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Sperm Storage, Internal Fertilization, and Embryonic Dispersal in Vent and Seep Tubeworms (Polychaeta: Siboglinidae: Vestimentifera)

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Abstract. Vestimentiferan tubeworms are ecologically important members of deep-sea chemosynthetic communities, including hydrothermal vents and cold seeps. Some are community dominants and others are primary colonists of new vent sites; they include some of the longest living and fastest growing marine invertebrates. Their mechanisms of propagation, dispersal, and genetic exchange have been widely discussed. Direct sperm transfer from males to females has been documented in one species, *Ridgeia piscesae,* but others are known to discharge what are apparently primary oocytes. Brooding of embryos has never been observed in any vestimentiferan. These observations have led to the supposition that fertilization might be external in most species. Here we report sperm storage at the posterior end of the oviduct in five species, including tubeworms from both vents and seeps. We show experimentally that most eggs are inseminated internally, that fertilization rate is typically lower than 100%, that meiosis is completed after eggs are released from the female, and that the dispersal phase includes the entire embryonic period.

Introduction

The discovery of hydrothermal vents in 1977 heralded the description of large vestimentiferan tubeworms that were initially placed as a class in the phylum Pogonophora (Jones, 1981) and then elevated to the phylum level (Jones, 1985). Genetic and embryological evidence now shows convincingly that these gutless, deep-sea tubeworms are polychaetes (Young *et al.,* 1996; McHugh, 1997; Rouse and Fauchald, 1997) currently classified within the polychaete family Siboglinidae (Rouse and Fauchald, 1997), which includes the frenulate, vestimentiferan, and moniliferan Pogonophora of previous authors.

Vestimentiferan tubeworms came to symbolize hydrothermal vents in the Pacific, and with their discovery at cold seeps in the Gulf of Mexico and elsewhere, became the icon of communities driven by chemosynthetic primary production. Species of vestimentiferans are known to be among the first colonizers of newly formed vents (Hessler *et al.,* 1988; Shank *et al.,* 1997), some of the fastest growing marine invertebrates (Lutz *et al.,* 1994), and the longest lived nonclonal animals known (Fisher *et al.,* 1997). Because of their ecological importance at vents and seeps, there has been considerable interest in their reproductive and dispersal biology.

In vestimentiferan tubeworms, small, yolky, and slightly buoyant eggs develop into nonfeeding trochophore larvae that are thought to disperse in the plankton for up to several weeks (Young *et al.,* 1996; Marsh *et al.,* 2001), facilitating genetic exchange and colonization of newly available seep and vent habitats. It has been estimated that larvae of the vent species *Riftia pachyptila* can disperse more than 100 km over a 5-week period (Marsh *et al.,* 2001). This estimate is based on the assumption that passive dispersal occurs during a 3-week embryonic period as well as a 2-week period when larvae are capable of independent ciliary movement (Marsh *et al.,* 2001). However, because the site of fertilization and the location of embryogenesis remain unresolved for all species of vestimentiferans, these dispersal estimates could be as much as 60% too high. Thus, the site of fertilization in vestimentiferans is of great interest

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and has been the subject of considerable debate. Internal fertilization and brooding of embryos appear to be the norm for frenulate siboglinids (Bakke, 1974; reviewed by Southward, 1999).

Free sperm have been observed in the oviduct of *Riftia pachyptila* (Gardiner and Jones, 1985), a species that apparently spawns its eggs (Van Dover, 1994). Moreover, transfer of large white spermatozeugmata (sperm masses) from male to female plumes has been documented in *Ridgeia piscesae* (Southward and Coates, 1989; MacDonald *et al.,* 2002) and *Tevnia jerichonana* (Southward, 1999). On the basis of these observations, Southward (1999) has proposed that eggs are fertilized either in the ovisac just before spawning or externally as the eggs are released. Internal fertilization is consistent with the unusual sperm morphology (sperm are elongate and have spiral mitochondria and nuclei) in all known species (Gardiner and Jones, 1985; Cary *et al.,* 1989), and also with the presence of external ciliary tracts (Fig. 1A, B) leading away from the male gonopores on the dorsal surface of the vestimentum (Gardiner and Jones, 1993). However, massive spermatozeugmata are known only in *Ridgeia piscesae* (Southward and Coates, 1989; MacDonald *et al.,* 2002) and *Tevnia jerichonana* (Southward, 1999); in other species, sperm appear to be released in smaller flame-shaped bundles (Fig. 1C, D) that are capable of swimming as coordinated units and that eventually break down in seawater (Gardiner and Jones, 1985; Cary *et al.,* 1989). The observations of modified sperm and direct sperm transfer are difficult to reconcile with numerous field and laboratory observations of apparent free spawning in both *Riftia pachyptila* and *Lamellibrachia luymesi* (Cary *et al.,* 1989; Van Dover, 1994; Young, unpubl. obs.). However, the clouds of presumed gametes observed in these "spawning events" could consist of zygotes, embryos, or even larvae if fertilization is internal.

