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Reproductive characteristics of a primitive bivalve from a deep-sea reducing environment: giant gametes and their significance in *Acharax alinae* (Cryptodonta: Solemyidae)

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ABSTRACT: Only 4 specimens of the cryptodont protobranch *Acharax alinae* have been found and collected to date, from the hydrothermal vent region of the Lau Basin (Fiji) in May 1989. The gonad organization and gametogenic cells of the 2 working specimens were investigated in the present study, using histology and transmission electron microscopy, in order to enhance our understanding of the reproductive biology of bivalves from deep-sea reducing habitats. The acini of the female were elongated and closely appressed to the ellipsoid-shaped mature oocytes, giving a tubular appearance. In both male and female the structural characteristics of the gametogenic cells were similar to those previously described for littoral species. The presence of female gametes in all stages of development suggests that spawning is continuous or at least repeatedly partial in this species. The mature female and male gametes were extraordinarily large: the equivalent spherical diameter for the mature oocytes was up to 600 µm; correction for fixation shrinkage would increase this size to approx. 660 µm. The mature spermatozoon presented a head+midpiece length of 28 µm and a flagellum length of approx. 100 µm. These dimensions for the male and female gametes are, to our knowledge, the greatest ever reported for any bivalve species. As the pericalymma larva of the Solemyidae is a non-feeding stage, the large oocyte size is probably an adaptation for an extended lecithotrophic strategy, which would favour either long-range dispersal or protracted benthic development. The unusually elongated spermatozoon is probably a consequence of the large oocyte size. Its morphology is distinct from those of the other bivalve subclasses, which however do present species showing some spermatozoon head elongation or curvature. More generally, we suggest that spermatozoon morphology in bivalve taxonomy is most useful in groups with homogeneous developmental strategies.

KEY WORDS: Reproduction · Deep-sea · *Acharax* · Solemyidae · Gametes

INTRODUCTION

Although considerable attention has recently been directed to the feeding biology of organisms inhabiting deep-sea hydrothermal vents and cold seeps (see Cavanaugh 1985, Page et al. 1990, Tunnicliffe 1991, Childress & Fisher 1992, and Le Pennec et al. 1995 for reviews and references), the reproductive biology of these organisms remains relatively obscure (Tunnicliffe 1991, Mullineaux & France 1995). Investigations of reproductive biology necessarily begin with the organization of the gonad and gametogenesis. The study of gametes allows insights into 2 fundamental areas: (1) From the gametogenesis and morphology of female gametes it is possible to deduce the nutritional strategy of early developmental stages (larvae). This is closely related to the amount of vitellus and size of the mature oocyte (Sastry 1979). (2) From the gametogenesis and morphology of male gametes it is possible to elucidate phylogenetic relationships with other taxa. The utility of spermatozoon characteristics for the study of phylogeny and taxonomy began to take root with

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the work of Franzén (1970), Popham (1979), Maxwell (1983) and Healy (1989). Interest in this concept has recently surged, with substantial progress in the understanding of phylogenetic relationships (for reviews, see Jamieson et al. 1995, Healy 1996). The morphology and ultrastructure of spermatozoa of deep-sea reducing environment fauna is particularly interesting, as the phylogenetic relationships among these organisms and with taxa from more conventional habitats remains obscure (Le Pennec & Beninger 1997).

One of the least well-known species from the deep-sea reducing habitats is the recently discovered protobranch *Acharax alinae* (Métivier & von Cosel 1993) Only 4 adults of this Solemyidae have been recovered to date in the vicinity of hydrothermal vents; 2 are reference specimens at the Muséum National d'Histoire Naturelle in Paris, France, and 2 working specimens have been used in the present study.

Of the 2 orders (Solemyioida and Praecardidioida) of the subclass Cryptodonta, only 2 genera of Solemyioida survived beyond the Palaeozoic to the present: *Solemya* and *Acharax*. All of the Solemyioida discovered to date inhabit reducing environments and contain endosymbiotic bacteria. Twelve species have been assigned to the genus *Acharax*, of which 7 are found at depths greater than 900 m. It is difficult to estimate their abundance, since they are deeply buried endobenthic organisms (Type 3 species from the standpoint of sulphide availability: Le Pennec et al. 1995), which until the 1989 Biolau expedition were only known by their shells, and in one case a single live specimen of the genus *Acharax* (see Métivier & von Cosel 1993). Knowledge of the reproductive biology of this species would thus contribute to our understanding of the intriguing deep-sea reducing ecosystems.

In this study we present the histological and ultrastructural characteristics of the gonad and gametes in *Acharax alinae*, including data on gametogenesis and the unusually large size of mature gametes.

