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Reproductive biology, sexual dimorphism, and population structure of the deep sea hydrothermal vent scale-worm, *Branchipolynoe seepensis* (Polychaeta: Polynoidae)

D. Jollivet*§, A. Empis*, M.C. Baker†, S. Hourdez*‡, T. Comtet†∫, C. Jouin-Toulmond*, D. Desbruyères∫ and P.A. Tyler†

*Station Biologique de Roscoff (UPMC-CNRS-INSU), BP 74, Place Georges Teissier, 29682, Roscoff cedex, France. †School of Ocean and Earth Science, University of Southampton (SOC), Southampton, SO14 3ZH. ‡Present address: Department of Biology, 208 Mueller Laboratory, Pennsylvania State University, University Park, PA 16802, USA. ∫IFREMER, Centre de Brest, DRO/EP, BP 70, 29280 Plouzané, France. §E-mail: jollivet@sb-roscoff.fr

The polychaete family Polynoidae (scale-worms) is well-represented at deep sea hydrothermal vents. Most species are free-living in a wide range of habitats: from high-temperature hydrothermal 'chimney' walls to diffuse venting areas. Conversely, species of the genus *Branchipolynoe* live inside the mantle cavity of vent and seep mytilids. Specimens, morphologically close to *Branchipolynoe seepensis*, have been reported from all the known vent areas on the Mid-Atlantic Ridge (MAR), with varying infestation rates (0–6 individuals per host). Reproductive tract, gametogenesis and population structures were examined for specimens from the Lucky Strike vent field (MAR) in order to test whether this species displays dwarf males, protandric hermaphroditism or differential mortality between males and females. Observations of histological sections reveal the presence of fully developed ovaries in females which originate ventrally in segments 7–9 and of an unusual genital tract in which both sperm and mature oocytes are stored. Oogenesis is intraovarian and quasi-continuous. The vitellogenic oocytes are only free in the coelom at their terminal growing stage and are then transferred into an ovisac through spermathecae. The species displays an external sexual dimorphism in the number of genital papillae and the shape of the pygidial appendages. Sex ratios showed significant deviations from a 1:1 expected ratio, in favour of females. The modal decompositions of size–frequency histograms show the occurrence of three modes in females and only two modes in males, indicating discrete breeding periods. The two first modes were not significantly different between males and females. These results indicate that *B. seepensis* forms heterosexual pairs and uses internal fertilization to reproduce during discrete spawning periods. Differential mortality between males and females is likely to shape size-histograms as observed by preventing males from reaching the female proportions. Such an observation could be a result of either cannibalism on larger males, small sizes facilitating the male escape, or natural predation when males move from one bivalve to another to breed.

INTRODUCTION

Polychaetes have the most diverse gametogenic mechanism of any class of animals and has been intensively studied in shallow-water species (Wilson, 1991; Giangrande, 1997). In contrast, the reproductive biology of polychaetes inhabiting deep sea environments, with few exceptions (e.g. Emson et al., 1993), has received relatively little attention. Polychaetes represent a significant component of the deep sea hydrothermal vent fauna and dominate the biological assemblages at most sites studied, particularly along the East Pacific Rise and the Juan de Fuca Ridge (Desbruyères et al., 1985; Tunnicliffe, 1991). These peculiar worms experience harsh environmental conditions and tremendous variations of venting with time (Johnson et al., 1988; Jollivet, 1993; Shank et al., 1998). However, it is not known yet whether such an unpredictable habitat may select typical reproductive features maximizing the colonization process.

In the eastern and north-eastern Pacific, the most documented and dominant polychaetes are species from the family Alvinellidae. McHugh (1989) compared the reproductive biology and population structures of *Paralvinella pandorae pandorae*, Desbruyères & Laubier and *Paralvinella palmiformis*, Desbruyères & Laubier from the Juan de Fuca Ridge (JdF). Additional studies have since been carried out on the reproductive biology of other alvinellid polychaetes (Zal et al., 1994, 1995; McHugh, 1995; Chevaldonnè, 1996; Jouin-Toulmond et al., 1997; Desbruyères et al., 1998; Copley, 1998) which showed important reproductive similarities between species (sexual dimorphism, sex ratio (1:1), gamete development, egg size and the fertilization mode) and profound differences in fecundity and sperm morphology. Such reproductive characteristics were compared by Tyler & Young (1999) and may suggest that alvinellid polychaetes have adapted to the unstable and harsh vent conditions by increasing their reproduction success using sperm storage and internal fertilization.

Another well-represented polychaete family at deep sea hydrothermal vents is the Polynoidae (scale-worms). Most species are free-living in habitats ranging from

high-temperature hydrothermal 'chimney' walls to diffuse venting areas (Tunnicliffe, 1991). In contrast to free-living counterparts, species of the genus *Branchipolynoe* Pettibone, 1984 live inside the mantle cavity of vent and seep mytilids (Pettibone, 1984, 1986; Miura & Hashimoto, 1991). The true nature of this association is still uncertain. However, the mytilid *Bathymodiolus* sp. could represent protection against numerous predators and *Branchipolynoe* species have therefore been classified as commensals.

Only three species of *Branchipolynoe* have been described to date, each one being found in a different biogeographic province: *Branchipolynoe symmytilida* Pettibone, 1984 in the eastern Pacific, *Branchipolynoe seepensis* Pettibone, 1986 in the Gulf of Mexico and *Branchipolynoe pettiboneae* Miura & Hashimoto, 1991 in the western Pacific. In addition, specimens, morphologically close to *B. seepensis*, have been reported from all the known vent areas of the Mid-Atlantic Ridge (MAR), with infestation rates varying from 0–6 individuals per host. This includes specimens from the Lucky Strike hydrothermal vent field. Although genetic differences exist between the mid-Atlantic specimens and true holotypes of *B. seepensis* from the Gulf of Mexico, the divergence seems to occur at the subspecies level (Chevaldonné et al., 1998; Jollivet et al., 1998). As a consequence, *B. seepensis* is still assigned to the mid-Atlantic specimens in this study. This small level of genetic divergence raises questions about how the worm reproduces and colonizes the vent habitat. The life cycle of these polynoid polychaetes is not very well known and their reproductive strategy has not been assessed, though there are a few preliminary observations on species from the East Pacific Rise and the MAR (Jollivet, 1996). Females produce limited numbers of very large eggs (400–500 μm diameter) indicative of lecithotrophy or possibly direct development (Jollivet, 1996; Jollivet et al., 1998). Conversely, DNA sequence data do not reveal any obvious genetic divergence between specimens collected along the MAR (Chevaldonné et al., 1998; Jollivet et al., 1998). In addition, the large specimens collected to date were all mature females; the presence of very few smaller males (S. Hourdez, personal observation) suggested sexually-related size differences and a male deficit that could indicate protandric hermaphroditism. The aim of our study was to describe the reproductive tract and gametogenesis and to analyse population structures of the deep sea vent polynoid *B. seepensis* from the Lucky Strike vent field (37°17′N/MAR) in order to test whether the occurrence of a few dwarf-males and numerous large females could rely on dwarfism or protandry hypotheses and whether direct development naturally occurs in this commensal species.

MATERIALS AND METHODS

Sampling

Specimens of *Branchipolynoe seepensis* were collected using the manned submersible 'Nautile' at the Lucky Strike vent field (Azores Triple Junction/MAR) during the June–July 1994 DIVA 2 and the August–September 1997 MARVEL cruises. A large number of mytilids (*Bathymodiolus* sp.) form dense beds on diffuse venting areas of the sites Bairro Alto (32°17.00′ W–37°17.37′ N; 1630 m) and Eiffel Tower (32°16.30′ W–37°17.22′ N; 1685 m). Samples were collected using a telemanipulated arm and brought back to the surface in an insulated basket. Individuals of *Branchipolynoe* were removed from freshly collected mytilids and, together with ones that had left their hosts, were fixed with 3–10% neutral formalin in seawater. Specimens for population analyses were transferred into 70% ethanol, whereas those used for reproduction studies were kept in formalin. To investigate size relationships between the scale-worm and its host, the greatest antero–posterior length of the shell of freshly collected worm-infested mytilids (single subsample: site Bairro Alto), and its associated *Branchipolynoe*, were measured to the nearest 0.01 mm using calipers.