Using histology, we have examined the female reproduc-

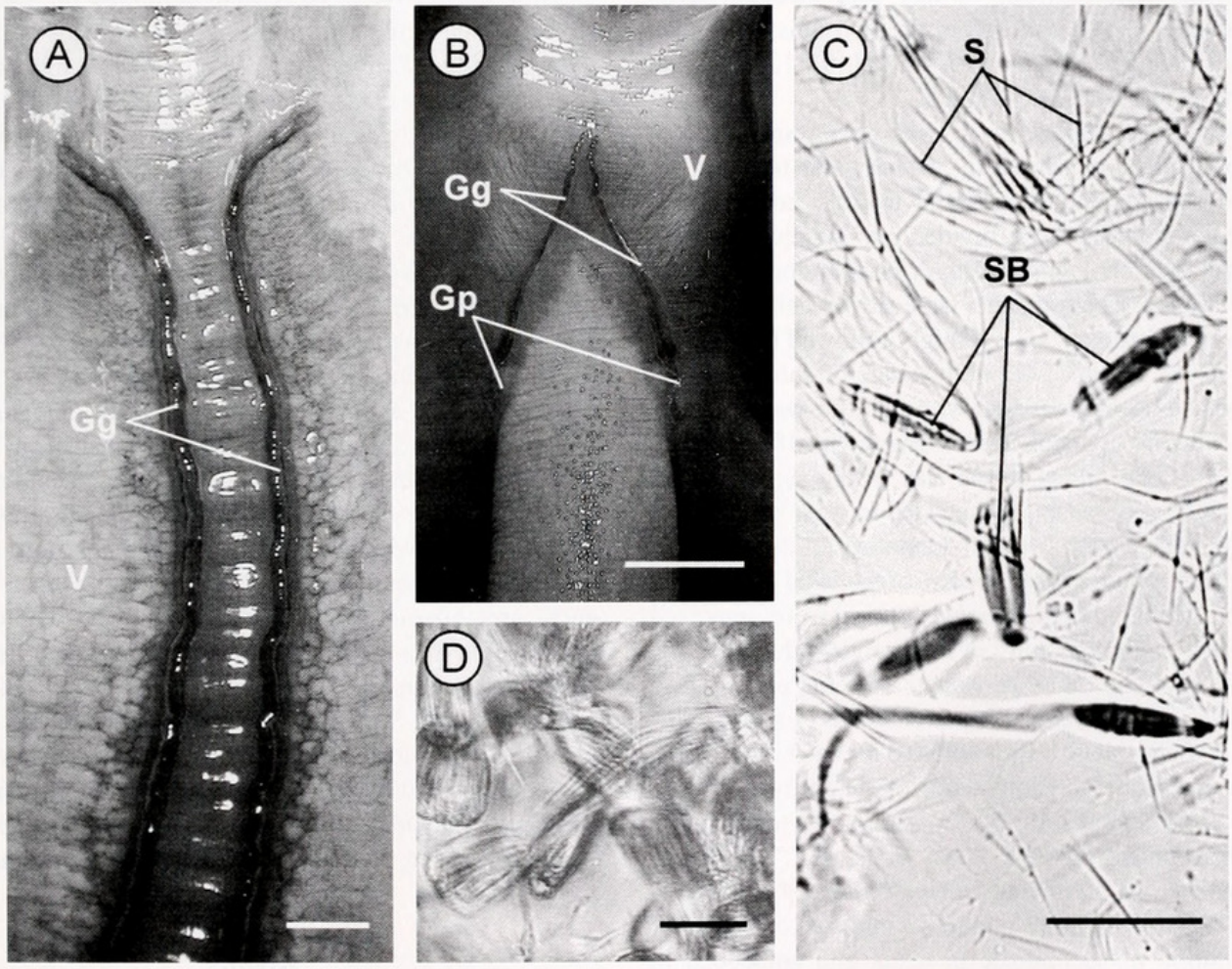

Figure 1. (A) Dorsal view of the vestimentum of a *Lamellibrachia luymesi* male, showing genital grooves leading away from paired gonopores and diverging near the anterior end (scale bar: 5 mm). (B) Dorsal view of the vestimentum in a *Riftia pachyptila* male, showing genital grooves converging (scale bar: 1 cm). (C) Actively swimming sperm bundles of *Lamellibrachia luymesi* dissected from the terminal portion of the male gonoduct. Some of the bundles have broken apart upon contact with seawater to release individual sperm (scale bar: 25 μm). (D) Sperm bundles of *Tevnia jerichonana* (scale bar: 25 μm). Gg, genital groove; Gp, gonopores; S, spermatozoa; SB, sperm bundles; V, vestimentum.

tive system of five vestimentiferan siboglinid species: *Riftia pachyptila, Ridgeia piscesae,* and *Tevnia jerichonana* from Pacific hydrothermal vents, and *Lamellibrachia luymesi* and *Seepiophila jonesi* from cold seeps in the Gulf of Mexico. In all five species we found a sperm storage region at the far posterior end of the female reproductive tract. We refer to this region as the spermatheca. We also report *in vitro* fertilization and field experiments showing that insemination is internal, that meiosis is completed after the eggs are released from the female, and that the dispersal phase of vestimentiferans includes the entire embryonic period.

Materials and Methods

Samples of *Riftia pachyptila, Ridgeia piscesae,* and *Tevnia jerichonana* were collected from a depth of 2500 m at hydrothermal vents on the East Pacific Rise (9°50′N), using the deep submergence vehicle *Alvin. Lamellibrachia luymesi* and *Seepiophila jonesi* were collected with *Johnson-Sea-Link* submersibles from various cold methane seeps between depths of 600 and 800 m on the Louisiana Slope. Major sites included GC-234 (27°44.7′N, 91°13.4′W) and Bush Hill (27°46.9′N, 91°30.5′W).

For histological studies, the worms were removed from their natural tubes, preserved in 5% seawater formalin for 48 h, and subsequently transferred to 70% ethanol. The trunk of each individual was divided into 10 equal regions, and a single section 1 mm in length was randomly chosen from each region. The segments were slowly dehydrated by transfer to 90% propan-2-ol overnight followed by a period of 9 h in 100% propan-2-ol, with change of solution every 3 h. Before being impregnated with paraffin wax at 70 °C for 12 to 24 h, the segments were cleared with 100% xylene for 6 to 12 h, depending on the size of the segment. The impregnated tissue was then embedded in wax, sectioned at 5 μm, and stained with Mayer's hematoxylin and eosin.

