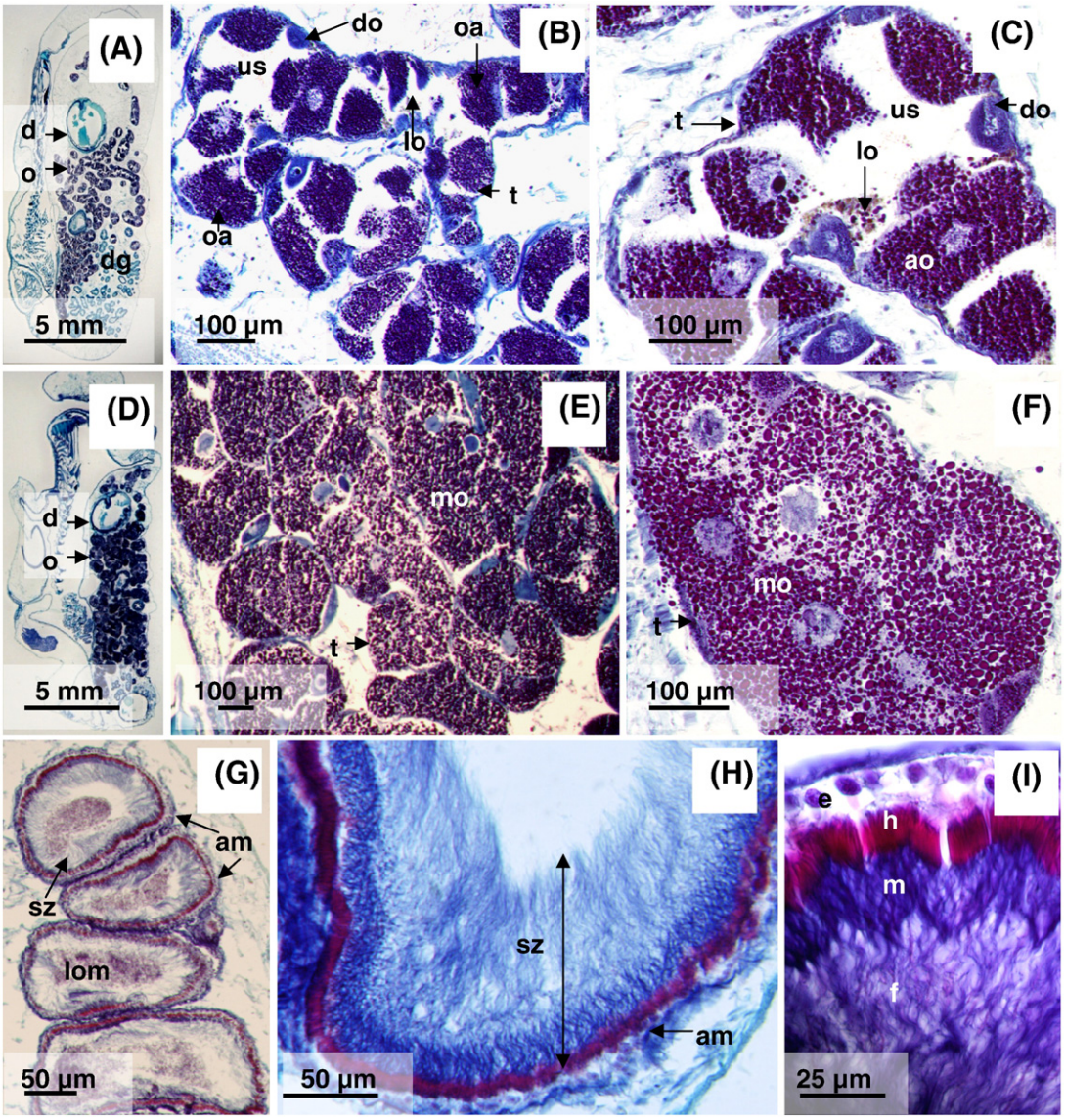

Fig. 6. Histological sections of the ovary and of the seminal receptacle of *Crepidula fornicata*. (A), (B), (C) Ovary (o) during the brooding periods (2006 and 2007) showing the location inside tubules (t) of unoccupied space (us), developing oocytes (do), oocytes undergoing atresia (ao) and lysed oocytes (lo). The digestive gland (dg) and digestive tracts (d) are indicated in (A) and (D). (D), (E), (F) Ovary during the period of oocyte maturation for the following brooding season (autumn 2006 to late winter 2007), showing dilated tubules filled with mature oocytes. (G), (H), (I) Seminal receptacle. (G) Ampullae (am) of the seminal receptacle with spermatozoa (sz) conjoined to the epithelium, and luminal organic matter (lom). (H), (I) Detail of the epithelium (e) of an ampulla showing a single layer of conjointed spermatozoa, forming regular superposed zones corresponding to the three spermatozoan sections: head (h), midpiece (m) and flagella (f). Note that the basal nuclei are visible in the ampulla epithelium.