Genital tract and gametogenesis

To describe the genital tract, dissections and histological sections of mature specimens were made. Paraffin transverse and sagittal 5 μm thick sections of chaetigers S7–S15, were stained using the trichrome one-step method (Gabe, 1968, p. 230) and subsequently observed with a Leitz microscope. Photographs were taken using a photoautomat. The gametogenic biology of 11 individuals from DIVA 2 and 39 individuals from MARVEL was examined. Ten of the larger specimens from each cruise collection (DIVA 2 or MARVEL), assumed to be female were bisected longitudinally from dorsal to ventral side. The half worm was processed through graded alcohols, dehydrated in absolute propan-2-ol, cleared in Histoclear™ and embedded in paraffin wax. Thin sections (7 μm) were cut, mounted, dried and stained with haemalum and eosin. Sections were observed under an Olympus BH 2 compound microscope fitted with a video camera. Images of the gonad were captured on video using a frame grabber and stored as .BMP files. The files were imported into SigmaScanPro. Outlines of oocytes containing nucleoli were traced using the mouse and the circumference was flood filled in order to automatically determine the oocyte area and calculate the ferret diameter (i.e. the diameter the oocyte would be if it were perfectly round). This method allows packed, irregularly shaped oocytes to be quantified. Sample sizes of measurable oocytes varied from 74 to 213 oocytes per individual. Oocyte size–frequency histograms were constructed using a 25 μm size-class intervals. Oocyte size–frequency distributions were compared between females using a Kolmogorov–Smirnov two-sample test. In addition, a multisample Kruskal–Wallis test was done to test whether groups of oocytes differ in 'location'.

Sexual dimorphism

The entire size range of formalin-preserved specimens was examined in order to seek morphological traits indicative of sexual dimorphism and to determine the size at which juveniles become sexually mature. Since external sexual dimorphism was already suspected, 76 specimens of various sizes were dissected and observed under a binocular microscope to be sexed by examination of both the genital tract and the gametes. The total length of each worm was measured ventrally to the nearest 0.01 mm from the anterior part of the prostomium (evaginated

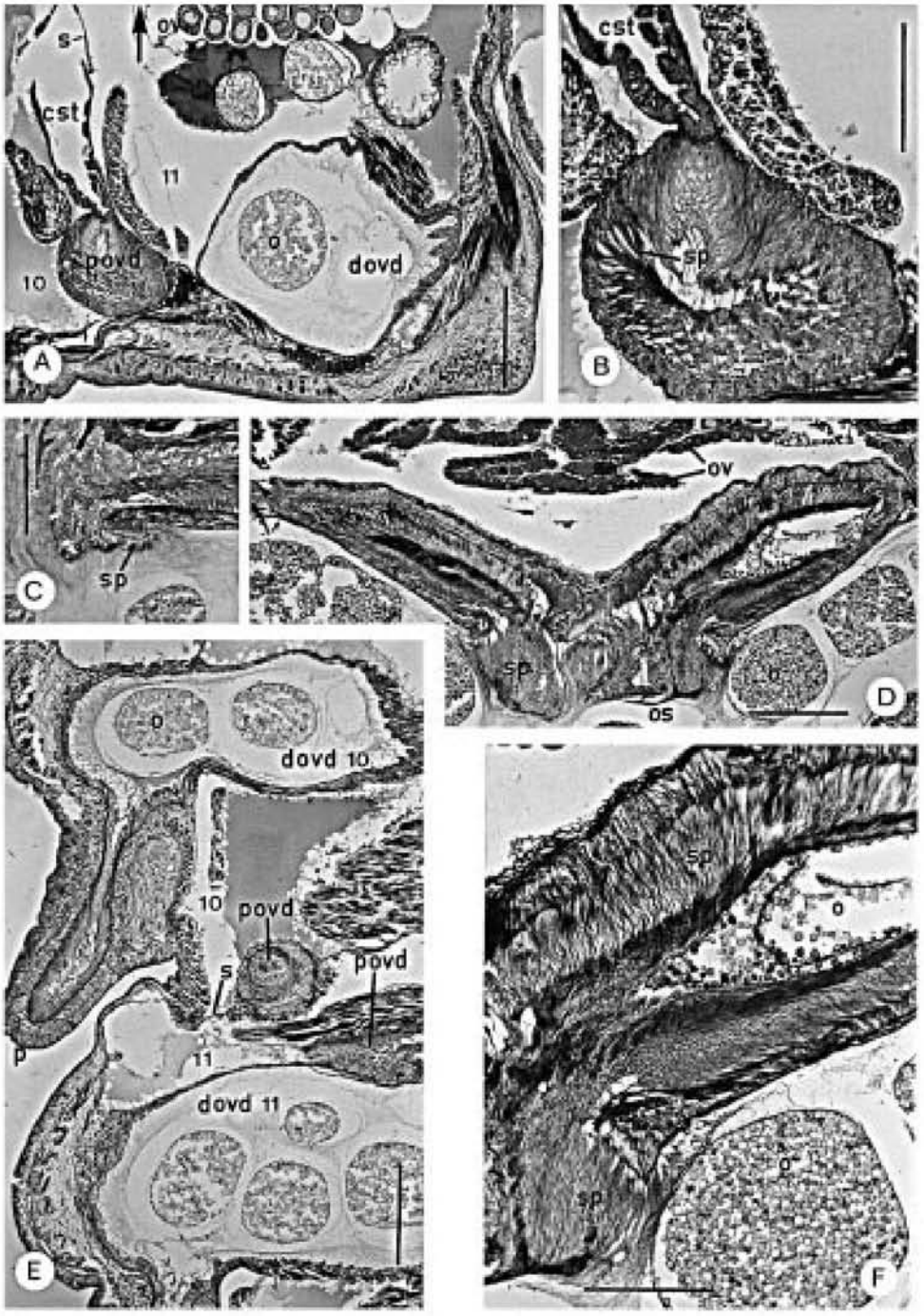

Figure 1. Female genital tract of *Branchipolynoe seepensis*. Parasagittal sections (A, B); frontal sections (C–F). (A) Section through chaetigers 10 and 11 showing, in the posterior part of S10, the coelomostome (cst) along the septum (s) and the proximal oviduct–spermatheca (povd), and in S11 the distal oviduct (dovd). (o) Oocyte, (ov) ovary, (arrow) points to dorsal part. (B) Detail showing sperm (sp) attached to the oviduct–spermathecal wall. (C) Opening of an oviduct–spermatheca into the ovisac, (sp) sperm attached to the ovisac wall. (D) Openings of the two oviduct–spermathecae into the ovisac (os) containing mature oocytes (o); (ov) ovary. (E) Chaetiger 10 with external papilla (p) connected to the distal oviduct (dovd10) and in its posterior part, the next proximal oviduct–spermatheca (povd) along the septum (s), prolonged in S11, close to the distal oviduct (dovd11). (F) Detail of a proximal oviduct–spermatheca, filled with abundant sperm (sp) attached to its walls, and showing an oocyte (o) crossing its lumen. Scales bars: A, C, D, E, 400 μm; B, F, 200 μm.

proboscis excluded) to the anus (pygidial appendages excluded) using an ocular micrometer in a binocular microscope. In addition, chaetigers were counted on each individual in order to establish whether the worm had reached its final number of chaetigers (i.e. a fully developed worm has 20 chaetigers). Small juveniles were progressively dehydrated in an ethanol series, dried at the critical point, mounted, gold-coated and examined under a JEOL JSM 5200 scanning electron microscope.

Size–frequency histograms and sex ratio

Sampling areas were selected *in situ* to take into account the spatial heterogeneity of the mytilid size distribution,

some sites being dominated by small mussels others by large ones (see Comtet & Desbruyères, 1998). Mytilid samples consisted of thousands of mussels of all the size-classes. As a result, a stratified subsampling was performed according to Frontier (1983). *Bathymodiolus* specimens were measured, sorted out and subdivided into size groups as previously (Comtet & Desbruyères, 1998). A proportionate number of *Bathymodiolus* specimens were opened in each size group, and carefully examined under a binocular microscope in order to collect all the scale-worms until 150 had been found. Polychaetes were sexed according to two criteria: (1) the number of elongated 'nephridial' (actually genital papillae); and (2) the morphology of the pre-pygidial and pygidial appendages. Deviations from the 1:1 expected sex ratio (*So*) were estimated following the formula of Christiansen et al. (1990): $So=(Mo-Fo)/(Mo+Fo)$ in which *Mo* is the total number of males and *Fo* is the total number of females. The sex ratios of samples were compared to the expected 1:1 sex ratio using a two-tailed χ^2 goodness of fit test. The total body length was also measured on each individual, following protocols described in the previous paragraph, in order to analyse the population structures. Size–frequency histograms were plotted using size-class intervals of 2 mm. This interval was chosen according to three criteria: (1) size-classes must have at least five individuals; (2) the number of adjacent empty classes must be minimized; and (3) the interval has to be much greater than the error of measurement. All size–frequency histograms were then smoothed using a weighted moving average at the third order to rule out spurious peaks (Frontier & Pichod Viale, 1991; Zal et al., 1995). Modal decompositions were first assessed according to the method of Ghéno & Le Guen (1968). This allows us to obtain a rough number of modes and their approximate position in the size distribution. Taking these data as an input, a fine-tuned decomposition was then performed using the MIX 2.3 program package (MacDonald & Pitcher, 1979; MacDonald & Green, 1986), assuming that sizes of *B. seepensis* follow a Gaussian distribution within each cohort. This iterative method uses the maximum likelihood criteria to provide the best mathematical fit between a theoretical mixture of normal distributions and the observed one and was preferred to other methods (such as those developed by Cassie/Harding and/or Bhattacharya) which tend to overestimate the number of modes (MacDonald & Pitcher, 1979). Size–frequency distributions were compared to a normal distribution using a Kolmogorov–Smirnov one-sample test. The overall and the pairwise comparisons of means were also performed either between males and females, locations and collection dates of a given population using both a one-way ANOVA test and a Student's *t*-test (Sokal & Rohlf, 1981). To perform these tests, mode of a given i^{th} size group (i.e. the i^{th} 'cohort') becomes the mean to be tested and is cross-compared with other ones using the standard deviations already provided by MIX 2.3.