For electron microscopy, pieces of gonadal tissue dissected from female *Lamellibrachia luymesi* were immersed for 1.5 h at room temperature in 2.5% glutaraldehyde buffered with Millonig's 0.4 *M* phosphate buffer at pH 7.4. Due to exigencies of ship scheduling, tissue was stored in Millonig's 0.4 *M* phosphate buffer with 0.6 *M* NaCl for 2 weeks prior to post-fixation and embedding. Tissue was post-fixed for 1 h at room temperature in 1% osmium tetroxide in 0.1 *M* phosphate buffer plus 1% NaCl at pH 7.2. Tissue was then dehydrated in ascending concentrations of ethanol to 100%, immersed in acetonitrile for 10 min, left overnight in a 50% solution of acetonitrile in resin, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H7000 transmission electron microscope.

Eggs of some vestimentiferans are stored in expanded distal ovisacs prior to release. We collected eggs from the ovisac or the distal region of the oviduct in all species studied and on many occasions to assess the gametogenic stage just prior to spawning. Immediately after they were removed, eggs were examined with a light microscope for the presence of visible germinal vesicles.

Oocytes were collected from the ovisacs of seven female specimens of *Lamellibrachia luymesi* and incubated without adding sperm to determine whether they had already been inseminated internally. New pipettes were used, taking special care to eliminate any possible contamination from extraneous sperm. These primary oocytes were incubated in 0.45-μm-filtered seawater for 24 h at 6 °C, after which at least 100 were examined from each individual.

To determine if tubeworms release eggs, zygotes, or embryos under natural conditions, we deployed inverted plankton nets over aggregations of *Riftia pachyptila* adults in the field (Fig. 2). Nets were 0.5 m in diameter and had 100-μm mesh. Disks of syntactic foam attached to the cod ends floated the nets upward, and polypropylene funnels at the mouths of the cod ends prevented escape of the buoyant eggs during recovery. Nets were deployed for various periods of time ranging from 3 to 10 days. They were recovered by using the submersible's manipulator and transported to the surface in closed plastic boxes to maintain temperature. Immediately after the dive, eggs and embryos were concentrated with nylon mesh filters and examined with a compound microscope to assess stages of gametogenesis or embryogenesis.

In broadcast spawners with external fertilization, fertilization rates virtually always decline as a function of distance from spawning males (*e.g.,* Pennington, 1985; Levitan, 1991; Levitan and Young, 1995; Metaxas *et al.,* 2002). We conducted a field experiment with *Lamellibrachia luymesi* eggs to determine whether distance from a dense aggregation ("bush") of tubeworms had any effect on the fertilization rate of oocytes removed from the ovisacs of females. Eggs were held 25 cm above the bottom in small plastic tubes (2-cm diameter, 2-cm length) capped on both ends with 50-μm nylon mesh. Three were deployed by submersible in a large bush of tubeworms, and the others were deployed in a straight line with three replicates each at 1.5 m, 3.0 m, 4.5 m, and 6.0 m downstream. The cultures were left to incubate for 24 h. As in the laboratory experiments mentioned above, we took special care to sterilize all containers, including the acrylic plastic box in which eggs were transported to the bottom, to eliminate spurious fertilization events.

Results

General anatomy of the female reproductive tract

The reproductive system of female vestimentiferans opens at the anterior end of the trunk, between the vestimental wings. A pair of meandering oviducts extend through the trunk, surrounded by trophosome tissue.