reported in other gastropods, from all higher taxonomic groups (Lind, 1973; Beeman, 1977; Trüb, 1990; Baur, 1994). In the present study, the seminal receptacle lining was observed to be uniformly covered with spermatozoa throughout the year. Our ongoing electron microscopic studies allow us to discard the possibility that the spermatheca is a site of excess spermatozoan resorption, as has been advanced for other gastropods (Luchtel et al., 1997). Two opposing scenarios may thus be proposed for the role of this organ. The first is that fertilization is effected by the female using the stored spermatozoa. In this scenario, the lack of an observed decrease in the spermatozoan covering would simply be due to the ephemeral nature of such an event, which was not observed on any of the sampling dates. Such a scenario would require rapid replacement of the sperm through a subsequent copulation, since the sampling period was two weeks, and the receptacle lining was always observed covered with sperm. The immediate advantage of such a scenario is that it allows the female to fertilize oocytes in accordance with the timing of their reproductive cycle, and in particular the timing of brooding periods, thus avoiding oviposition when already incubating. The second scenario is that fertilization is effected primarily by the male immediately following copulation. In this scenario, the spermatozoa of the spermatheca would function only as a reserve in the event of subsequent non-copulation (e.g. mortality of males on the chain).

A third possible scenario also emerges from our observations and from previous studies in *C. fornicata*. The seminal receptacle may function as a reservoir not only for stored sperm from a previous copulation, but also as a theatre of sperm competition among spermatozoa from several males which have copulated with the same female. Evidence from many invertebrates possessing a seminal receptacle shows that this organ participates in diverse and complex ways in sperm competition (e.g. Elner and Beninger, 1995; den Boer et al., 2010). Multiple paternities of single broods has been previously documented in *C. fornicata* (Gaffney and McGee, 1992; Dupont et al., 2006), and it is possible that the site of sperm mixing and competition

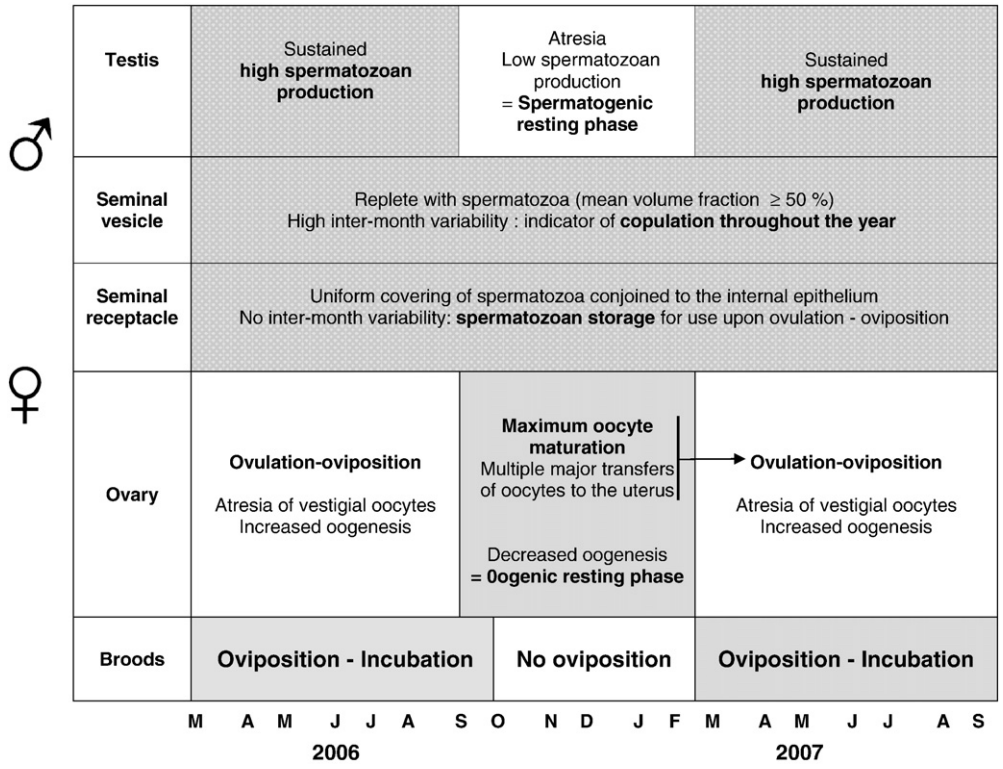

Fig. 7. Summary of the field reproductive cycle of *C. fornicata* in Bougneuf Bay.

is the spermatheca, especially given the limited available space on the epithelial lining against which all spermatozoa abut. Molecular genetic studies on the spermatozoa found within the spermatheca should allow the elucidation of this interesting possibility.

