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RESEARCH ARTICLE

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Reproductive strategies of two deep-sea gastropod species from the Porcupine Seabight (Northeast Atlantic)

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Abstract Two species of small gastropods (< 6 mm in length), *Amphissa acutecostata* (Philippi, 1844) and *Gymnobela subaraneosa* (Dautzenberg and Fischer, 1896), widely distributed in the northeast Atlantic, were found in large numbers in the Porcupine Seabight (Northeast Atlantic). Except for some aspects of taxonomy and distribution, as well as some data on larval development, the biology of these species is unknown. This study describes basic aspects of the life-history strategies of both species. Histological studies showed that oocyte and sperm development in both species was similar to the gametogenetic patterns observed in other deep-sea gastropods. In females, oogonia proliferated in the germinal epithelium and developed into previtellogenic oocytes (30–40 μm), which grew into vitellogenic primary oocytes. Vitellogenic oocytes were covered by a thin layer of follicle cells involved in the vitellogenic processes. The maximum size for mature oocytes was 99.06 μm for *A. acutecostata* and 114.82 μm for *G. subaraneosa*. In *A. acutecostata* most of the volume of the ovary was occupied by previtellogenic and early vitellogenic oocytes, whereas in *G. subaraneosa* most of the volume was filled by large vitellogenic oocytes. Both species showed quasi-continuous production of oocytes. The oocyte size-frequency diagrams suggested a continuous release of a small number of oocytes throughout the year for *A. acutecostata*, and asynchronous periodic spawning events for *G. subaraneosa*. Gonad development and gametogenesis could be strongly affected by presence of parasites in one of the populations of *G. subaraneosa*.

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Introduction

The deep sea supports a highly diverse benthic invertebrate fauna (Hessler and Sanders 1967; Grassle 1989), and community structure is thought to vary at scales from centimetres to hundreds of kilometres (Grassle and Maciolek 1992; Rex et al. 1993, 2000). Understanding the structure and dynamics of these assemblages is, however, not possible without knowledge of the autoecology of the component species. Reproductive patterns such as gametogenesis, age at maturity, timing of spawning, egg size, fecundity and larval development affect the adaptation of populations to the environment (e.g. Eckelbarger 1994; Ramirez-Llodra et al. 2000). A good understanding of the reproductive biology of an organism is therefore crucial to determine the selective forces that shape the evolution of life histories and produce the patterns of diversity in different habitats (Eckelbarger 1994). Despite a large number of studies on reproduction of diverse deep-sea benthic infaunal invertebrates (e.g. Lightfoot et al. 1979; Gage and Tyler 1982; Tyler and Gage 1984; Harrison 1988; Tyler et al. 1992; Young 2003), many aspects of life histories of deep-sea organisms remain poorly understood. Gametogenetic processes play a central role in the life cycle of an organism. Within gametogenesis, the vitellogenic pathways (autosynthetic, heterosynthetic and mixed) are phylogenetically constrained and therefore species-specific (Eckelbarger 1983, 1994; Jaeckle 1995). Previous studies suggest that deep-sea species have retained ancestral life-history traits shared with their shallow-water counterparts and they have not developed specific specializations for reproduction in the deep sea (Eckelbarger and Watling 1995). For example, modes of development and mechanisms and rate of egg production, are phylogenetically constrained, and therefore

intrinsic to the species and are not affected by environmental factors (Healy et al. 1988; Heller 1993; Bouchet and Warén 1994; Eckelbarger and Watling 1995). In contrast, number and quality of eggs, i.e. reproductive output, may be affected by environmental factors such as food availability and quality, stress in physical factors or toxicity (Eckelbarger 1994; Ramirez-Llodra 2002).

Molluscs constitute the third most dominant group of animals of the macrofauna in the deep sea (Gage and Tyler 1991). In general, the deep-sea gastropod fauna is dominated by very small organisms, with the Turridae being the largest family of deep-sea gastropods (Bouchet and Warén 1980). With the exception of a number of studies published on reproductive periodicity, gametogenesis and gamete structure of some species (for review, see Young 2003) and on larval development (e.g. Scheltema 1972; Bouchet 1977; Bouchet and Warén 1979, 1994; Colman et al. 1986), the reproductive biology of deep-sea gastropods remains poorly understood.

Between November 1977 and December 1986 the Institute of Oceanographic Sciences Deacon Laboratory (IOSDL) conducted an intensive survey of the benthic biology of the Porcupine Seabight to the southwest of Ireland (Rice et al. 1991). Two species of small gastropods [< 6 mm in length (personal observations)], *Amphissa acutecostata* (Philippi, 1844) and *Gymnobela subaraneosa* (Dautzenberg and Fischer, 1896), widely distributed in the northeast Atlantic (Bouchet and Warén 1980, 1985), were found in large numbers in the Porcupine Seabight. *A. acutecostata* (family Columbellidae) was found at depths of 940–2,775 m whereas *G. subaraneosa* (family Turridae) was found at depths of 1,300–4,050 m.