MATERIALS AND METHODS

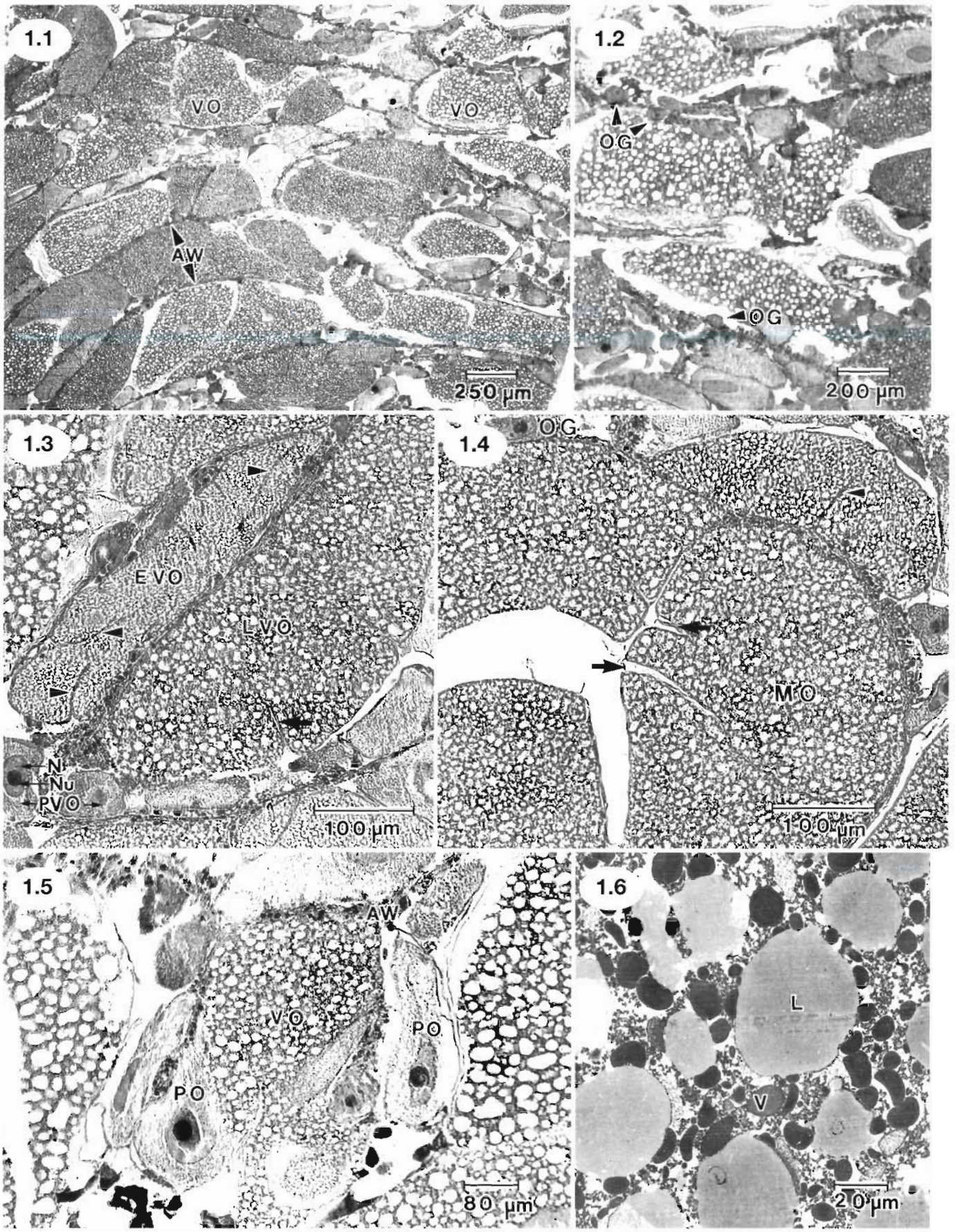
The specimens of *Acharax alinae* were obtained at −1890 m using the IFREMER submersible 'Nautile' and mother ship 'Nadir', in May 1989 during the Biolau mission in the Lau Basin near the Fiji islands. The location of the sampling site, baptized 'Hine-Hina', was 22° 32' S, 176° 43' W. At sampling, the specimens were buried in coarse sediment in proximity to a hydrothermal vent, although no temperature anomaly was recorded at the sampling site. The female specimen measured 91 mm and the male 102 mm along the antero-posterior axis. Upon arrival shipboard, the specimens were fixed whole in 10% formalin seawater. Two were retained as reference specimens at the Muséum National d'Histoire Naturelle, Paris, France, and 2 (1 male and 1 female) were transferred to the Laboratoire de Biologie Marine, Brest, France. Approx. 5 mm^3 pieces of the gonad were dissected out and re-fixed in aqueous Bouin's medium and processed for paraffin histology, using the Goldner variation of the Masson trichrome staining procedure on 5 µm sections. Small (1 to 2 mm) pieces of the gonad were also dissected out and post-fixed in 3% glutaraldehyde-sodium cacodylate buffer (0.4 M, adjusted to 1300 mosM with 7% NaCl, pH 7.25). Previous studies had shown that while this procedure was not optimal, it did not induce distortion in sensitive structures such as mitochondria (Le Pennec & Beninger 1997). The pieces were then rinsed in cacodylate buffer, dehydrated in an ascending ethanol gradient, embedded in Spurr resin, and cut using an ultramicrotome. Thin sections (1 to 2 µm) were stained with toluidine blue, while ultrathin sections (100 nm) were contrasted with uranyl acetate and lead citrate for transmission electron microscopy (TEM).