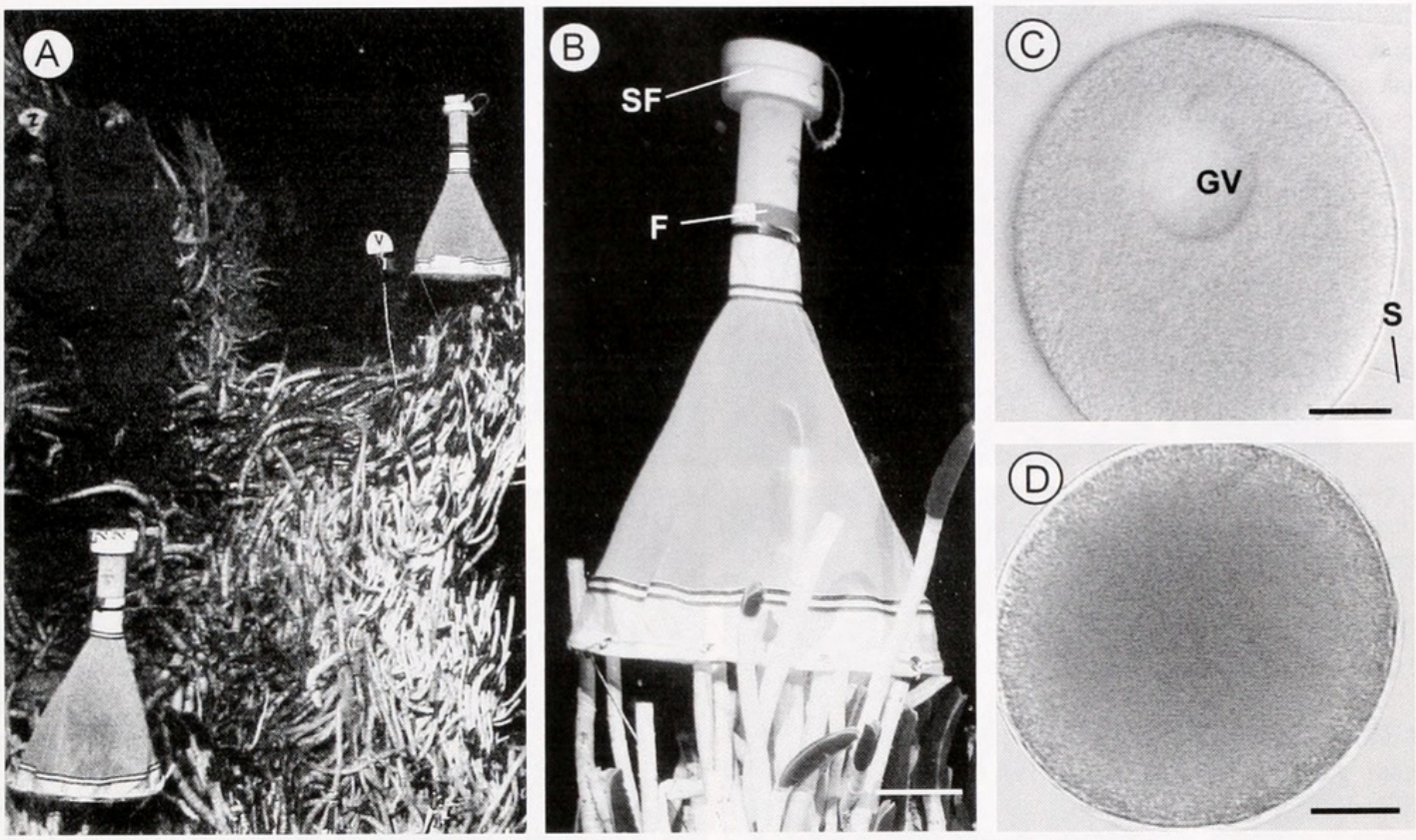

Figure 2. (A) Two egg traps deployed in a large aggregation of *Riftia pachyptila* at 9°50′N on the East Pacific Rise. Depth is 2500 m. The downward-facing net openings are 50 cm in diameter. (B) Close-up of an egg trap deployed over a clump of *R. pachyptila* (scale bar: 10 cm). SF, syntactic foam; F, position of the funnel on the inside of the cod end. (C) Light micrograph of a primary oocyte removed from the female ovisac of *R. pachyptila* and artificially inseminated with free sperm. Several filament-like sperm heads are attached to the egg surface (scale bar: 25 μm). GV, germinal vesicle; S, sperm. (D) Typical uncleaved *R. pachyptila* zygote captured in an egg trap (scale bar: 25 μm).

Whereas in *Riftia pachyptila, Ridgeia piscease,* and *Tevnia jerichonana* the gonad runs through the whole length of the trunk, in *Lamellibrachia luymesi* and *Seepiophila jonesi* it can only be found in the anterior two-thirds of the trunk. The paired gonocoels, which contain the ovaries, run parallel and dorsal to the oviducts. At the posterior end, the gonocoels bend and pass anteriorly as paired oviducts. In *Riftia pachyptila, Ridgeia piscesae,* and *Lamellibrachia luymesi,* the terminal portion of each oviduct is enlarged as an egg storage compartment known as the ovisac.

A sheet of connective tissue separates the gonocoels from each other and from the ventral blood vessel. A narrow strip of germinal epithelium grows from this connective tissue sheet into each gonocoel, filling the gonocoel cavity with rows of developing eggs. Webb (1977) described structures called "transverses," situated at irregular intervals along the length of the trunk, where the gonocoel, the oviduct, and the ventral blood vessel cross from right to left, and where the oviduct opens into the gonocoel by a nonciliated funnel. These structures were also reported by Malakhov *et al.* (1996) in *Ridgeia piscesae.* Although we have observed transverses in this same species, histological sections revealed no openings that would permit the passage of oocytes from the gonocoels to the oviducts. Thus, all eggs must pass through the posterior ends of their respective gonocoels before entering the oviducts.

The spermatheca

Through most of its length, the wall of the oviduct is composed of a ciliated cuboidal epithelium surrounded by a thin layer of circular muscle. However, in all five species examined, the oviducal epithelium is folded into a series of loops and sacs at the far posterior end of the reproductive tract, where the ovarian gonocoel joins the oviduct (Fig. 3). In every female examined, these small sacs contained clusters of spermatozoa, with the heads all aligned towards the wall of the sac (Fig. 3A–D). These sperm are no longer packaged in the discrete flame-shaped bundles released by the males (Gardiner and Jones, 1985; Cary *et al.,* 1989). We refer to this region of sperm storage as the spermatheca. In *Lamellibrachia luymesi* and *Seepiophila jonesi* the spermatheca appears as a white, hook-shaped structure easily visible with the naked eye through the body wall of the