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References

Barillé, L., Cognie, B., Beninger, P.G., Decottignies, P., Rincé, Y., 2006. Feeding responses of the gastropod *Crepidula fornicata* to changes in seston concentration. Mar. Ecol. Prog. Ser. 322 (1), 169–178.

Barillé-Boyer, A.-L., Haure, J., Baud, J.-P., 1997. L'ostréiculture en baie de Bourgneuf. Relation entre le croissance des huîtres *Crassostrea gigas* et le milieu naturel : Synthèse de 1986 à 1995. Station Ifremer Bouin, Rapport Scientifique et Technique de la Direction des Ressources Vivantes de l'IFREMER.

Baur, B., 1994. Multiple paternity and individual variation in sperm precedence in the simultaneously hermaphroditic land snail *Arianta arbustorum*. Behav. Ecol. Sociobiol. 35 (6), 413–421.

Beeman, R.D., 1977. Gastropoda: Opisthobranchia. In: Giese, A.C., Pearse, J.S. (Eds.), Reproduction of Marine Invertebrates, Vol. IV. Academic Press, New York, pp. 115–179.

Beninger, P.G., Cannuel, R., Blin, J.-L., Pien, S., Richard, O., 2001. Reproductive characteristics of the archaeogastropod *Megathura crenulata*. J. Shellfish Res. 20 (1), 301–307.

Beninger, P.G., Decottignies, P., Guiheneuf, F., Barillé, L., Rincé, Y., 2007. Comparison of particle processing by two introduced suspension feeders: selection in *Crepidula fornicata* and *Crassostrea gigas*. Mar. Ecol. Prog. Ser. 334 (2), 165–177.

Blanchard, M., 1995. Origine et état de la population de *Crepidula fornicata* (Gastropoda Prosobranchia) sur le littoral français. Haliotis 24, 75–86.

Blanchard, M., 1997. Spread of the slipper limpet *Crepidula fornicata* (L. 1758) in Europe: current state and consequences. Sci. Mar. 61 (2), 109–118.

Blanchard, M., Ehrhold, A., 1999. Cartographie et évaluation du stock de crépidules (*Crepidula fornicata* L.) en baie du Mont Saint-Michel. Haliotis 28, 11–20.

Briarty, L.G., 1975. Stereology: methods for quantitative light and electron microscopy. Sci. Prog. 62 (245), 1–32.

Chipperfield, P.N.J., 1951. The breeding of *Crepidula fornicata* (L.) in the river Blackwater, Essex. J. Mar. Biol. Assoc. 30, 49–71.

Coe, W.R., 1936. Sexual phases in *Crepidula*. J. Exp. Zool. 72 (3), 455–477.

Collin, R., 2003. Worldwide patterns in mode of development in calyptraeid gastropods. Mar. Ecol. Prog. Ser. 247, 103–122.

Conklin, E.G., 1897. The embryology of *Crepidula*. J. Morphol. 13, 1–230.

Corlay, J.-P., Robert, J.-M., 1986. Les cultures marines en baie de Bourgneuf. Cah. Nantais 27, 103–169.

Coum, A., 1979. La population de crepidules (*Crepidula fornicata*) en rade de Brest, écologie et dynamique. Thèse de doctorat, Université de Bretagne Occidentale, France.

Davis, M.A., Thompson, K., 2000. Eight ways to be a colonizer; two ways to be an invader: a proposed nomenclature scheme for invasion ecology. Bull. Ecol. Soc. Am. 81, 226–230.

de Montaudouin, X., Sauriau, P.G., 1999. The proliferating Gastropoda *Crepidula fornicata* may stimulate macrozoobenthic diversity. J. Mar. Biol. Assoc. U.K. 79 (6), 1069–1077.

Decottignies, P., Beninger, P.G., Rincé, Y., Riera, P., 2007a. Trophic interactions between two introduced suspension-feeders, *Crepidula fornicata* and *Crassostrea gigas*, are influenced by seasonal effects and qualitative selection capacity. J. Exp. Mar. Biol. Ecol. 342, 231–241.