*A. acutecostata*and *G. subaraneosa* (Bouchet 1977; Bouchet and Warén 1980; Bouchet and Fontes 1981) have larval shells characteristic of planktotrophic development. Both species, as other species of the same families, are supposed to be predators (Bouchet and Warén 1980, 1985). Except from this information, the life history of these species is unknown. This paper describes the reproductive biology of *A. acutecostata* and *G. subaraneosa* from time-series samples in the Porcupine Seabight.

Materials and methods

Samples were collected between July 1979 and April 1983 during several cruises in the Porcupine Seabight (Table 1). The samples were taken from different areas at latitudes from 49° 11′ N to 51° 47′ N and depths between 940 m and 2,700 m. Specimens were collected using the IOS epibenthic sledge based on a steel frame approximately 1.9-m long, 2.3-m wide and 1.2-m high (see Rice et al. 1982). Two tiers of net were attached to this frame, both tiers being closed while the sledge was in mid-water and opened mechanically when the gear reaches the seafloor. The lower tier was designed to sample the benthic epifauna and, to some extent, the infauna (Rice et al. 1991). The material was washed through a 0.42-mm mesh screen, fixed in 5% formaldehyde for 5–7 days, and then transferred to 70% isopropanol for storage. Although samples were originally collected to determine variation in community structure and not explicitly to study reproductive patterns, most specimens analysed were in good condition allowing analysis of gametogenesis.

Oogenesis and spermatogenesis were described from light microscopy. All the individuals were decalcified using a proprietary rapid decalcifying solution (Cellpath), dehydrated in graded alcohols, cleared in xylene and embedded in paraffin wax. Sections were cut at 5 µm and stained with haematoxylin and eosin. All the oocytes that had been sectioned through the nucleus ($n = 20$–140) were measured (feret diameter) with an image analysis program (Matrox-Rainbow Runner/Jandel Scientific SigmaScan Pro 4). Two slides per male ($N = 20$) were prepared to describe spermatogenesis. Fifty spermatogonia, 50 spermatocytes, and 50 spermatids were measured for each individual and the data averaged for each species.

Variation from the 1:1 male/female ratio for the two species was tested with χ^2 tests. Oocyte sizes were grouped into 10-µm (*A. acutecostata*) and 15-µm (*G. subaraneosa*) classes and oocyte size-frequency diagrams constructed for each individual and for the composite data for each sample. In addition, variation of mean oocyte sizes among samples of each species was compared with a nested-design analysis of variance (ANOVA) for uneven sample sizes (Sokal and Rohlf 1995). With this design it is possible to obtain two independent measures of the following: (1) the levels (samples, individuals) at which there is significant variability in oocyte sizes, and (2) the components of variation among individuals of a sample and among samples. Prior to the analyses, data were transformed via the $\sqrt{(x+1)}$ function to reduce heterogeneity (Sokal and Rohlf 1995).

Results

Gross morphology

In both species the gonad was located on the upper part of the body whorl overlying the digestive gland. This

Table 1 Location of the sampling sites of the two species of gastropods

Species	Station	Date	Position	Depth (m)	No. of individuals
Amphisa acutecostata	50606 # 5	July 1979	50° 43.1′ N, 13° 56.1′ W	1,130	34
	10109 # 8	September 1979	49° 11.7′ N , 12° 19.4′ W	1,120	14
	10111 # 8	September 1979	49° 32.6′ N, 13° 07.1′ W	1,635	15
	51103 # 5	May 1981	51° 47′ N, 13° 13.1′ W	940	29
	51403 # 5	March 1982	51° 37.8′ N, 12° 58.9′ W	1,290	44
	51420 # 4	April 1982	51° 37.9′ N, 12° 59.5′ W	1,283	47
Gymnobela subaraneosa	10108 # 1	September 1979	49° 20.6′ N, 12° 49.2′ W	1,500	23
	10112 # 1	September 1979	50° 25′ N, 13° 19.1′ W	2,600	10
	51403 # 2	March 1982	51° 37.4′ N, 12° 59.2′ W	1,300	7
	51416 # 1	March 1982	50° 16.8′ N, 13° 31.4′ W	2,700	15
	51715 # 2	April 1983	51° 29.4′ N, 12° 58.8′ W	1,500	10

comprised the visceral coils, lying mainly on the columellar side. Gonads were covered with a layer of pallial epithelium overlying a layer of muscle-fibre tissue. In immature individuals, gonadal tissue in histological sections was insufficient for sex determination.

A. acutecostata

Sex ratio

A. acutecostata is gonochoristic. The overall sex ratio of this species showed significant variation from a 1:1 sex ratio ($\chi^2 = 6.12$, $df = 1$, $P < 0.05$), with a total of 63 females to 94 males (approximately 0.6:1). When samples were analysed separately, only two populations [populations collected at 940 and 1,283 m, respectively (Table 1)] showed a significant variation from 1:1 sex ratio. Only 12 specimens from the 940-m station were available for analysis, of which 10 were males and 2 females ($\chi^2 = 5.33$, $df = 1$, $P < 0.05$). The total number of individuals sexed from the 1,283-m station was 43, with 28 males and 15 females ($\chi^2 = 3.93$, $df = 1$, $P < 0.05$).