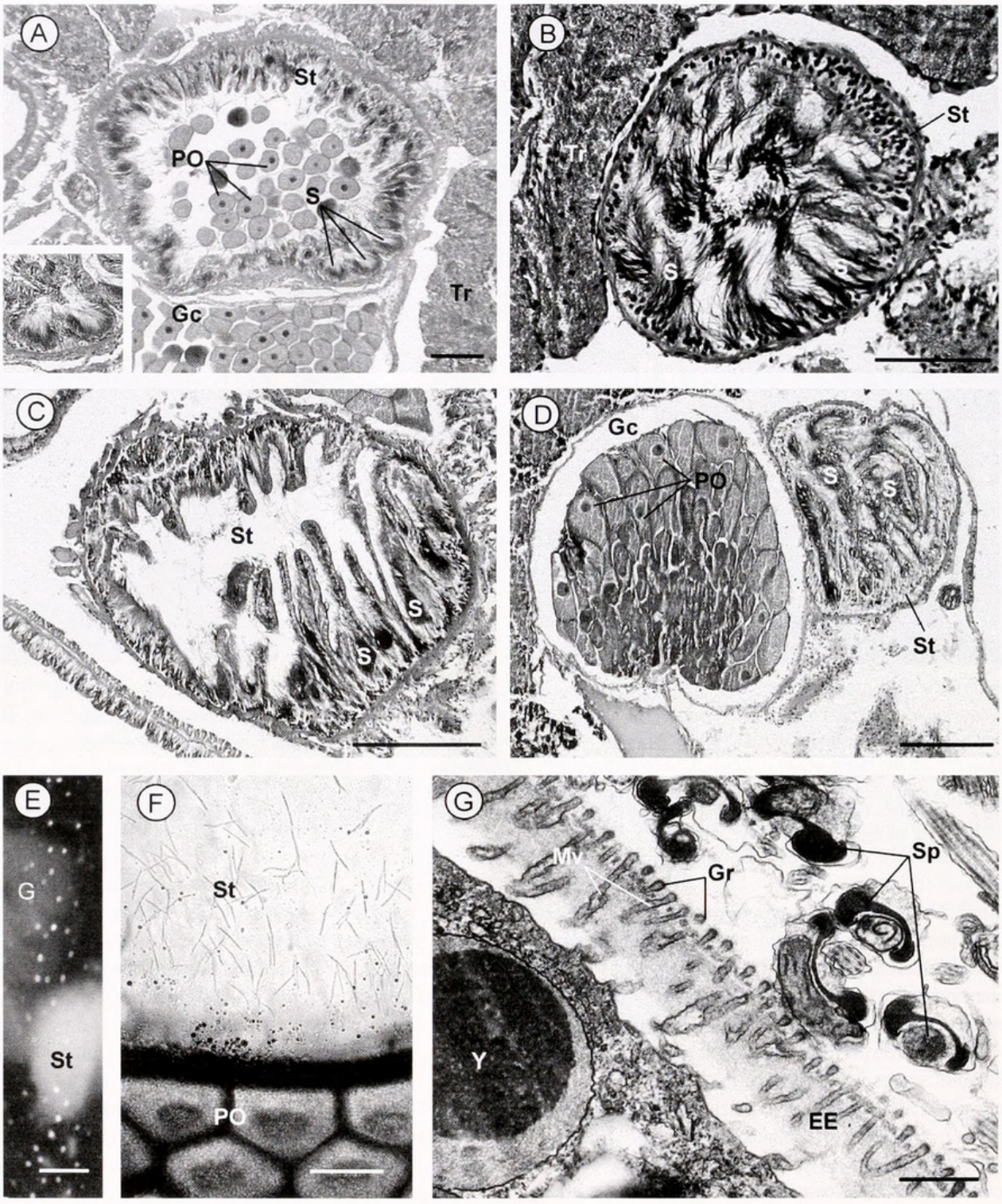

Figure 3. **(A–D)** Light micrographs of histological sections through the spermatheca regions of females of four species: *Riftia pachyptila* (A), *Tevnia jerichonana* (B), *Lamellibrachia luymesi* (C), and *Seepiophila jonesi* (D). The insert in A shows detail of the spermatheca of *Riftia pachyptila* with clusters of spermatozoa. Scale bars for A–D: 200 μm. (E) Spermatheca of *Lamellibrachia luymesi* as seen through the wall of the body (scale bar: 1 mm). (F) Squash preparation of the spermatheca from a *Lamellibrachia luymesi* female, showing isolated sperm in the lumen lying close to primary oocytes. Scale bar: 75 μm. (G) Transmission electron micrograph of the spermatheca of *Lamellibrachia luymesi,* showing an oocyte and several spermatozoa in proximity (scale bar: 0.5 μm). EE, egg envelope; G, gonad; Gc, gonocoel; Gr, granule with filamentous glycocalyx; Mv, microvilli; PO, primary oocyte; S, clusters of spermatozoa, Sp, sperm; St, spermatheca; Tr, trophosome; Y, yolk granule.

Table 1

Percentage of Riftia pachyptila *eggs, zygotes, and embryos that had not cleaved by the time they were recovered in inverted plankton nets (egg traps) deployed over adult aggregations*

Recovery Date	Site	Days Deployed	Embryos Captured	% Uncleaved
7 May 2000	Q-Vent	3	13	100.00
12 May 2000	Q-Vent	8	377	88.86
14 May 2000	Tica Vent	6	247	78.94
20 May 2000	M-Vent	6	126	61.11
21 May 2000	Biovent	7	28	71.42

worm (Fig. 3E). Free sperm are readily observed in proximity to primary oocytes in light-microscope squash preparations of the posterior oviduct (Fig. 3F). In the paraffin sections, the sperm heads do not appear to be buried in the walls of the spermatheca, but the magnifications are too low to reveal whether there are intracellular junctions that might indicate nutrient exchange between the spermatozoa and the female epithelium.

Ultrastructural examination of oocytes collected from the spermatheca shows that the egg envelope is formed of branched microvilli that terminate in a monolayer of granules situated along the outer surface of the envelope and underlying a filamentous glycocalyx (Fig. 3G). Sperm heads are often found in direct contact with the glycocalyx (Fig. 3G). However, we did not find any sperm that had penetrated the egg envelope.

Status of the oocytes at "spawning"

In all five species examined, oocytes in the spermatheca, the ovisac, and all along the length of the oviduct had large germinal vesicles (Fig. 2C), suggesting that they were primary oocytes in the first prophase of meiosis. In no instance did we encounter embryos already developing in the ovisac or in any other region of the oviduct.

Collections of eggs spawned naturally by *Riftia pachyptila* and collected in egg traps contained many uncleaved eggs (Table 1), most (but not all) of which lacked germinal vesicles (Fig. 2D) by the time they were collected. After deployments of several days, nets also contained cleaving embryos, but the high percentage of uncleaved eggs in all samples (Table 1) indicates that embryos do not begin cleavage before release from the parent. In the shortest (3-day) deployment, only uncleaved eggs were found (Table 1). As first cleavage in *R. pachyptila* occurs more than 2 days after eggs are diluted with seawater (Marsh *et al.*, 2001), these egg-trap data are completely consistent with our other observations suggesting that eggs are released as primary oocytes.