Decottignies, P., Beninger, P.G., Rincé, Y., Robins, R.J., Riera, P., 2007b. Exploitation of natural food sources by two sympatric, invasive suspension-feeders: *Crassostrea gigas* and *Crepidula fornicata*. Mar. Ecol. Prog. Ser. 334, 179–192.

den Boer, S.P.A., Baer, B., Boomsma, J.J., 2010. Seminal fluid mediates ejaculate competition in social insects. Science 327, 1506–1509.

Dupont, L., Richard, J., Paulet, Y.M., Thouzeau, G., Viard, F., 2006. Gregariousness and protandry promote reproductive insurance in the invasive gastropod *Crepidula fornicata*: evidence from assignment of larval paternity. Mol. Ecol. 15 (10), 3009–3021.

Dutertre, M., Beninger, P.G., Barillé, L., Papin, M., Haure, J., 2009. Rising water temperatures, reproduction and recruitment of an invasive oyster, *Crassostrea gigas*, on the French Atlantic coast. Mar. Environ. Res. 69 (1), 1–9.

Ehrhold, A., Blanchard, M., Auffret, J.-P., Garlan, T., 1998. Conséquences de la prolifération de la crépidule (*Crepidula fornicata*) sur l'évolution sédimentaire de la baie du Mont-Saint-Michel (Manche, France). C. r. Acad. Sci., Ser. IIA, Earth Planet. Sci. 327 (9), 583–588.

Elner, R.W., Beninger, P.G., 1995. Multiple reproductive strategies in snow crab, *Chionoecetes opilio*: Physiological pathways and behavioral plasticity. J. Exp. Mar. Biol. Ecol. 193 (1–2), 93–112.

Gabe, M., 1968. Techniques Histologiques. Masson, Paris.

Gaffney, P.M., McGee, B., 1992. Multiple paternity in *Crepidula fornicata* (Linnaeus). The Veliger 35 (1), 12–15.

Gould, H.N., 1917a. Studies on sex in the hermaphrodite mollusc *Crepidula plana*. I. History of the sexual cycle. J. Exp. Zool. 23 (1), 1–69.

Gould, H.N., 1917b. Studies on sex in the hermaphrodite mollusk *Crepidula plana*. II. Influence of the environment on sex. J. Exp. Zool. 23 (1), 225–250.