RESULTS

The female genital tract

Observation of sagittal sections of females from *Branchipolynoe seepensis* reveals the presence of three ovaries which originate ventrally in segments 7–9. Oogenesis is intra-ovarian and seems to be quasi-continuous. The vitellogenic oocytes are only free in the coelom at their terminal growing stage.

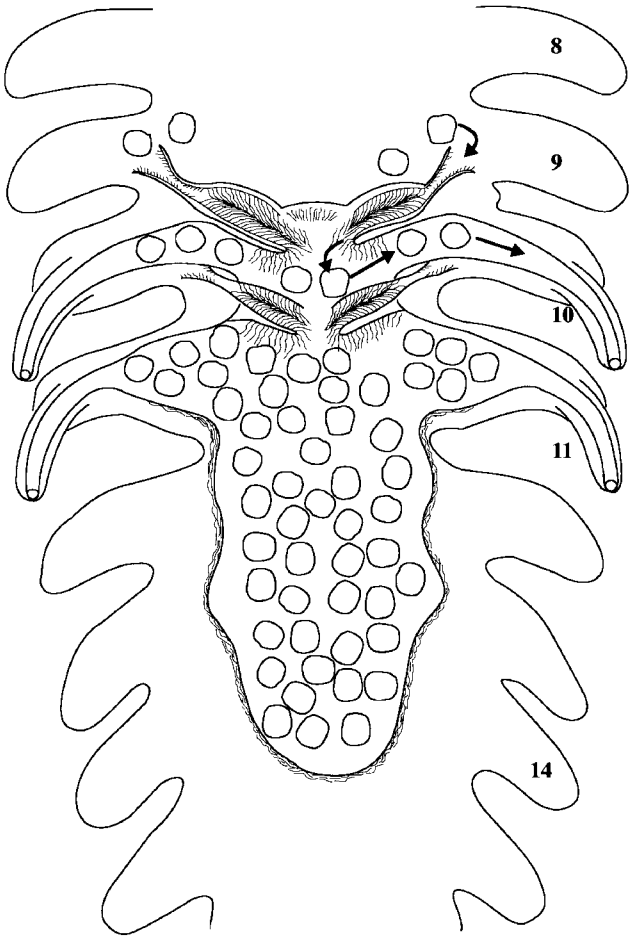

Figure 2. Semidiagrammatic representation of a frontal section of a *Branchipolynoe seepensis* female, through chaetigers 8–14. (Not to scale.) The genital tract is partly shown (ovaries not represented). Two pairs of oviducts in S10 and S11 are connected to a large median ovisac in which mature oocytes accumulate before spawning. The proximal parts of the oviducts (povd) are transformed in spermathecae: they both transfer the mature oocytes, from the coelom into the ovisac, and store the sperm. The distal oviducts (dovd) open to the outside at the genital pore openings located at the end of the genital papillae on S10 and S11. They are spawning organs (fertilized oocytes) and also transfer the sperm, released in bundles by the male at the female genital pore, up to the spermathecae.

Coelomostomes and proximal parts of the oviduct

The large vitellogenic oocytes, once free in the coelom, are collected by two pairs of ciliated coelomostomes located ventro–laterally in the posterior part of chaetigers S9 and S10 (Figure 1A,B). These coelomostomes extend along the posterior septa of S9 and S10, which are also ciliated. Following the coelomostomes and crossing the septa, there are two pairs of oviducts (called proximal oviducts), which drive the oocytes in the next part of the genital tract.

Spermathecae

The proximal oviducts are enlarged and form conspicuous spermathecae, full of sperm (Figures 1 & 2). These

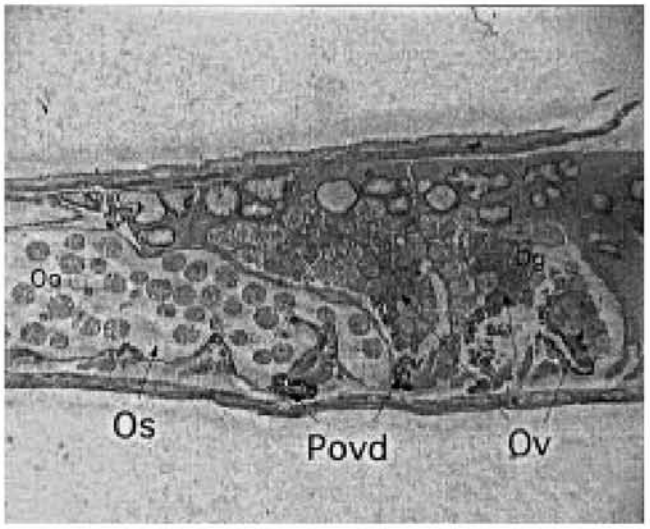

Figure 3. Female genital tract of *Branchipolynoe seepensis*: longitudinal section of the ovisac (os) containing mature oocytes (oo), ovaries (ov) containing developing oocytes (og: from ovogonia towards mature oocytes) and spermathecae (povd).

seminal receptacles have a reduced lumen, through which the oocytes are transferred from the coelomostome to the next part of the tract (Figure 1F). The spermathecae have a thick wall, in which spermatozoa are inserted by their head, while their long flagella are free in the lumen of the organ (Figure 1F). In segment 10, the two 'oviduct–spermathecae' merge into a large central cavity, here called the ovisac; the same occurs in S11 where the two other 'oviduct–spermathecae' merge also with the ovisac (Figure 2).

The ovisac

This unpaired and median cavity extends longitudinally from S10 to S14, and from the ventral part of the body to the dorsal part, leaving only a small longitudinal space for the digestive tract. The ovisac is full of mature oocytes (reaching at most, 400 μm in diameter). This specific and unusual organ receives and stores the large vitellogenic oocytes, driven from the coelom through the coelomostomes and the 'oviduct–spermathecae', and the spermatozoa which are abundant and also implanted at the surface of the ovisac lumen where the two pairs of spermathecae open (Figures 1 & 2).

The distal oviducts

Laterally, within S10 and S11, two pairs of ducts with a rather large lumen, here called the distal oviducts, penetrate into the two pairs of ventral genital papillae (Figure 1E). Large oocytes can be seen laterally in the ovisac, approaching the distal oviduct lumen.

Sperm transfer

The spermathecae do not have their own external opening. As a consequence, the huge number of spermatozoa, inserted into the epithelium of both the spermathecae and the ovisac, must have to be transferred through the female genital papillae at the end of which bundles of spermatozoa have been observed. So, the sperm invade the anterior part of the ovisac and accumulate in the four spermathecae.

Spawning and fertilization

Although spermatozoa are abundant all around the mature oocytes, in the spermathecae and the ovisac, no fertilized oocytes have been observed in the female genital tract. We infer from this that fertilization occurs just at spawning, when the oocytes are expelled from the ovisac and the sperm detach from the ovisac and spermatheca walls (see schematic view of the genital tract in Figure 2).

Gametogenesis

In *B. seepensis*, numerous oocytes of various sizes develop in ovaries in segments 7–9 (Figure 3). The sizes of mature oocytes found inside the ovisac are large, up to 400 μm. Fecundity is low (100–300 mature oocytes per female). Initial observations suggest that there are distinct cohorts of oocytes in each ovary. The oocyte size–frequency data show that, in most females, there is a distinct cohort of oocytes with a diameter of less than 125 μm (Figure 4). In all the females examined there was evidence of at least one, if not two, smaller cohorts of larger oocytes. Although not consistent, this second cohort had oocyte diameters of 300–350 μm in the females examined. Conversion of these oocyte diameter/frequency data to volume demonstrate that the larger oocytes form a significant volume of the gametogenic output. There was no significant difference in the oocyte size between individuals from a single site and between individuals from different sites or from different cruises (MARVEL samples: Kruskal–Wallis H^* statistic $=7.55$ with 9 df, $P=0.5805$; DIVA-2 samples: $H^*=3.46$ with 9 df, $P=0.9431$). However, equality of the oocyte size–frequency distributions was rejected since 25–30% of Kolmogorovov–Smirnov two-samples test indicated significant differences between females within each cruise (DIVA-2, 45 tests, 29% of pairwise combinations are highly significant ($\alpha=0.01$); MARVEL, 45 tests, 13% and 31% of pairwise combinations are significant ($\alpha=0.05$) and highly significant ($\alpha=0.01$), respectively). This suggests that gametogenesis is not totally synchronized at the population level.