Oogenesis

The ovary consisted of a large number of individual follicles separated from each other by thin layers of connective tissue. No reserve tissue was observed between the follicles. Around the inner periphery of each follicle there was a thin layer of germinal epithelium. Oogonia appeared as small round cells (4.30 ± 0.064 μm diameter) with a very large nucleus. These grew to 9–12 μm on the germinal epithelium and then developed into previtellogenic oocytes. Previtellogenic oocytes were found close to the germinal epithelium. They were characterised by a high nucleus/cytoplasm ratio and a basophilic cytoplasm that stained purple with haematoxylin (Fig. 1A). Vitellogenesis started at 30–35-μm oocyte diameter. The cytoplasm of growing vitellogenic oocytes became granular in appearance with the accumulation of yolk granules and there was a change to acidophilia, staining pink with eosin (Fig. 1A). Vitellogenic oocytes lay free in the lumen of the follicle, packing the ovary when they reached the maximum size. In most individuals analysed, the ovary contained mainly previtellogenic and early vitellogenic oocytes (30–50 μm). The overall median size of oocytes was 29.6 μm and the maximum oocyte size observed was 99.06 μm in a specimen collected at 1,300 m in July 1979.

Oocyte size-frequency distribution

The oocyte size-frequency diagrams showed no evidence of synchronous oogenesis. There was a wide range of oocyte sizes at any given time, which suggests a quasi-continuous production of oocytes. In most specimens, there was a pool (35–90%) of previtellogenic and early vitellogenic oocytes (< 40 μm) and a low percentage (< 5%) of large vitellogenic oocytes (Fig. 2). Although there was high variability among individuals in the mean oocyte sizes [$F_{47,3755} = 25.22$, $P < 0.001$ (see Table 2)], there was a common pattern among samples [$F_{5,47} = 0.84$, $P > 0.05$ (Table 2)]. Most of the variance was caused by a wide range of oocyte sizes within each individual, i.e. residual, and by among-individual differences, rather than by variation among samples (Table 2). This variation among individuals was shown, for example, in some samples collected at 1,290 m (March 1982), 940 m (May 1981), 1,130 m (July 1979) and 1,283 m (April 1982), where some individuals had a very high percentage (> 70%) of small oocytes (< 30 μm), whereas other specimens showed a wider range of oocyte sizes [e.g. individuals from the 1,130-m station, July 1979 (Fig. 3)].

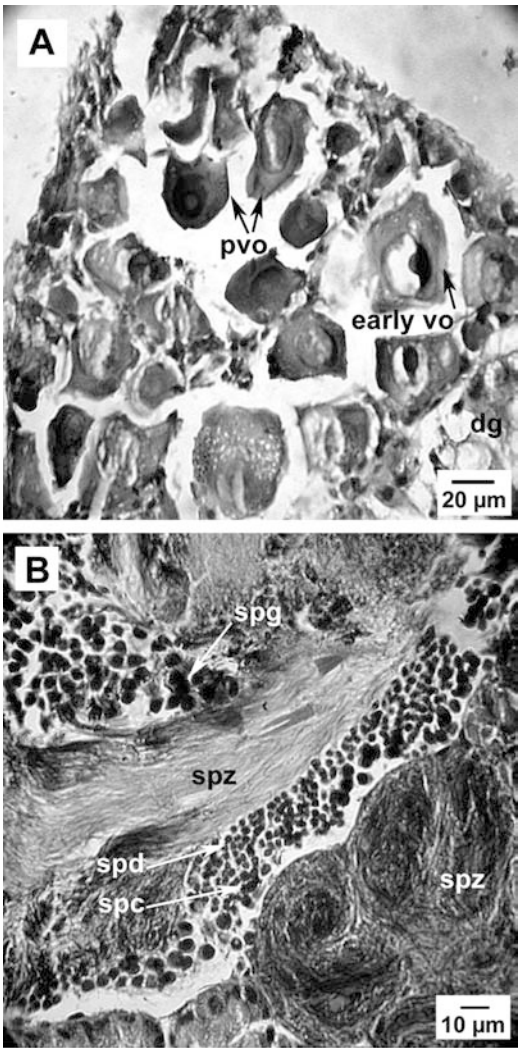


Fig. 1A, B *Amphissa acutecostata*. Light histology of gonads stained with haematoxylin and eosin. **A** Ovary of adult female showing previtellogenic (*pvo*) and vitellogenic oocytes (*vo*); *dg* digestive gland. **B** Seminiferous tubule of adult male showing spermatogonia (*spg*), spermatoctyes (*spc*), spermatids (*spd*) and spermatozoa (*spz*)