During November 2003, oocytes carefully dissected from seven female specimens of *Lamellibrachia luymesi* were incubated *in vitro* without adding additional sperm. After 24 h, a mean of 90.09% (SD: 8.45) of embryos had attained 2-cell to 16-cell embryonic stages, showing that a large percentage of oocytes had already been inseminated by the time they reached the ovisac. During a cruise in July 2004, this experiment was repeated qualitatively, using very small individuals of *L. luymesi.* Oocytes from three females yielded a high percentage of embryos without addition of sperm, but oocytes from two very small females did not develop, suggesting the possibility that these individuals, though reproductively active, had not received any sperm.

Fertilization rates

For species that fertilize internally, it is commonly assumed that all eggs are fertilized (Thorson, 1950). However, in rearing the embryos of three species of vestimentiferans in both the laboratory and the field, we found that the number of eggs developing was always less than 100%, suggesting that the internal fertilization mechanism may not function with perfect efficiency in these worms.

In laboratory pressure vessels (Marsh *et al.*, 2001), the percentage of *Riftia pachyptila* embryos cleaving ranged from 40% to 84% (Table 2), with an average of 65%. When cultures were incubated *in situ* (Table 2), about 20% more embryos cleaved; the highest value for cleaving embryos was 91%.

Fertilization rates were high (74.32% to 97.60%) in incubations of *Lamellibrachia lumesi* eggs held near the sea floor and in the laboratory (Table 3). A single field culture

Table 2

Percentage of Riftia pachyptila *embryos undergoing cleavage in laboratory pressure vessels at 250 atm (laboratory) and in mesh-covered vials outplanted to the field at a depth of 2500 m*

				Embryos Cleaving	
Location	Date	Females	Eggs	Percentage	Mean ± SD
Laboratory	12 Dec 1995	2	38	84.21	
	14 Dec 1995	1	124	60.48	
	5 Dec 1998	1	692	40.75	
	5 Dec 1998	1	300	41.66	
	15 May 1999	5	202	84.16	
	4 May 2000	6	120	77.50	
	6 May 2000	6	107	82.24	
	16 May 2000	9	100	49.00	65.00 ± 19.26
Field	25 April 1999	2	73	86.30	
	16 May 1999	2	212	90.57	
	13 Dec 1999	6	426	78.64	
	4 May 2000	6	55	76.36	
	9 May 2000	5	260	90.38	
	13 May 2000	3	232	86.21	
	17 May 2000	9	456	83.77	85.55 ± 5.71

All eggs were removed by dissection from the female ovisac and additional sperm were added *in vitro* prior to incubation.

Table 3

Percentage of Lamellibrachia lumesi *embryos undergoing cleavage at 1 atm in the laboratory and in mesh-covered vials outplanted to the field at a depth of 700 m*

			Embryos Cleaving	
Location	Date	Eggs	Percentage	Mean ± SD
Laboratory	11 Sept 1995	66	92.42	
	13 Sept 1995	222	74.32	
	17 Aug 1997	167	97.60	88.11 ± 12.23
Field	19 Aug 1997	637	96.54	NA

All eggs were removed by dissection from the female ovisac, and additional sperm were added *in vitro* prior to incubation. Eggs from numerous females were combined for each experiment.

of *Tevnia jerichonana* with combined eggs from three females yielded 87.8% development.

In a field experiment with *L. luymesi,* distance from a tubeworm bush (0 m to 6 m) had no significant effect on the fertilization rate of oocytes taken from the ovisacs of females (Fig. 4); the mean percentages of development ranged from 85% to 95%. This lack of correlation with distance strongly suggests that fertilization had already occurred before the experimental deployment. Similar experiments with freely spawning invertebrates that fertilize externally always show a strongly declining relationship between fertilization rate and distance from males (Pennington, 1985; Levitan, 1991; Levitan and Young, 1995).

Discussion

The reproductive anatomy of vestimentiferans has been studied in *Lamellibrachia luymesi* (Van der Land and Norrevang, 1977), *Lamellibrachia barhami* (Webb, 1977), and mainly in the male in *Riftia pachyptila* (Gardiner and Jones, 1985; Jones and Gardiner, 1985; Gardiner *et al.,* 1992). Early development has been described for *Riftia pachyptila* (Marsh *et al.,* 2001) and for *Lamellibrachia* sp. and *Escarpia* sp. from the Gulf of Mexico (Young *et al.,* 1996); the morphology of very young juveniles of *Ridgeia piscesae* has been described by Jones and Gardiner (1989).

Apparent spawning events have been observed in *Riftia pachyptila* (Van Dover, 1994) and *Lamellibrachia luymesi* (our unpublished observations). However, in none of these observations was it possible to ascertain whether the "spawn" consisted of unfertilized oocytes, zygotes, developing embryos, bundles of sperm, or free sperm.

Gardiner and Jones (1985) suggested that direct sperm transfer and internal fertilization might occur in *Riftia pachyptila,* because this species has modified sperm. They also reported an anecdotal observation of sperm in the female genital tract. In *Ridgeia piscesae* and *Tevnia jerichonana,* sperm masses have been found attached to the female vestimentum and within the oviduct, strongly suggesting active sperm transfer when the plumes of closely juxtaposed animals brush against each other (Southward and Coates, 1989; Southward, 1999; MacDonald *et al.,* 2002), followed by internal fertilization. These observations also raise the possibility that embryos are brooded.

Except in *Ridgeia piscesae* (Southward and Coates, 1989; MacDonald *et al.,* 2002), we do not know how sperm or sperm bundles are transferred to the female, but it seems likely, on the basis of spawning observations, that sperm bundles may be released into the water column, from which they are either collected by the females or find their way into the female gonopores. The presence of spermatozoa at the posterior end of the female gonoduct demonstrates that sperm either swim or are transported deep into the reproductive system. Further work is required to determine if sperm bundles swim downward as cohesive bundles against a ciliary current that carries oocytes upward in the oviduct, or if they are transported by ciliary action or peristalsis. It is interesting that the positioning of external ciliary tracts on the male vestimentum varies from species to species and that the size and shape of sperm bundles also vary in a species-specific manner (Fig. 1). Further work is needed to determine if these characters are correlated and if they are related to the mode of sperm release or transfer.