Sexual dimorphism, sex ratio and size at first reproduction

Morphological characters of *B. seepensis* were examined on 76 individuals with total length ranging from 800 to 24 μm. This sample was subdivided into 47 fully developed adults (19 females and 28 males) and 29 juveniles for which the number of chaetigers varied from 10 (Figure 5A) to 16 (Figure 5B). In most juveniles, the morphology was similar to that of adults, but pygidial appendages and genital papillae were frequently missing. The largest juveniles (total length: 5–8 mm) display early stages of papillae on chaetigers S10 and/or S11 (Figure 5C). All the adults exhibit either one or two pairs of elongated genital papillae which are present latero–ventrally on S11 or both on S10 and S11 (Figure 6A,B). These two categories of adults also display two different morphologies of the two last body segments (parapodial cirri) and pygidial appendages. In specimens of type A, parapodial cirri are hypertrophied and together with the pygidial appendages are curved, whereas in other specimens (type B), the two last parapodial cirri and the

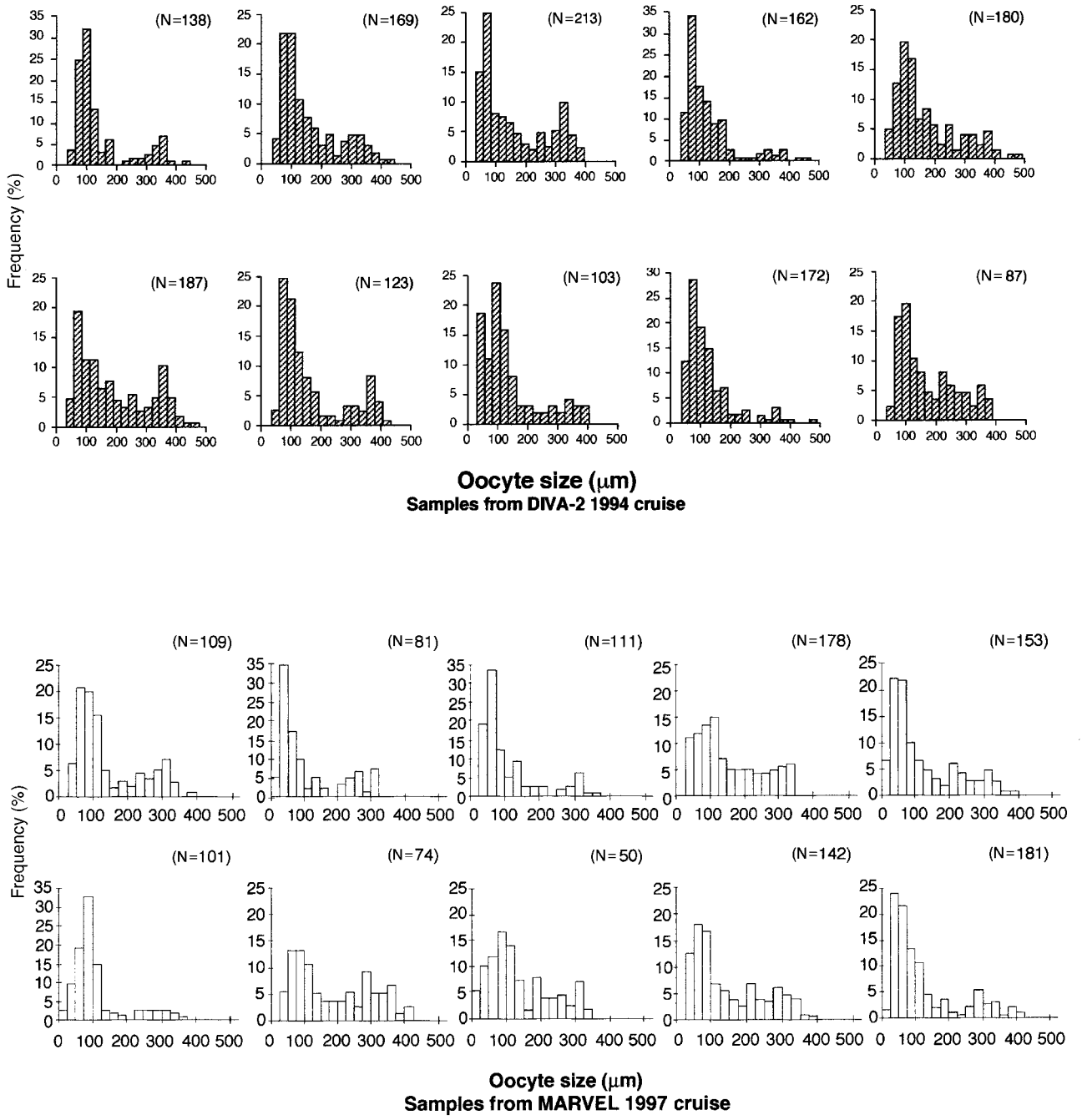

Figure 4. Oocyte size frequency histograms obtained from longitudinally-bisected individuals collected during the 1994 DIVA-2 (A) and the 1997 MARVEL (B) cruises (ten females per cruise).

pygidial appendages are straight and shorter than type A (Figure 6C,D). A subsample of 50 specimens exhibiting both papillae and typical posterior bodyparts (types A or B) were dissected in order to sex them according to gonoducts. This analysis showed, in all cases, that: (1) individuals which display one pair of papillae on S11 possess a pygidium of type A and are male; (2) individuals, which display two pairs of papillae on S10 and S11, possess a pygidium of type B and are female.

The examination of gonoducts allowed us to distinguish several stages of maturation both for males and females (see legend of Figure 7). In males, four stages could be distinguished whereas in females two stages were identified (Figure 7). The frequency of each stage was estimated for each 1 mm size-class over the size range of subsampled individuals. Males become mature at a size of 8 mm (Figure 7A) whereas females mature at a size of 14 mm (Figure 7B). Although oocytes are developing in

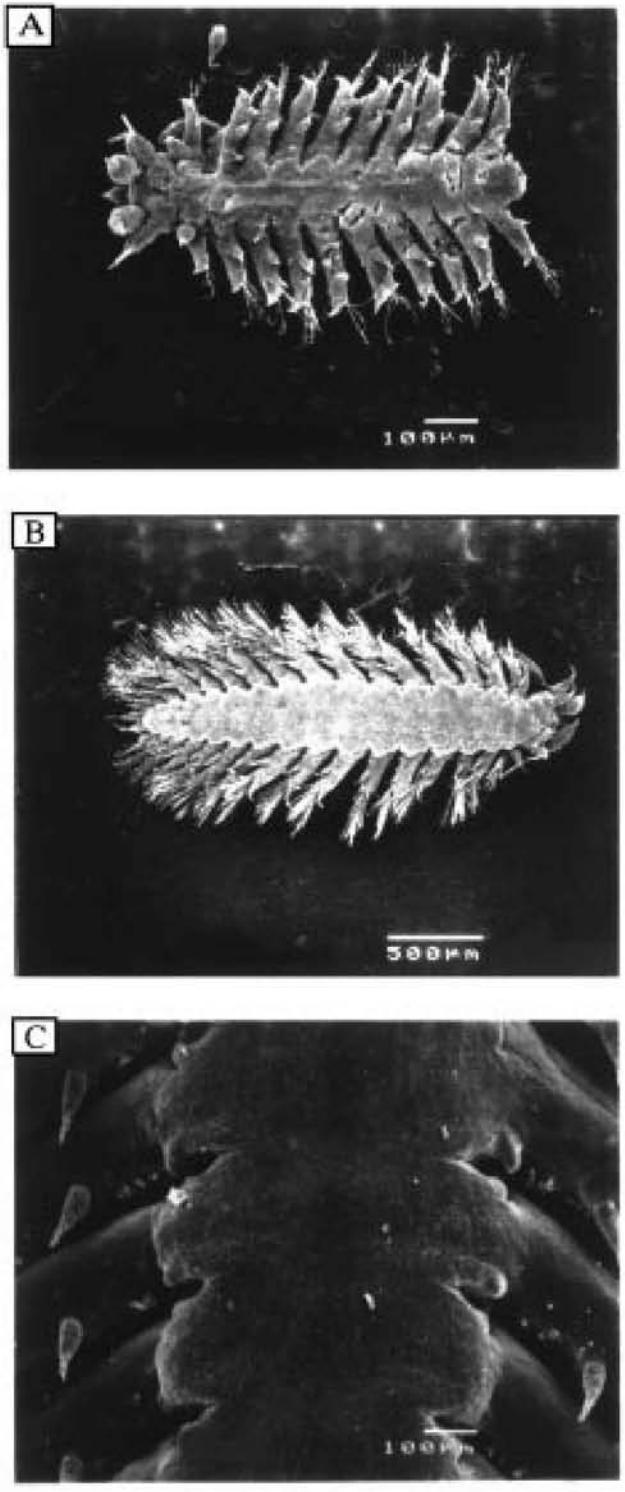

Figure 5. Scanning electron microscope views of *Branchipolynoe seepensis* not fully developed juveniles. (A) Ventral view of a juvenile with 10 chaetigers; (B) ventral view of a juvenile with 16 chaetigers; and (C) early stage of papillae on S10 and S11 on a young female of 18 chaetigers.

females of 14–16 mm, histological sections showed that the ovisac is a thin structure containing no oocytes and not yet functional.