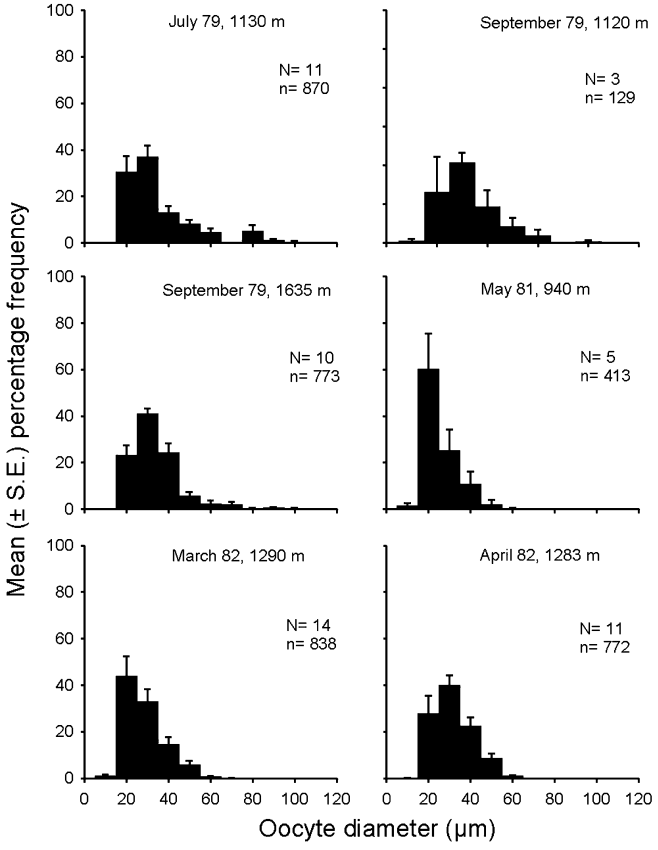


Fig. 2 *A. acutecostata*. Mean percentage (±SE) of the oocyte size-frequency distribution of individuals for each sample. *N* Number of individuals analysed,*n* number of oocytes measured

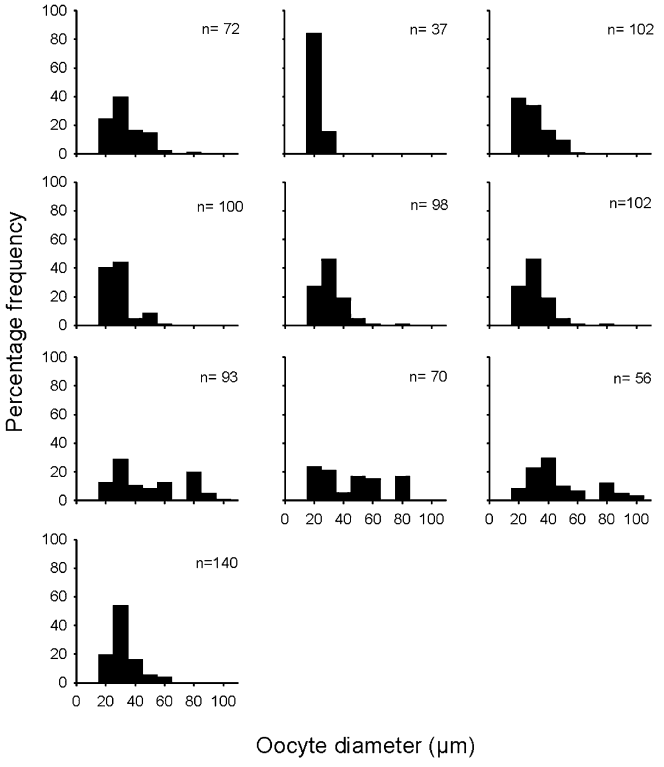


Fig. 3 *A. acutecostata*. Oocyte size-frequency distribution of individuals collected at 1,130 m in July 1979 (50606#5). A total of 10 individuals was analysed; *n* indicates number of oocytes measured

Spermatogenesis

The testis was formed by a large number of elongated seminiferous tubules separated from each other by thin walls of connective tissue. The tubules were pentagonal (in transverse section) with the germinal layer around the periphery. All stages of sperm development were found at any one time, indicating a continuous production of spermatozoa. The germinal layer gave rise to spermatogonia (6.78 ± 0.11 µm in diameter), which divided mitotically and developed into spermatocytes with a diameter of 4.82 ± 0.06 µm. Spermiogenesis occurred at this stage. Firstly, spermatids (2.21 ± 0.04 µm in diameter) arose by reduction division and then differentiated into mature sperm, which accumulated as densely packed aggregations in the lumen of the tubules (Fig. 1B). Spermatozoa were orientated with their heads located amongst the developing spermatocytes and their tails trailing into the lumen of the tubule. Mature sperm was accumulated at the ventral side of the body.