Webb (1977), in describing the reproductive anatomy of *Lamellibrachia barhami,* noted that the gonocoel of the gonad opens into the gonoduct by nonciliated funnels situated at irregular intervals along the length of the trunk. These connections between the gonoduct and the gonocoels were not found in any of the species studied here, which suggests that all primary oocytes released from the ovary must pass through the spermatheca before they enter the

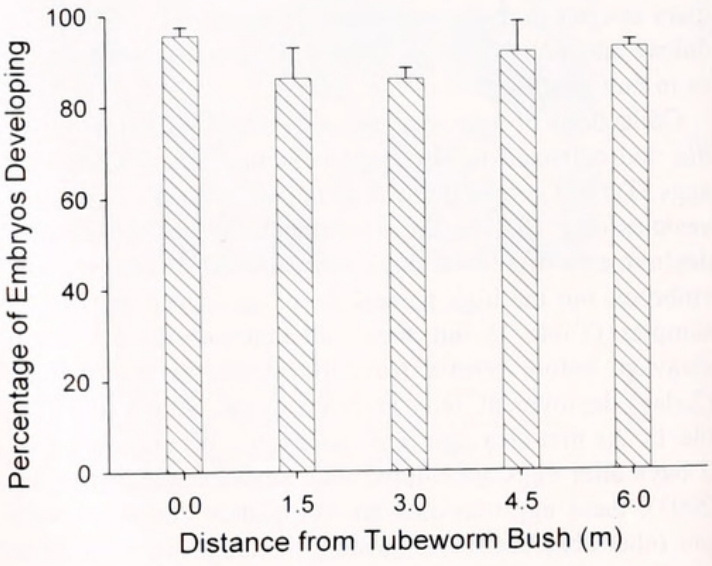

Figure 4. Percentage of fertilized eggs (mean ± 1 SD) of *Lamellibrachia luymesi* after a 24-h *in situ* deployment at various distances from a large bush of conspecifics.

gonoduct. In eggs of other polychaetes, granulated glycocalyces similar to the one found in oocytes collected from the spermatheca of *Lamellibrachia luymesi* are known to possess sperm receptors (Eckelbarger, 1992). We hypothesize that as primary oocytes pass through the spermatheca, sperm bind to the primary oocytes, which are arrested at the first meiotic prophase until they are released by the female. This hypothesis is supported by electron micrographs (Fig. 3G) showing sperm heads in direct contact with the glycocalyces of primary oocytes in the spermatheca. Westheide (1988) has categorized the sperm storage organs of polychaetes into three major types. The spermathecae of vestimentiferans, which are outpocketings of the oviduct, fall clearly into his Type 2a classification.

The breeding strategy of internal fertilization followed by zygote release rather than brooding assures a high level of fertilization without sacrificing dispersal potential. Moreover, sperm storage is an ideal strategy in an environment where periodic cues for gametogenesis and spawning synchrony are limited (Young, 1999). Indeed, hydrothermal vent polychaetes from many different families are now known to possess specialized organs for sperm storage (Zal *et al.,* 1995; Jollivet *et al.,* 2000).

Not all oocytes taken from the ovisacs of female vestimentiferans undergo development, an observation that suggests imperfect internal fertilization. However, the number of embryos undergoing development is often higher for cultures reared in the field than in the laboratory (Tables 1, 2). This raises the possibility that the percentage of eggs developing is not the same as the percentage fertilized; thus, observed rates of development lower than 100% may be caused by culture conditions rather than fertilization failure.

Previous estimates of dispersal times and distances based on lipid stores, larval metabolism, and current speeds (Marsh *et al.,* 2001) have assumed external fertilization and dispersal during the entire embryonic period. Even though the presumed location of fertilization was clearly erroneous in these studies, the dispersal estimates themselves remain valid, since embryogenesis does not begin until after inseminated oocytes are released into the water column.

Acknowledgments

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Literature Cited

Bakke, T. 1974. Settling of the larvae of *Siboglinum fjordicum* Webb (Pogonophora) in the laboratory. *Sarsia* **56:** 57–70.

Cary, S. C., H. Felbeck, and N. D. Holland. 1989. Observations on the reproductive biology of the hydrothermal vent tube worm *Riftia pachyptila. Mar. Ecol. Prog. Ser.* **52:** 89–94.

Eckelbarger, K. J. 1992. Polychaeta: Oogenesis. Pp. 109–127 in *Microscopic Anatomy of Invertebrates,* Vol. 7, F. W. Harrison and S. L. Gardiner, eds. Wiley-Liss, New York.

Fisher, C. R., I. A. Urcuyo, M. A. Simpkins, and E. Nix. 1997. Life in the slow lane: growth and longevity of cold-seep vestimentiferans. *Mar. Ecol.* **18:** 83–94.

Gardiner, S. L., and M. L. Jones. 1985. Ultrastructure of spermiogenesis in the vestimentiferan tube worm *Riftia pachyptila* (Pogonophora: Obturata). *Trans. Am. Microsc. Soc.* **104:** 19–44.

Gardiner, S. L., and M. L. Jones. 1993. Vestimentifera. Pp. 371–460 in *Microscopic Anatomy of Invertebrates,* Vol. 12, F. W. Harrison and M. E. Rice, eds. Wiley-Liss, New York.

Gardiner, S. L., S. E. Shrader, and M. L. Jones. 1992. Preliminary observations on oogenesis in the tube worm *Riftia pachyptila* Jones (Vestimentifera). *Am. Zool.* **32:** 124A.

Hessler, R. R., W. M. Smithey, M. A. Boudrias, C. H. Keller, R. A. Lutz, and J. J. Childress. 1988. Temporal change in megafauna at the Rose Garden hydrothermal vent (Galapagos Rift; eastern tropical Pacific). *Deep-Sea Res.* **35:** 1681–1709.