Sex ratio deviations (*So*) estimated from four samples collected during DIVA-2 and MARVEL at Eiffel Tower and Bairro Alto are presented in Table 1. All the sex ratios showed significant deviations from a 1:1 expected ratio ($P(\chi^2)<0.01$ in most cases). This deviation varies greatly between samples but is always in favour of females (male-biased sex ratio). The overall *So* obtained as an average of the four samples is also significant when compared to the expected one using Student's *t*-test ($P<0.05$).

Size–frequency histograms

The demographic structure of the male and female samples of *B. seepensis* was analysed using the total length of the worm as an index of size. This measure is well-correlated with the individual wet weight of *B. seepensis* (regression coefficient: $r=0.978$, $P<0.0001$; $N=50$ individuals; Figure 8). The total length ranged from 0.8 to 33 mm. Juvenile, male and female lengths of *B. seepensis* were also compared to the lengths of their hosts (Figure 9). Graphs showed a positive relationship between the female length of *B. seepensis* and the length of *Bathymodiolus* sp. whereas no apparent correlation existed between the male or the juvenile lengths of *B. seepensis* and those of their mytilid hosts. This indicates that juveniles and males are mobile and do not exhibit any size-segregating behaviour during the infestation. Sample sizes, size range, average lengths and standard deviations are presented in Table 2. All the distributions significantly differed from the normal distribution (Kolmogorov–Smirnov one-sample test, $P<0.01$) and are assumed to be polymodal. Size–frequency histograms were plotted within sexes (Figure 10). The modal decompositions of these histograms indicate the occurrence of three modes in females (*M1*, *M2* and *M3*; Figure 10A,C,E,G) and only two modes in males (*M1* and *M2*; Figure 10B,D,F,H). These numbers of modes gave the best fits for the eight samples (Table 3). A multisample ANOVA compared data extracted from a given Gaussian component between the eight samples (*M1*s and *M2*s) and between the four female samples (*M3*s). Such a statistical analysis was performed since the position of a given mode does not vary very much between samples and the risk to confound *M1*s with *M2*s or *M2*s with *M3*s is negligible. The result of the analysis showed that highly significant differences exist between modes of each sample (*M1*: $F=23.9$, N=287 and $P<0.001$; *M2*: $F=32.9$, N=210 and $P<0.001$; *M3*: $F=12.2$, N=95 and $P<0.001$). To explain the observed difference between samples, pairwise comparisons of the modal values were performed using a Student's *t*-test according to three main null hypotheses: (*H1*) males and females do not differ in distributions; (*H2*) Eiffel Tower and Bairro Alto samples do not differ in distributions and, finally; (*H3*) samples from a given locality do not differ in distributions between 1994 and 1997. Results of these tests are summarized in Table 4.

As female distributions seem to display an additional mode when they are compared to the male distributions, a Kolmogorov–Smirnov two-samples test was performed on the overall male and female distributions. The difference $D_{obs}=0.384$ is greater than $D_{0.01}=0.113$, indicating that males and females display significantly different distributions. Conversely, most observed differences between modes are not significant (six Student's *t*-tests out of eight, $P>0.05$; Table 4). These two analyses therefore strengthen the hypothesis that the difference between

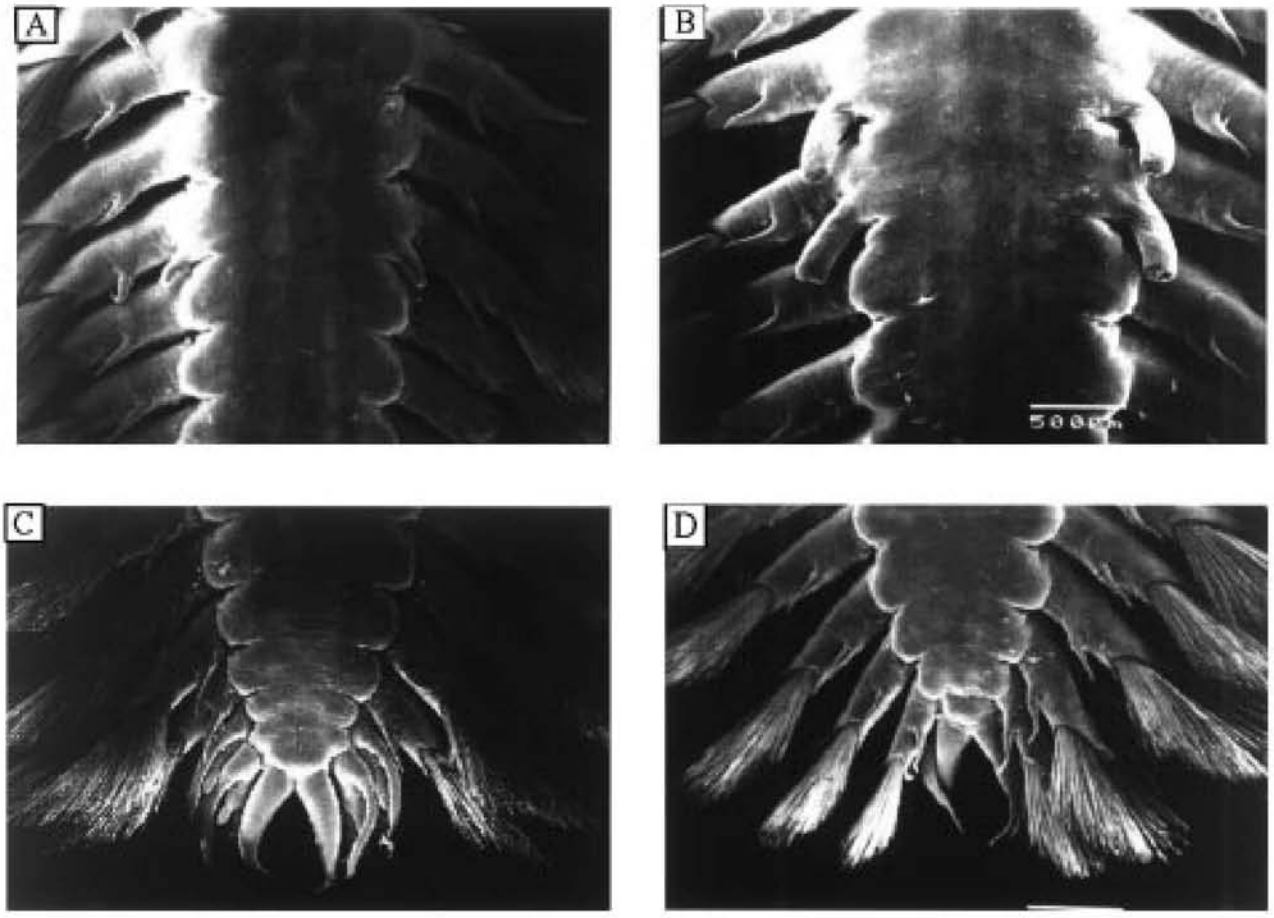

Figure 6. Scanning electron microscope views of morphological characters involved in the sexual dimorphism of *Branchipolynoe seepensis*. (A) Ventral view of the male papillae (S11); (B) ventral view of the female papillae (S10 and S11); (C) ventral view of the male pygidium; and (D) ventral view of the female pygidium.

male and female distributions is a result of the occurrence of a third mode in females.

Comparisons between the two sites Eiffel Tower and Bairro Alto showed two different patterns of spatial variation at the Lucky Strike vent field. These patterns seem to be related to the sampling date as distributions from the 1994 DIVA 2 samples do not differ from each other (all Student's *t*-tests, $P > 0.05$) whereas most of the 1997 MARVEL samples display highly significant differences between modes (three Student's *t*-tests out of five, $P < 0.001$). Comparisons between the two sampling periods (1994 and 1997) indicate that male and female distributions significantly differ between 1994 and 1997 only at the site Bairro Alto (three Student's *t*-test out of five, $P < 0.01$). The complete examination of pairwise comparisons indicates that male and female distributions from only one sample (*BA97*) lead to these significant differences.