Table 2 *A. acutecostata*. Nested analysis of variance of intra- and inter-sample comparison of oocyte sizes. Variance components and percentage of variance are also explained for each source of variation

Source	*df*	MS	*F*ratio or (*F'*)	Variance components	Percentage of total
Samples	5	17.56	0.96 ns (1.14)[a] ns	−0.01[b]	0.00 ± 0.00
Individuals	47	18.16	25.22*	0.25	25.59 ± 0.50
Residuals	3,755	0.72		0.72	74.41 ± 0.85
Total	3,807			0.97	±0.98

*$P < 0.001$
ns Not significant
[a] Correction for unequal sample size
[b] Value is negative and is estimated by zero

G. subaraneosa

Sex ratio

G. subaraneosa is also gonochoristic. The overall sex ratio of this species did not differ significantly from unity. The total number of sexed specimens was 58, with 32 males and 26 females ($\chi^2 = 0.62$, $df = 1$, $P > 0.05$). However, the sex ratio in the sample collected at the 1,500-m station (April 1983) differed significantly from unity ($\chi^2 = 6.40$, $df = 1$, $P < 0.05$) with a total of 1 female to 9 males.

Oogenesis

The ovary was formed by a large number of elongated follicles. Oogonia developed from a layer of germinal

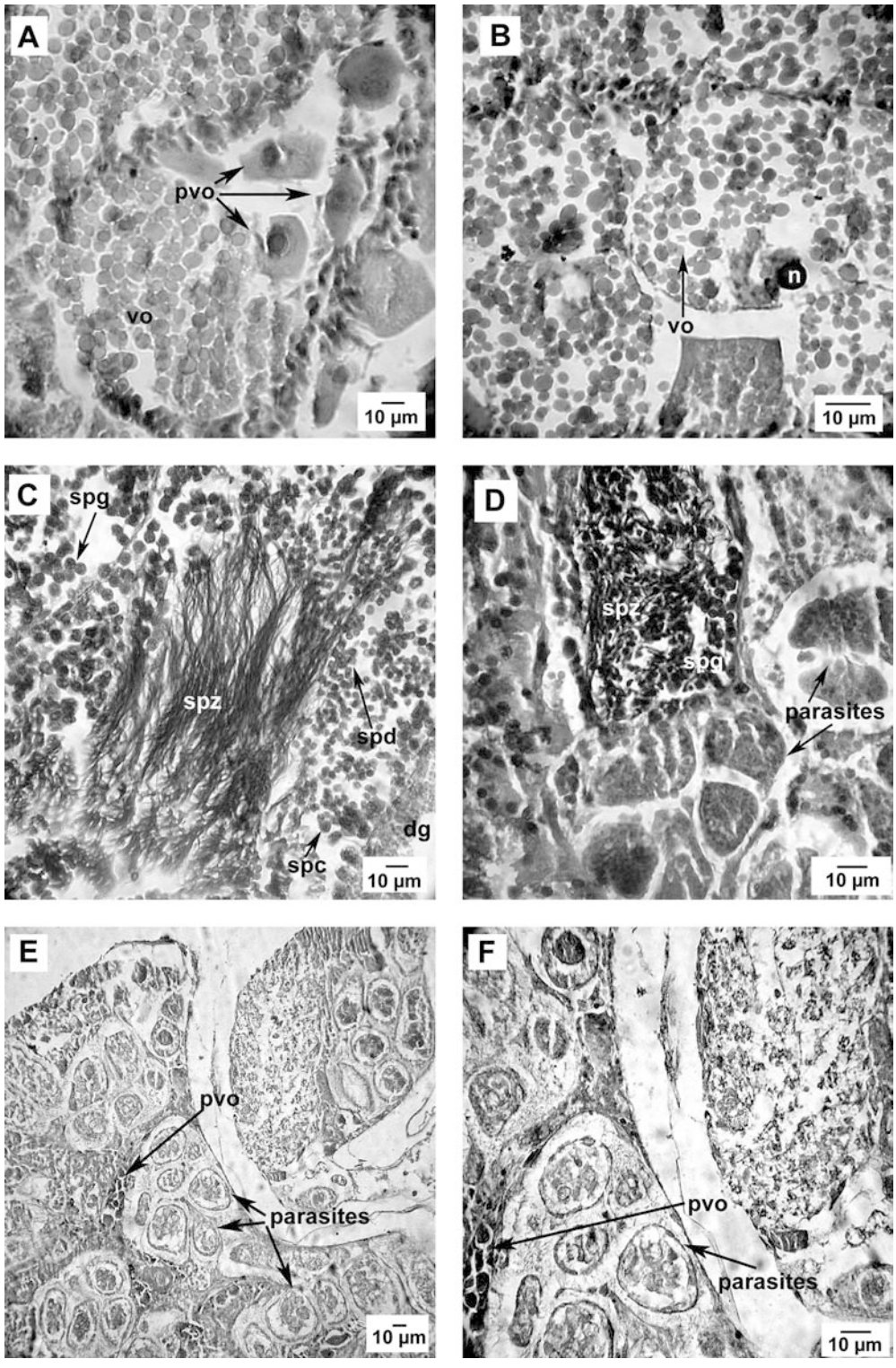


Fig. 4A–F *Gymnobela subaraneosa*. Light histology of gonads stained with haematoxylin and eosin. **A** Ovary of adult female showing previtellogenic (*pvo*) and vitellogenic (*vo*) oocytes. **B** Ovary of adult female showing vitellogenic oocytes (*vo*); *n* nucleus. **C**Seminiferous tubule of adult male showing spermatogonia (*spg*), spermatocytes (*spc*), spermatids (*spd*) and spermatozoa (*spz*); *dg* digestive gland. **D** Male with parasitic infestation in the gonad (redia phase?) showing anomalous spermatogenesis. **E** Female with parasitic infestation (sporocyst phase) in gonads and digestive gland, showing very few small previtellogenic oocytes. **F** Ovary of an infested female (sporocyst phase) showing few previtellogenic oocytes