Jollivet, D., A. Emphis, M. C. Baker, S. Hourdez, T. Comtet, C. Jouin-Toulmond, D. Debruyères, and P. A. Tyler. 2000. Reproductive biology, sexual dimorphism, and population structure of the deep sea hydrothermal vent scale-worm, *Branchipolynoe seepensis* (Polychaete: Polynoidae). *J. Mar. Biol. Assoc. UK* **80:** 55–68.

Jones, M. L. 1981. *Riftia pachyptila* Jones: observations on the vestimentiferan worm from the Galapagos Rift. *Science* **213:** 333–336.

Jones, M. L. 1985. On the Vestimentifera, new phylum: six new species, and other taxa, from hydrothermal vents and elsewhere. *Bull. Biol. Soc. Wash.* **6:** 117–158.

Jones, M. L., and S. L. Gardiner. 1985. Light and scanning electron microscopic studies of spermatogenesis in the vestimentiferan tube worm *Riftia pachyptila* (Pogonophora: Obturata). *Trans. Am. Microsc. Soc.* **104:** 1–18.

Jones, M. L., and S. L. Gardiner. 1989. On the early development of the vestimentiferan tube worm *Ridgeia* sp. and observations on the nervous system and trophosome of *Ridgeia* sp. and *Riftia pachyptila. Biol. Bull.* **177:** 254–276.

Levitan, D. R. 1991. Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. *Biol. Bull.* **181:** 261–268.

Levitan, D. R., and C. M. Young. 1995. Reproductive success in large populations: empirical measures and theoretical predictions of fertilization in the sea biscuit *Clypeaster rosaceus. J. Exp. Mar. Biol. Ecol.* **90:** 221–241.

Lutz, R. A., T. M. Shank, D. J. Fornari, R. M. Haymon, M. D. Lilley, K. L. Von Damm, and D. Desbruyères. 1994. Rapid growth at deep-sea vents. *Nature* **371:** 663–664.

MacDonald, I. R., V. Tunnicliffe, and E. C. Southward. 2002. Detection of sperm transfer and synchronous fertilization in *Ridgeia piscesae* at Endeavour Segment, Juan de Fuca Ridge. *Cah. Biol. Mar.* **43:** 395–398.

Malakhov, V. V., I. S. Popelyaev, and S. V. Galkin. 1996. Microscopic

anatomy of *Ridgeia phaeophiale* Jones, 1985 (Pogonophora, Vestimentifera) and the problem of the position of Vestimentifera in the system of the Animal Kingdom. IV. Excretory and reproductive systems and coelom. *Russ. J. Mar. Biol.* **22:** 249–260.

Marsh, A. G., L. S. Mullineaux, C. M. Young, and D. T. Manahan. 2001. Larval dispersal potential of the tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents. *Nature* **411:** 77–80.

McHugh, D. 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. *Proc. Natl. Acad. Sci. USA* **94:** 8006–8009.

Metaxas, A., R.E. Scheibling and C.M. Young. 2002. Estimating fertilization success in marine benthic invertebrates: a case study with the tropical sea star *Oreaster reticulatus*. *Mar. Ecol. Prog. Ser.* **226:** 87–101.

Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. *Biol. Bull.* **169:** 417–430.

Rouse, G. W., and K. Fauchald. 1997. Cladistics and polychaetes. *Zool. Scr.* **26:** 139–204.

Shank, T. M., D. J. Foranri, K. L. Von Damm, M. D. Lilley, R. M. Haymon, and R. A. Lutz. 1997. Temporal and spatial patterns of biological community development at nascent deep-sea hydrothermal vents (9° 50′N, East Pacific Rise). *Deep-Sea Res. II* **45:** 465–515.

Southward, E.C. 1999. Development of Perviata and Vestimentifera (Pogonophora). *Hydrobiologia* **402:** 185–202.

Southward, E. C., and K. A. Coates. 1989. Sperm masses and sperm transfer in a vestimentiferan, *Ridgeia piscesae* Jones, 1985 (Pogonophora: Obturata). *Can. J. Zool.* **67:** 2776–2781.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25:** 1–45.

Van der Land, J., and A. Norrevang. 1977. Structure and relationships of *Lamellibranchia* (Annelida, Vestimentifera). *K. Dan. Vidensk. Selsk. Biol. Skr.* **21(3):** 1–102.

Van Dover, C. L. 1994. *In situ* spawning of hydrothermal vent tubeworms (*Riftia pachyptila*). *Biol. Bull.* **186:** 134–135.

Webb, M. 1977. Studies on *Lamellibrachia barhami* (Pogonophora) II—The reproductive organs. *Zool. Jahrb. Anat. Bd.* **97:** 455–481.

Westheide, W. 1988. Genital organs. Pp. 263–279 in *Ultrastructure of the Polychaeta*, W. Westheide and C.O. Hermans, eds. Gustav Fischer, Stuttgart.

Young, C. M. 1999. Synchrony and sociality: breeding strategies in constant and variable environments. Pp. 1–4 in *Aquatic Life Cycles Strategies: Survival in a Variable Environment*, M. Whitfield, J. Matthews, and C. Reynolds, eds. Mar. Biol. Assoc., Plymouth, UK.

Young, C. M., E. Vásquez, A. Metaxas, and P. A. Tyler. 1996. Embryology of vestimentiferan tube worms from deep-sea methane/sulfide seeps. *Nature* **381:** 514–516.

Zal, F., P. Chevaldonné, and D. Debruyères. 1995. Reproductive biology and population structure of the deep-sea hydrothermal vent worm *Paralvinella grasslei* (Polychaeta: Alvinellidae) at 13°N on the East Pacific Rise. *Mar. Biol.* **122:** 637–648.