DISCUSSION

An unusual reproductive tract for oocyte and sperm storage

Histological examination of *Branchipolynoe seepensis* showed that females display an unusual hypertrophied genital tract. The complex female genital tract from the ovary to the genital papillae (two pairs of oviducts) can be divided into four parts: coelomostome, proximal oviduct (=spermathecae), ovisac, and distal oviduct. This tract appears to be used for the storage of both mature oocytes and sperm prior to spawning. The spermathecae (two pairs) belong to a type derived from the type II (part of the oviduct) as defined by Westheide (1988). This type of seminal receptacle is known from the polychaete families Hesionidae, Histriobdellidae, Pisionidae, Sacconcirridae (see Westheide, 1988) and Alvinellidae (Zal et al., 1994; Jouin-Toulmond et al., 1997), but this is the first record of spermathecae in the family Polynoidae. In Alvinellidae, the spermathecae are located near the genital opening, in the distal part of the oviduct. By contrast, in *B. seepensis*, they are located along the genital tract, since they correspond to the anterior part of the oviduct, just following the coelomostome. This proximal oviduct, though transformed into a seminal receptacle, keeps its role in the transfer of oocytes towards the ovisac. As a consequence, mature oocytes driven by the coelomostomes have to cross the inseminated spermathecae prior to arrival in the ovisac. The sperm are always immobilized with their heads implanted in the spermathecal wall. We have never observed sperm in the coelomostomes nor in the coelomic cavity of the females.

One of the most astonishing observations in *B. seepensis* is the presence of an ovisac, an organ unique in polychaetes. It corresponds to an enlargement of the two pairs of oviducts, fused together to form a permanent, large, single cavity, in which mature oocytes are stored. The ovisac communicates

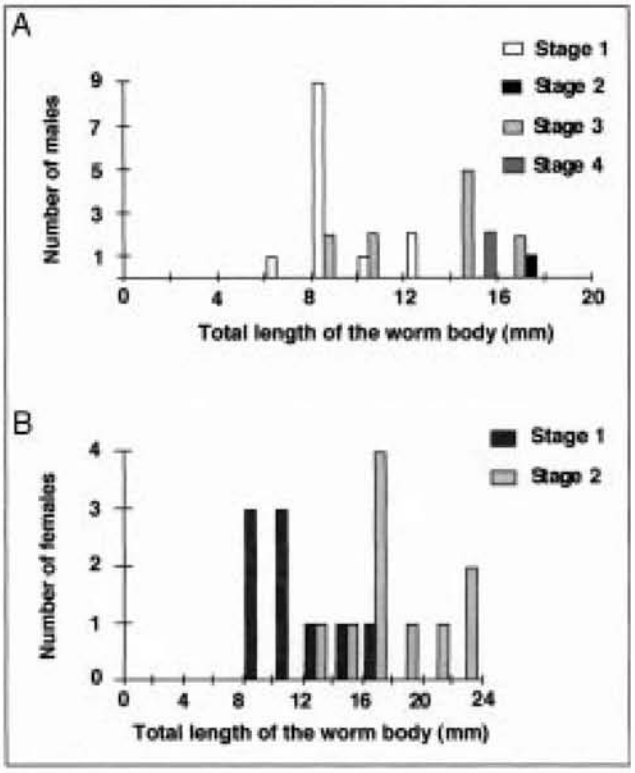

Figure 7. Distribution of the developmental stages of gonoducts for 28 small males (A) and 19 small females (B). In males, stage 1 corresponds to short, whitish and coiled spermiducts, stage 2 to short, slightly yellow coloured spermiducts with few spermatozoae, stage 3 to long, orange-yellow coloured spermiducts full of spermatozoae which extend from S11 to S16 and, stage 4 to long and whitish spermiducts without spermatozoae which extend from S11 towards S16 (sexual pause?). In females, only 2 stages are distinguished: stage 1 in which ovaries develop without oocytes and stage 2 in which a large ovisac with small to mature oocytes is present.

with the ambient seawater by two pairs of distal oviducts extending through the genital papillae and opening outside through the genital pores. The sperm transfer occurs through the genital pores: bundles of sperm, are deposited

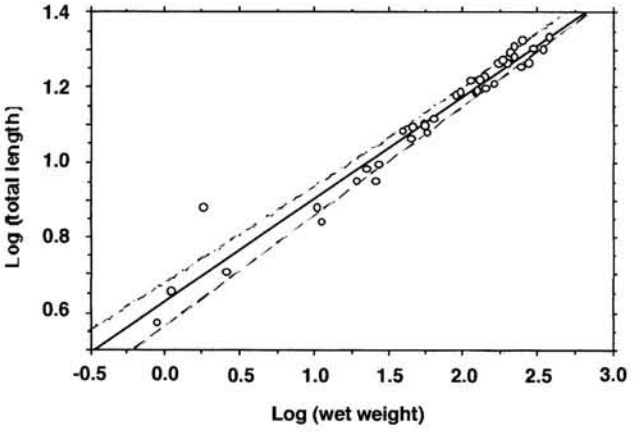

Figure 8. Relationship between the total length (L) and wet weight (W) of *Branchipolynoe seepensis*. (W=0.63+0.27L with r^2=0.956).

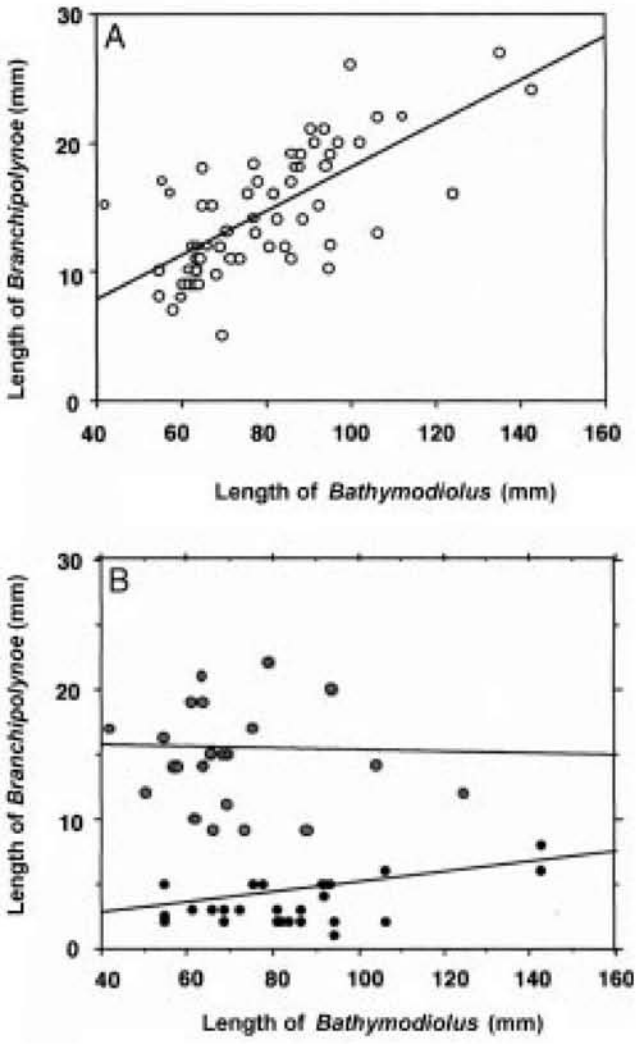

Figure 9. Relationships between total length (Y) of *Branchipolynoe seepensis* and the antero-posterior length (X) of the *Bathymodiolus* shell. (A) ○, *B. seepensis* female, Y=11.08+0.17X with r^2=0.491; (B) ○, *B. seepensis* male, Y=16.09–0.01X with r^2=0.001; ●, *B. seepensis* juvenile, Y=1.34+0.04X with r^2=0.092.

by the male on the female genital papillae. This implies a pseudocopulation process, since the males have no penis. The bundles of sperm then have to pass up from the distal oviduct to the ovisac, and then up to the spermathecae.

A related reproductive apparatus has been described in other Polynoidae. In *Harmothoe imbricata* (L.), during gamete maturation, the nephridia enlarge in the fertile region of the body and the gametes accumulate in these swollen nephridia before spawning (Daly, 1972). In the commensal scale-worm *Arctonoe vittata* (Grube), gametogenesis takes place in gonads located around the segmental blood vessels and, before spawning, oocytes accumulate also within an enlarged nephridial cavity (Britayev, 1991). Thus, gamete storage in nephridial sacs seems to be usual in Polynoidae, but is generally transient, nephridial swelling appearing only a few days before the spawning period.