epithelium situated in the inner periphery of these follicles. Oogonia (6.5 ± 0.072 µm in diameter) grew to 10–15 µm and developed into previtellogenic oocytes. Previtellogenic oocytes were elongated in outline and had a large nucleous/cytoplasm ratio and basophilic cytoplasm (Fig. 4A). These oocytes grew to a size of 35–40 µm before undergoing vitellogenesis. Vitellogenesis was characterised by the presence of yolk granules spreading in the cytoplasm (Fig. 4B). The yolk globules were oval in shape and stained bright red with esosin. The formation of these granules might be an artefact caused by the dissolution of lipids during histological processing (Colman 1987). The overall median size of oocytes was ~57.93 µm and the maximum oocyte size observed was 114.82 µm in one specimen found at 2,600 m. In the majority of the examined females, most

of the gonad volume was occupied by large mature vitellogenic oocytes. Also, most mature females showed mature sperm in the gonadal duct, suggesting that part of this duct might act as a seminal receptacle as shown in other gastropod species (Fretter and Graham 1994).

The specimens collected from the station at 2,700 m showed poorly developed gonads and this could be due to parasitic infestation (see below).

Oocyte size-frequency distribution

The sample collected in April 1983 was not analysed because all the individuals were males except for only one female with very few previtellogenic and early vitellogenic oocytes. The oocyte size-frequency diagrams of *G. subaraneosa* showed a bimodal distribution of oocyte sizes. This suggested the existence of two cohorts of developing oocytes, suggesting periodic asynchronous spawning events. Although all samples present a pool of previtellogenic and large vitellogenic oocytes in most individuals, this distribution varied among samples and within samples (Fig. 5). For example, samples collected from 1,500 and 1,300 m in September 1979 and March 1982, respectively, showed a high percentage (~15–30%) of previtellogenic oocytes and a peak (~40–80%) of vitellogenic oocytes, mainly in the size classes of 75 and 90 μm. In contrast, the sample collected from 2,700 m showed a very different pattern, with a high percentage (>80%) of previtellogenic oocytes and a very low percentage of small vitellogenic oocytes (Fig. 5). The lack of large vitellogenic oocytes in this population was most probably caused by the parasite infection of the gonads in most specimens (85.71% of females). Mean oocyte size varied significantly among populations [$F_{3,20} = 39.70$, $P < 0.001$ (Table 3)] and among individuals [$F_{20,2028} = 5.18$, $P < 0.001$ (Table 3)]. The majority of the variance was, however, caused by the wide range of oocyte sizes within each individual, i.e. residuals and by among-sample differences (see percentage of total variation in Table 3). Because of the large size but small number of mature vitellogenic oocytes, only a few of them had been sectioned through the nucleus and therefore included in the measures. This caused an underestimated number of vitellogenic oocytes to appear in the largest size classes as an artefact of processing, and could therefore account for part of the variability in oocyte size distribution within this species.

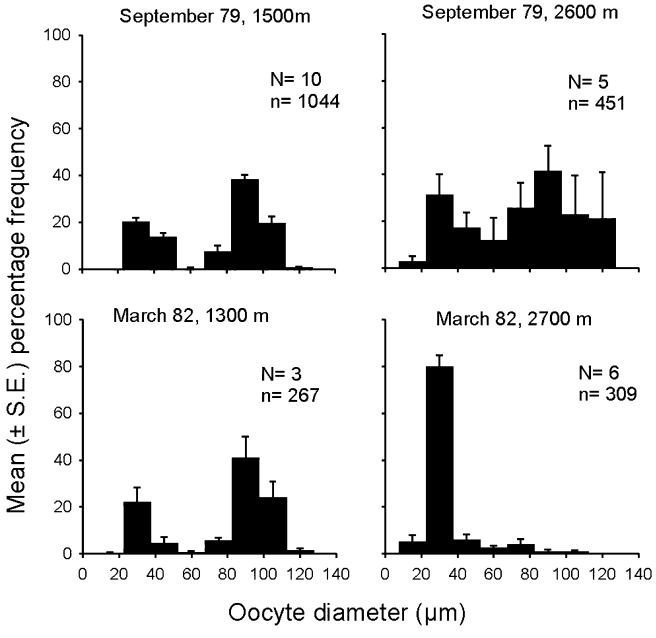


Fig. 5 *G. subaraneosa*. Mean percentage (±SE) of the oocyte size-frequency distribution of individuals for each sample. *N* Number of individuals analysed, *n* number of oocytes measured