Gould, H.N., 1952. Studies on sex in the hermaphrodite mollusk *Crepidula plana*. IV. Internal and external factors influencing growth and sex development. J. Exp. Zool. 119 (1), 93–163.
Goulletquer, P., Bachelet, G., Sauriau, P.-G., Noel, P., 2002. Open Atlantic coast of Europe — a century of introduced species into French waters. In: Leppäkoski, E., et al. (Ed.), Invasive Aquatic Species of Europe. Kluwer Academic Publishers, Netherlands, pp. 276–290.
Grall, J., Hall-Spencer, J.M., 2003. Problems facing maerl conservation in Brittany. Aquat. Conserv. 13 (1), 55–64.
Hamon, D., Blanchard, M., 1994. Etat de la prolifération de la crépidule (*Crepidula fornicata*) en baie de Saint-Brieuc. Rapport Ifremer-DEL 94 14.
Hoagland, K.E., 1975. Reproductive strategies and evolution in the genus *Crepidula* (Gastropoda: Calyptraeidae). Thesis, University of Harvard, Cambridge, Massachusetts.
Hoagland, K.E., 1973. Protandry and the evolution of environmentally-mediated sex change : a study of the Mollusca. Malacologia 17, 365–391.
Lind, H., 1973. The functional significance of the spermatophore and the fate of spermatozoa in the genital tract of *Helix pomatia*. J. Zool. 169 (1), 279–301.
Luchtel, D.L., Martin, A.W., Deyrup-Olsen, I., Boer, H.H., 1997. Gastropoda: Pulmonata. In: Harrison, F.W., Kohn, A.J. (Eds.), Mollusca II. Microscopic Anatomy of Invertebrates, Vol. VI B. Wiley-Liss, New York, pp. 459–718.
Martin, M.-C., 1985. Etude expérimentale de l'inversion sexuelle et de la morphogenèse génitale femelle chez un mollusque hermaphrodite protandre *Crepidula fornicata* L. (mésogastéropode). Thèse de doctorat, Université de Caen, France.
Martoja, R., Martoja-Pierson, M., 1967. Initiation aux techniques de l'histologie animale. Masson, Paris.
Mayhew, T.M., 2000. 3D structure from thin sections: applications of stereology. Eur. Micro. Anal. 11, 17–20.
Morales-Alamo, R., Mann, R., 1989. Anatomical features in histological sections of *Crassostrea virginica* as an aid in measurements of gonad area for reproductive assessment. J. Shellfish Res. 8 (1), 71–82.
Morvan, C., Ansell, A.D., 1988. Stereological methods applied to reproductive cycle of *Tapes rhomboides*. Mar. Biol. 97 (3), 355–364.
Pazos, A.J., Román, G., Acosta, C.P., Abad, M., Sánchez, J., 1996. Stereological studies on the gametogenic cycle of the scallop, *Pecten maximus*, in suspended culture in Ria de Arousa (Galicia, NW Spain). Aquaculture 142 (1–2), 119–135.
Pechenik, J.A., 1979. Role of encapsulation in invertebrate life histories. Am. Nat. 114 (6), 859–870.
Richard, J., Huet, M., Thouzeau, G., Paulet, Y.-M., 2006. Reproduction of the invasive slipper limpet, *Crepidula fornicata*, in the Bay of Brest, France. Mar. Biol. 149 (4), 789–801.
Runham, N.W., 1988. Mollusca. In: Adiyodi, K.G., Adiyodi, R.G. (Eds.), Reproductive biology of invertebrates. Volume 3: Accessory sex glands. Wiley, Chichester UK, pp. 113–188.
Sauriau, P.-G., Pichocki-Seyfried, C., Walker, P., de Montaudouin, X., Palud, C., Héral, M., 1998. *Crepidula fornicata* L. (mollusque, gastéropode) en baie de Marennes-Oléron: cartographie des fonds par sonar à balayage latéral et estimation du stock. Oceanol. Acta 21 (2), 353–362.
Sauriau, P.-G., Walker, P., Barillé, L., Barillé, A.-L., Davenne, E., Gruet, Y., Escaravage, C., 2006. La crépidule en baie de Bourgneuf: état du stock quarante ans après son introduction et enjeux pour l'ostréiculture de demain. In: Chaussade, J., Guillaume, J. (Eds.), Pêche et Aquaculture. Pour une exploitation durable des ressources vivantes de la mer et du littoral. Presses Universitaires de Rennes, pp. 241–252.
Silberzahn, N., 1978. Aspect cytologique du réceptacle séminal de la crépidule. Haliotis 9, 49–52.
Thieltges, D.W., Strasser, M., van Beusekom, J.E.E., Reise, K., 2004. Too cold to prosper — winter mortality prevents population increase of the introduced American slipper limpet *Crepidula fornicata* in Northern Europe. J. Exp. Mar. Biol. Ecol. 311 (2), 375–391.
Thieltges, D., Strasser, M., Reise, K., 2006. How bad are invaders in coastal waters? The case of the American slipper limpet *Crepidula fornicata* in Western Europe. Biol. Invasions 8 (8), 1673–1680.
Trüb, H., 1990. Züchtung von Hybriden zwischen *Viviparus ater* und *V. contectus* (Mollusca: Prosobranchia) im Zürichsee und ökologische Untersuchungen in einer gemischten Population im Gardasee. Thesis, University of Zurich, Switzerland.
Valdizan, A., Beninger, P.G., Cognie, B., Decottignies, P., 2009. External fertilization and excapsular development in *Crepidula fornicata*: evaluating the risk of invasion control by dredging, crushing, and on-site rejection. Aquat. Living Resour. 22 (1), 21–28.
Voltzow, J., 1994. Gastropoda: Prosobranchia. In: Harrison, F.W., Khon, A.J. (Eds.), Microscopic Anatomy of Invertebrates, V: Mollusca. Wiley and Sons, New York, pp. 111–252.
Weibel, E.R., Kistler, G.S., Scherle, W.F., 1966. Practical stereological methods for morphometric cytology. J. Cell Biol. 30 (1), 23–38.
Werner, B., 1948. Die amerikanische Pantoffelschnecke *Crepidula fornicata* L. im nordfriesischen Wattenmeer. Zoologische Jahrbücher. Abt. Syst. Oekol. Geogr. Tiere. 77, 449–488.
Wonham, M.J., O'Connor, M., Harley, C.D.G., 2005. Positive effects of a dominant invader on introduced and native mudflat species. Mar. Ecol. Prog. Ser. 289, 109–116.