However, in shallow-water species there are no spermathecae and fertilization is external (Daly, 1972;

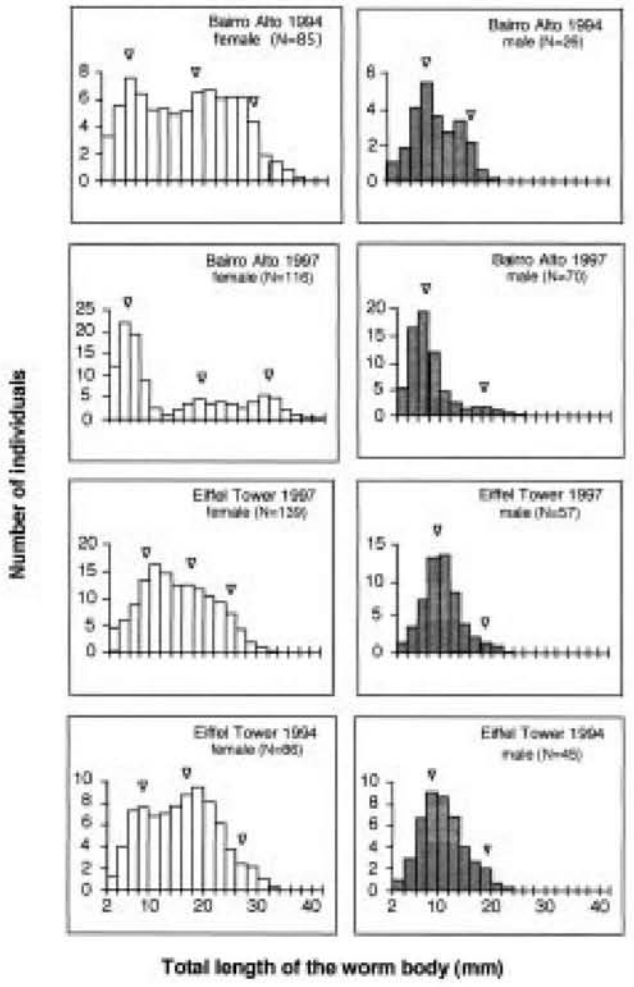

Figure 10. Size–frequency histograms of the total length of *Branchipolynoe seepensis* from four samples collected in populations of Eiffel Tower and Bairro Alto during the cruises 1994 DIVA-2 and 1997 MARVEL cruises. N, sample size; ▽, modal-classes.

Britayev, 1991). In contrast, fertilization is internal in *B. seepensis*. This is deduced from the observation of numerous spermatozoa all around the mature oocytes. Moreover, the spermatozoa possess an elongate head and nucleus as well as a long flagellum and fit well with the introsperm type defined by Jamieson & Rouse (1989). However, the spermatozoa appear to be inactive since we never observed fertilized oocytes inside the ovisac. Internal fertilization probably occurs during a discrete period, just prior to spawning. Our data also suggest that *B. seepensis* displays pairing behaviour. Pairing is not unusual in polychaetes (Schroeder & Hermans, 1975) and has been described in *Harmothoe imbricata* (Daly, 1972). Similarly, regular distribution of heterosexual pairs has been reported in the commensal polynoid *Harmothoe hyalonema* (Martin et al., 1992), *Harmothoe commensalis* (Rozbaczylo & Canete, 1993), *Bathynoe cascadiensis* (Ruff, 1991) and *Gastrolepidia clavigera* (Schmarda) (Britayev & Zamishliak, 1996). Sperm storage, internal fertilization and pairing might also be an adaptive response of the vent polychaetes to their unstable environment since it is also a general trend in Alvinellidae (Zal et al., 1994; Jouin-Toumond et al., 1997; Desbruyères et al., 1998; Jouin-Toulmond & Mozzo, unpublished data).

Continuous egg production with discrete unsynchronized spawning events

In *B. seepensis*, gametogenesis takes place in ovaries located around the segmental blood vessels. A close association of the blood vessels and developing oocytes is indicative of fast egg-producing species which generally undergo continuous reproduction (Eckelbarger, 1983). As a matter of fact, oocytes are found from the whole size range (previtellogenic to fully mature) but, although not systematic, significant differences exist in the oocyte size–frequency distribution between individuals within the two cruise samples. As such, this would indicate a non-synchronous quasi-continuous egg production. However, the oocyte size–frequency histograms suggest this distribution may be bimodal. This result does not seem unusual since the commensal polychaete *Benthoscolex cubanus* (Hartman) displays sexually mature individuals at all seasons of the year with a bimodal recruitment (Emson et al., 1993). Similarly, the shallow-water polynoid *Harmothoe imbricata* (L.) produces two cohorts of oocytes per individual each season, with the second cohort developing 2.5 times faster than the first (Daly, 1972). Daly interpreted his results as either two groups of the same oocyte pool entering vitellogenesis at different times or two distinct periods of oocyte proliferation. In the case of *B. seepensis*, cohorts are not completely separated from each other, which suggests that the inter-spawning period is short. Individuals with a large proportion of oocytes $<150\,\mu$m in diameter, but few oocytes

Table 1. *Observed deviations from the 1:1 expected sex ratio (So) in* Branchipolynoe seepensis. t *corresponds to the critical value obtained from a Student's* t*-test between the overall So mean and zero (theoretical expected deviation in the 1:1 sex ratio case).*

	Eiffel Tower		Bairro Alto		Overall estimate
	1994	1997	1994	1997	
Juveniles	42	25	25	87	179
Females	72	121	66	62	321
Males	38	50	20	37	145
So	−0.309**	−0.415***	−0.535***	−0.253*	−0.378* (t=3.04)
χ^2	10.5	29.4	24.6	6.3	

Levels of significance: *, $P<0.05$; **, $P<0.01$; and ***, $P<0.001$.

Table 2. *Sample size (N), size range, mean and standard deviations (SD) of the total length (mm) for the eight samples of* Branchipolynoe seepensis.

Samples	N	Range	Mean ±SD
ET94 (Males)	45	5.0–18.1	9.86 ±3.27
ET94 (Females)	86	6.4–28.1	15.53 ±5.52
BA94 (Males)	26	5.3–16.5	10.05 ±3.15
BA94 (Females)	85	5.7–32.6	18.26 ±6.71
ET97 (Males)	57	4.0–18.5	9.38 ±3.06
ET97 (Females)	139	4.3–29.0	14.39 ±5.63
BA97 (Males)	70	4.0–20.0	8.74 ±4.70
BA97 (Females)	116	5.2–38.0	20.31 ±9.08

N, number of individuals; SD, standard deviation; ET, Eiffel Tower, BA, Bairro Alto.

Table 3. *Modal decomposition of the eight size-frequency histograms using the MIX 2.3 software package. Most probable values obtained from decompositions after runs with various numbers of modes.*

	Modal peaks		
Samples	M1	M2	M3
DIVA-2 1994			
Males (Eiffel Tower)	χ^2=0.123, p(χ^2)=0.998, df=8		
Means (mm)	7.83	15.14	
Proportions (%)	77	23	
SD	3.07	2.56	
Females (Eiffel Tower)	χ^2=0.295, p(χ^2)=1.000, df=8		
Means (mm)	6.43	15.87	23.9
Proportions (%)	31	57	12
SD	2.64	3.91	3.19
Males (Bairro Alto)	χ^2=0.400, p(χ^2)=0.980, df=4		
Means (mm)	6.60	13.28	
Proportions (%)	67	33	
SD	2.70	2.13	
Females (Bairro Alto)	χ^2=1.996, p(χ^2)=0.996, df=10		
Means (mm)	6.09	16.66	24.26
Proportions (%)	38	32	30
SD	3.40	3.97	4.31
MARVEL 1997			
Males (Eiffel Tower)	χ^2=0.580, p(χ^2)=0.996, df=6		
Means (mm)	7.66	12.80	
Proportions (%)	80	20	
SD	2.73	4.15	
Females (Eiffel Tower)	χ^2=1.060, p(χ^2)=1.000 df=13		
Means (mm)	6.12	14.95	22.31
Proportions (%)	28	52	20
SD	2.47	3.87	3.6
Males (Bairro Alto)	χ^2=490, p(χ^2)=0.992, df=5		
Means (mm)	4.61	14.90	
Proportions (%)	83	17	
SD	2.57	4.01	
Females (Bairro Alto)	χ^2=1.460, p(χ^2)=0.999, df=11		
Means (mm)	3.96	16.42	27.90
Proportions (%)	56	16	28
SD	2.22	3.22	4.97

SD, standard deviation; df, degree of freedom.

Table 4. *t-values and levels of significance (P<0.05*, P<0.01** and P<0.001***) obtained between pairwise combinations of modal subsamples of* Branchipolynoe seepensis.

	M1	M2	M3
	Male/Female		
Bairro Alto 94	0.53	2.64**	–
Bairro Alto 97	1.40	1.17	–
Eiffel Tower 94	1.84	0.59	–
Eiffel Tower 97	2.09*	1.70	–
	Eiffel Tower/Bairro Alto		
Males of 94	1.37	1.74	–
Males of 97	5.39***	1.23	–
Females of 94	0.42	1.16	0.38
Females of 97	4.15***	1.52	4.69***
	DIVA-2 1994/MARVEL 1997		
Males of Eiffel Tower	0.24	1.59	–
Males of Bairro Alto	2.73**	1.09	–
Females of Eiffel Tower	0.46	1.28	1.23
Females of Bairro Alto	3.19**	0.49	2.81**

M1, mode l; M2, mode 2; M3, mode 3.