Spermatogenesis

The testis was composed of subquadrangular tubules separated from each other by thin walls of connective tissue. The germinal epithelium gave rise to a dense layer of spermatogonia. Spermatogonia (4.54 ± 0.09 μm in diameter) developed into spermatocytes with a diameter of 3.21 ± 0.07 μm. Spermatids (1.66 ± 0.06 μm in diameter) arose by reduction division and then differentiated into mature sperm, which accumulated as packed aggregations in the lumen of the tubules. As in *A. acutecostata*, most specimens showed all stages of spermatogenesis, indicating a continuous pattern of sperm production (Fig. 4C).

Parasites

An unidentified parasite-like structure (Fig. 4D–F) was visible in some individuals (18.96%) of *G. subaraneosa*. This parasite was particularly abundant at the 2,700-m station (March 1982), with an occurrence of 81.81%. The parasites were found infesting the mantle cavity, digestive gland and gonads. We suggest that the observed structures belong to two different phases—sporocysts and redia—of the life cycle of a digenetic trematode parasite that uses snails as intermediate hosts. Most infected females had very few previtellogenic oocytes and no vitellogenic oocytes were observed

Table 3 *G. subaraneosa*. Nested analysis of variance of intra- and inter-sample comparison of oocyte sizes. Variance components and percentage of variance are also explained for each source of variation

Source	*df*	MS	*F*ratio or (*F'*)	Variance components	Percentage of total
Samples	3	664.80	39.70 (37.07*)[a]	1.43	29.64 ± 1.19
Individuals	20	16.75	5.18*	0.16	3.34 ± 0.40
Residuals	2,028	3.23		3.23	67.02 ± 1.80
Total	2,051			4.82	±1.85

*$P < 0.001$
[a] Correction for unequal sample size

(Fig. 4E, F). Similarly, males showed anomalous shorter sperm (Fig. 4D). The infestation of the mantle cavity and gonads by parasites appear to disrupt deeply and even to inhibit the processes of gamete development in both males and females of the population studied.

Discussion

The reproductive biology and life-history strategies of most deep-sea species are poorly understood (Young 2003). The oogenesis of *A. acutecostata* and *G. subaraneosa* is described for the first time. The two species are dioecious with a quasi-continuous pattern of reproduction. The gonads were located on the upper part of the body whorl overlying the digestive gland. Histological studies showed that oocyte maturation processes in both species was similar to that observed in other deep-sea gastropods (e.g. Rex et al. 1979; Colman et al. 1986; Colman 1987).

Oogonia proliferated in the germinal epithelium and developed into previtellogenic oocytes, which grew into vitellogenic primary oocytes. These previtellogenic oocytes grew to around 30–40 μm before undergoing vitellogenesis. Vitellogenic oocytes were covered by a thin layer of follicle cells involved in the vitellogenic processes. The maximum size of vitellogenic oocytes was smaller in *A. acutecostata* (~90 μm) than in *G. subaraneosa* (~115 μm). All stages of spermatogenesis were found in all mature males, indicating a continuous production of sperm throughout the year. Spermatogonia rose from the germinal epithelium and developed into spermatocytes that differentiated into spermatids. The mature spermatozoa lay free in the lumen of the testis in both species. In some females of *G. subaraneosa* there was evidence of storage of sperm in the gonadal duct.

Furthermore, the population of *G. subaraneosa* collected at 2,700 m showed evidence of parasitic infestation, with disruption of gametogenesis. Individuals from this sample presented a very high percentage (80%) of previtellogenic oocytes (< 30 μm) and very few vitellogenic oocytes. The effects of parasites on different invertebrate species are very variable (Barber 1996; Gorbushin and Levakin 1999; Tetreault et al. 2000; Bollache et al. 2002), but most studies have shown that parasites may prevent or delay gametogenesis in molluscs (e.g. Barber 1996; Tetreault et al. 2000). For example, infected females had a higher proportion of oocytes that failed to reach complete maturity and also presented altered structures, i.e. gonadal tissue, that suggest major disruption to vitellogenetic processes. Similarly, the sperm of infected males seemed abnormal and shorter in length, indicating disrupted spermatogenesis.

The oocyte size-frequency diagrams suggest quasi-continuous reproduction for both species, but with differences in spawning events. In the case of *G. subaraneosa*, results should be interpreted cautiously because of the low number of samples available. In most individuals of the two species, all stages of developing oocytes, from oogonia to mature vitellogenic oocytes were present in the gonad at a single time. In *A. acutecostata*, the ovary volume was mainly occupied by previtellogenic and early vitellogenic oocytes (30–50 μm) at any time and there was a low percentage ($< 5\%$) of oocytes in the larger size classes (80–100 μm). This quasi-unimodal distribution (see Fig. 3) suggests a continuous and slow maturation of a small number of oocytes throughout the year. In contrast, most females of *G. subaraneosa* (but see sample collected at the 2,700-m station) showed a bimodal distribution corresponding to two different cohorts of developing oocytes, with a first cohort of previtellogenic oocytes followed by a cohort of vitellogenic oocytes and the presence of oocytes (5–10%) in the largest size classes (120 μm). This bimodal distribution of oocyte sizes, together with the presence of two cohorts of developing oocytes in most of specimens, suggests lack of synchrony in reproduction for the population as a whole (see Fig. 5) and a periodic release of mature gametes. Both strategies of reproduction are asynchronous, with no seasonality in the oocyte development or spawning events. These quasi-continuous patterns of reproduction are widespread in deep-sea fauna, where the environment is vast and relatively constant (Young 2003). The presence of mature gametes in the gonads at any one time would be beneficial in environments where population density is low, since it would increase the chance of encounters between mature males and females and therefore enhance fertilisation success (Rokop 1979). *A. acutecostata* and *G. subaraneosa* inhabit similar environments and are often collected together in the Porcupine Seabight. The differences observed in the oogenesis patterns and overall egg size might be related to the phylogenetic history of each species as shown in other deep-sea species (e.g. Bouchet and Warén 1994; Giangrande 1997; Eckelbarger and Young 1999; Eckelbarger et al. 2001).

Reproduction in deep-sea molluscs shows a wide variety of patterns (Young 2003). Some bivalve species from the North Atlantic such as *Ledella pustulosa* and *Yoldiella jeffreysi*(Tyler et al. 1992), and from hydrothermal vents and cold seeps such as *Calyptogena kilmeri* and *Bathymodiolus childressi* (Young 2003), present a seasonal pattern of reproduction. In contrast, a few studies conducted on gastropods from the North Atlantic (Rex et al. 1979; Colman et al. 1986; Colman 1987) show a continuous pattern of egg-production. Continuous reproduction is the most common strategy in deep-sea fauna and is also widespread within molluscs (see Young 2003). Furthermore, variability in patterns of gonad and egg production within species is very common in the deep sea (e.g. Rex et al. 1979; Tyler et al. 1992; Ramirez-Llodra et al. 2002) and has been related to food supply, mainly quality and/or quantity of food (Tyler et al. 1982; Tyler et al. 1992; Levin et al. 1994; Ramirez-Llodra et al. 2000; Ramirez-Llodra 2002; Niehoff 2003). Several studies have shown that, apart from environmental factors such as food availability and

temperature, genetic control also plays a very important role in shaping intra-specific differences in patterns of reproduction of marine species (Barnes and Barnes 1976; Tyler and Sumpter 1996; Sato 1999). Intra-sample variation in both species could be a bias caused by sampling methods. The epibenthic sledge is usually towed for about 1 km and individuals from the same sample will come from potentially different patches of habitat, i.e. different food supply, different predation pressure. Intra-sample variability could also reflect difficulties in the preserving and histological processes, caused by the small size of the individuals and the relative large size and small number of vitellogenic oocytes in *G. subaraneosa*.

The predominant mode of larval development of some deep-sea molluscs such as bivalves at both bathyal and abyssal depths appears to be lecithotrophy, where the larval phase is only sustained by the energy reserves that were accumulated in the egg during vitellogenesis (Scheltema 1994). Recent studies show, however, a high incidence of species with planktotrophic development within gastropods, such as some turrids (for review, see Rex and Warén 1982; Colman et al. 1986; Scheltema 1994). Rex and Warén (1982) indicated that planktotrophic development in gastropods increases with depth in the western North Atlantic. Although prosobranchs show no clear correlation between egg size and type of larva, the morphology of larval shells of *A. acutecostata* and *G. subaraneosa* (Bouchet 1977; Bouchet and Warén 1985; Colman et al. 1986), together with the small oocyte sizes (< 115 μm), suggest that both species undergo planktotrophic larval development. Rex and Warén (1982) suggested that low vagility of adults and feeding strategies such as predation, might favour planktotrophy in deep-sea gastropods. Mode of larval development has important consequences for the biogeography and evolution of deep-sea molluscs, i.e. bivalves and gastropods (Bouchet and Warén 1994; Scheltema 1994). Species with lecithotrophic development usually have a lower dispersal potential than species with planktotrophic larvae. The latter show little morphological variation, and have high degree of genetic homogeneity and broad geographic and stratigraphic ranges. In fact, both species were very common in the northeast Atlantic (Bouchet and Warén 1980, 1985) and were found across a wide bathymetric range.

In summary, the present study has revealed several previously unknown aspects of the reproductive biology of *A. acutecostata* and *G. subaraneosa*. Both species share some common patterns such as a continuous production of gametes and small egg sizes, but also differ in aspects such as patterns of gametogenesis, oocyte size distribution and maximum egg size. Phylogenetic history could play an important role in the reproductive biology of both species, being the ultimate cause of gametogenetic pathways during vitellogenesis. Variability within each species might reflect temporal and/or spatial variability in the habitat.

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