>300 μm in diameter, suggest that they may have spawned recently. Similarly, significant numbers of oocytes in the upper size cohort are indicative of individuals approaching spawning. Oocyte-size bimodalism has also been reported for other vent polychaetes such as *Paralvinella pandorae* and *Amphisamytha galapagensis* which undergo a semi-continuous egg production (McHugh, 1989; McHugh & Tunnicliffe, 1994) and may be widespread in polychaetes (McHugh, 1995). Most asynchronously breeding non-vent deep sea invertebrates maintain a cohort of small oocytes of which a number develop through to maximal size at any one time (Tyler & Young, 1992). From our data we can see no evidence of reproductive periodicity or synchrony. We can only propose that *B. seepensis* either starts its spawning period during the summer period, since a great proportion of mature oocytes still remain inside the ovisac at the beginning of September, or it repeatedly breeds all through the year at discrete periods.

Egg size and dispersal

One of the most striking observations on *B. seepensis* is the surprisingly large (for polychaetes) size of the mature oocytes particularly for such a sedentary species for which the expected mode of dissemination would be as a larva. Egg size above 250 μm is indeed generally considered to indicate lecithotrophy in Polychaeta (Blake, 1975; 1991), and oocyte size >400 μm has only been reported for Maldanidae, Terebellidae and Onuphidae in species undergoing direct development (Giangrande, 1997). It therefore seems that *B. seepensis* probably undergo a lecithotrophic development. A direct development with brooding is possible since the size of the smallest *Branchipolynoe* juvenile found inside the pallial cavity of the mytilid bivalve (800 μm) is twice the maximum oocyte size in the coelom (400 μm). Brooding is not unusual in polynoids (Schroeder & Hermans, 1975; Blake, 1975). However, most free-living and commensal polynoid

species have a planktotrophic development with a maximum oocyte diameter varying from 75 to 135 μm (Britayev, 1991; Giangrande, 1997). Lecithotrophy and brooding do not seem therefore associated with commensalism in polynoids. *Branchipolynoe seepensis* also displays a low overall fecundity which may be related to the size of the oocytes (size limitation of the ovisac). In contrast, coastal polynoid polychaetes usually display great fecundities (50,000 eggs per female in *Harmothoe imhricata*: Daly, 1972; 1,000,000 eggs per female in *Arctonoe vittata*: Britayev, 1991). The more likely explanation would be that the large yolky eggs are an adaptive response to the vent environment as most of the vent polychaetes examined so far produce few relatively large oocytes (McHugh, 1989; McHugh & Tunnicliffe, 1994; Zal et al., 1995). Although planktotrophic development is generally correlated with broad dispersal in marine species, lecithotrophic development may have adaptive benefit in the oligotrophic deep sea conferring the possibility of widespread dispersal whereas a planktotroph is limited by planktonic food availability (Shilling & Manahan, 1994; Young, 1994). Whatever the larval development mode, metamorphosis and subsequent settlement occur generally in Polynoidae when the larva reaches nine chaetigers which, in *H. imhricata,* roughly corresponds to a size of 700–800 μm (Daly, 1972). Such a settlement size is not very different from our smallest *Branchipolynoe* juvenile stage (ten chaetigers) and therefore we cannot rule out the possibility of sequential infestations within the same host from newly settling 9-chaetiger postlarvae. Such an observation was reported for juveniles of *Arctonoe vittata* which are driven away or eaten by large individuals and force to infest uninhabited hosts (Britayev, 1991).

Sexual difference in size: protandry, dwarf males or differential mortality?

Many polynoid polychaetes live commensally with other marine invertebrates (Britayev, 1991; Ruff, 1991; Rozbaczylo & Canete, 1993; Britayev & Zamishliak, 1996). Polychaetes in general, and free-living Polynoidae, do not display either a peculiar size dimorphism or a biased sex ratio (Daly, 1972; Rozbaczylo & Canete, 1993). However, commensal polynoids belonging to the genera *Arctonoe*, *Bathynoe* and *Gastrolepidia*, are characterized by a marked size dimorphism with few small males (Ruff, 1991; Britayev & Zamishliak, 1996). In *Branchipolynoe seepensis*, comparisons of the length distributions between sexes were performed in order to test whether previous observations, reporting a male deficiency in populations and a great size difference between sexes, males being much smaller than females was valid (Jollivet, 1996). Analysis of the four samples collected at the sites Bairro Alto and Eiffel Tower showed a male-biased sex ratio. In addition, significant differences between the length distributions of males and females were also observed at both sites, with three size-classes for females and only two for males. This difference could be partially the reflection of a sampling bias related to male foraging behaviour, males living mostly outside the pallial cavity of the mytilid bivalves whereas females remain inside. Such a behaviour would therefore increase the number of females compared to the males during the collection of mytilid bivalves. Polynoids could also escape from the mytilids when they are brought up to the surface inside the insulated basket of 'Nautile'. However, if some males did this, they should have been recovered from the basket's washings (already taken into consideration in this study) and all size-classes should be affected rather than the largest ones. Male-biased sex ratios and size dimorphism, males being smaller than females, have also been reported from other commensal polynoids such as *Bathynoe cascadiensis* and *Gastrolepidia clavigera* (Ruff, 1991; Britayev & Zamishliak, 1996), who interpreted this size dimorphism as the result of a longer life span for females. Size dimorphism and male-biased sex ratio may also be explained by protandry, dwarf males, differential migration or differential mortality between males and females, owing peculiar sexual behaviours (Wenner, 1972). A close examination of the data has allowed us to reject two hypotheses at least.

Protandrous hermaphroditism is restricted to polychaete families such as Syllidae, Dorvilleidae, Sabellidae or Serpulidae and is uncommon in Polynoidae with only one suspected species: *Macellicephala violacea* (Schroeder & Hermans, 1975). Nevertheless, in general, a male-biased ratio shifted towards small sizes is consistent with protandry. However, neither the size–frequency histograms of males nor those of females were opposed in size, as it would be expected if protandry occurs naturally. In all the protandric deep sea species so far examined (P.A. Tyler, personal observation), developing small oocytes are always found in the late male phase and some relict sperm in the early female phase. Careful examination of *Branchipolynoe* specimens under the microscope found no co-occurrence of male and female germinal tissue in the smallest females. The early stages of nephridial papillae were always observed to develop first on S10 in females whereas they develop on S11 in males.

Dwarf males are often found in species with sedentary and territorial females (Vollrath & Parker, 1992; Vollrath, 1998). Dwarfing seems to have emerged from natural and sexual selection, larger females having a higher investment in eggs and smaller males being less subject to attack by the female prior to or after copulation (Hedrick & Temeles, 1989). Although size dimorphism between sexes is poorly documented in polychaetes, it exists in the interstitial polychaete *Dinophilus* in which very small males are able to fertilize female embryos within the cocoon before hatching (Westheide, 1990) and in an interstitial sabellid (Berrill, 1977). In *Branchipolynoe*, females do not seem to move outside the pallial cavity of mussels, whereas dwarf-males or juveniles appear to travel from one mytilid bivalve to another (personal observation onboard). However, dwarfing is often a result of differential growth rates during development with, as an extreme, differing metamorphosis (Crisp, 1983). This is not the case in *Branchipolynoe* as males do not differ morphologically from females and do not display a distribution skewed towards small sizes as would be expected from dwarfing. In contrast, modal lengths do not differ significantly between sexes, males only lacking the third Gaussian component found in females.

As a consequence, the male-biased sex ratio and size dimorphism in *B. seepensis* appear to only be explained by

either a differential mortality between sexes or a migration of the largest males. A large emigration of males seems unlikely as male drifting mainly occurs in the small sizes. On the contrary, differential predation is likely to shape the observed size–frequency histograms by preventing males from reaching the size of the largest females. Males could indeed easily suffer from fish predation when they travel from one bivalve to another in order to breed. Intraspecific aggression also seems to occur widely in commensal scale-worms leading to trauma or the death of some worms (Britayev, 1991; Britayev & Zamishliak, 1996) and we cannot rule out cannibalism, especially because *Branchipolynoe* possesses a powerful muscular pharynx armed with jaws (Desbruyères et al., 1985). At present, we cannot discriminate between cannibalism and natural predation in *Branchipolynoe*. However, preliminary data of population genetics using microsatellite markers (D.J., unpublished data) revealed the occurrence of strong heterozygote deficiencies in populations of *B. seepensis* that could suggest sexual selection in which smaller males would be safer from female attack than larger ones.

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