RESULTS

Female gonad

Two anatomical indices suggest that the acini of the female were greatly elongated and almost tubular: (1) the vitellogenic and mature oocytes were ellipsoid in shape, and (2) the germinal epithelium walls were closely appressed to these oblong gametes (Fig. 1.1–1.3). No reserve tissue was observed between the acini (Fig. 1.1, 1.2). Some oogonia (approx. 80 µm diameter)

Fig. 1. *Acharax alinae*. Gonadal structure and details of female gametes. (1.1) General histological organization of the female gonad, showing vitellogenic oocytes (VO) within elongated acinal walls (AW). 1 µm section, toluidine blue stain. (1.2) Detail of female gametes, showing oogonia (OG) adhering to acinal walls. 1 µm semithin section, toluidine blue stain. (1.3, 1.4) Detail of vitellogenic oocytes, showing deep, tightly appressed folds of cell membrane in early vitellogenic oocytes (arrowheads, EVO), and looser folds in late vitellogenic and mature oocytes (arrows, LVO, MO). Previtellogenic oocytes (PVO) have round nuclei (N) and nucleoli (Nu). 1 µm semithin sections, toluidine blue stain. (1.5) Very early, pedunculated oocytes (PO) adhering to acinal wall (AW), surrounded by vitellogenic oocytes (VO). 1 µm semithin sections, toluidine blue stain. (1.6) Transmission electron micrograph detail of cellular inclusions in mature vitellogenic oocyte (L, lipid droplets; V, vitelline inclusions)


1.1
VO
VO
AW
250 µm
1.2
OG
OG
200 µm
1.3
EVO
LVO
Ni
Nu
PVO
100 µm
1.4
OG
MO
100 µm
1.5
AW
VO
PO
PO
80 µm
1.6
L
V
20 µm

were observed adhering to the acinal walls, presenting a round nucleus and nucleolus (Fig. 1.2, 1.4). Both previtellogenic and vitellogenic oocytes were observed in the acini. The previtellogenic oocytes adhered to the acinal wall and measured approx. 80 to 130 µm in diameter, with a round nucleus and nucleolus (Fig. 1.3). Early vitellogenic oocytes and their nuclei showed a tendency to elongation; late vitellogenic oocytes and their nuclei were markedly elongated (Fig. 1.3). These oocytes contained an abundant cytoplasm with numerous vitelline and lipid inclusions (Fig. 1.1–1.6). The largest of the vitellogenic oocytes measured 900 × 400 µm in longitudinal section. Deep, tightly appressed invaginations of the cell membrane were common in early vitellogenic oocytes, widening in later vitellogenic and mature oocytes (Fig. 1.3, 1.4).

Although no bacteria were observed in the female gametes at any stage, the resolution limits imposed by the fixation technique preclude any conclusive statement.

Male gonad

The male gonad lacked independent anatomical features which would allow definitive judgement concerning acinal elongation as seen in the female. The germinal epithelium was surrounded by loose connective tissue (Fig. 2.1), and progressively anastomosed and emptied into evacuating ducts comprising a pseudostratified ciliated epithelium (Fig. 2.2). The acini were lined with spermatogonia (diameter 7 µm), characterized by a relatively pale nucleus and cytoplasm when stained with Masson's trichrome (Fig. 2.3). The chromatin was clumped both in the interior of the nucleus and as a discontinuous border around the nuclear periphery (Fig. 2.5). Toward the lumen the following cell types were progressively encountered: spermatocytes, spermatids, and spermatozoa (Fig. 2.3, 2.4).

Several stages of primary spermatocytes (diameter approx. 5 µm) were readily visible (Figs. 2.5, 2.6 & 3.1). The majority of these were in the first stage of prophase and located immediately adjacent to the spermatogonia, with chromatin more densely clumped than in the spermatogonia (Fig. 2.5). Toward the lumen, pachytene-stage cells were visible (Fig 2.6). No secondary spermatocytes were observed.

The spermatids were notably smaller than the spermatocytes, and depending on their stage of development and plane of section were either round (diameter approx. 3.5 µm) or oval (approx. 3.7 × 2.2 µm) (Fig. 2.3, 2.4). The chromatin was progressively more condensed in spermatids closer to the acinal lumen. In addition, the nucleus began to elongate, the acrosome began to form as a small cone, and the mitochondria migrated to the basal pole of the cell (Figs. 2.4 & 3.2). Spermiogenesis continued with constitution of the single flagellum, further chromatin condensation, and an extreme elongation of both the nucleus and acrosome. The mature spermatozoon had a curved, rod-shaped head measuring 26 to 28 µm from the tip of the acrosome to the base of the 4 mitochondria (diameter approx. 5 µm each) constituting the midpiece (Fig. 3.3). The nucleus contained extremely dense and homogeneous chromatin, with rare vestiges of nucleoplasm; it measured approx. 19.6 × 0.8 µm. The acrosome had the same width as the nucleus in its basal region, tapering to a point over its approx. 7 µm length. The acrosome was electron-transparent except for the distal extremity, which was notably more electron-opaque, and the 2 small pyramidal loci at its basal region (Fig. 3.4).

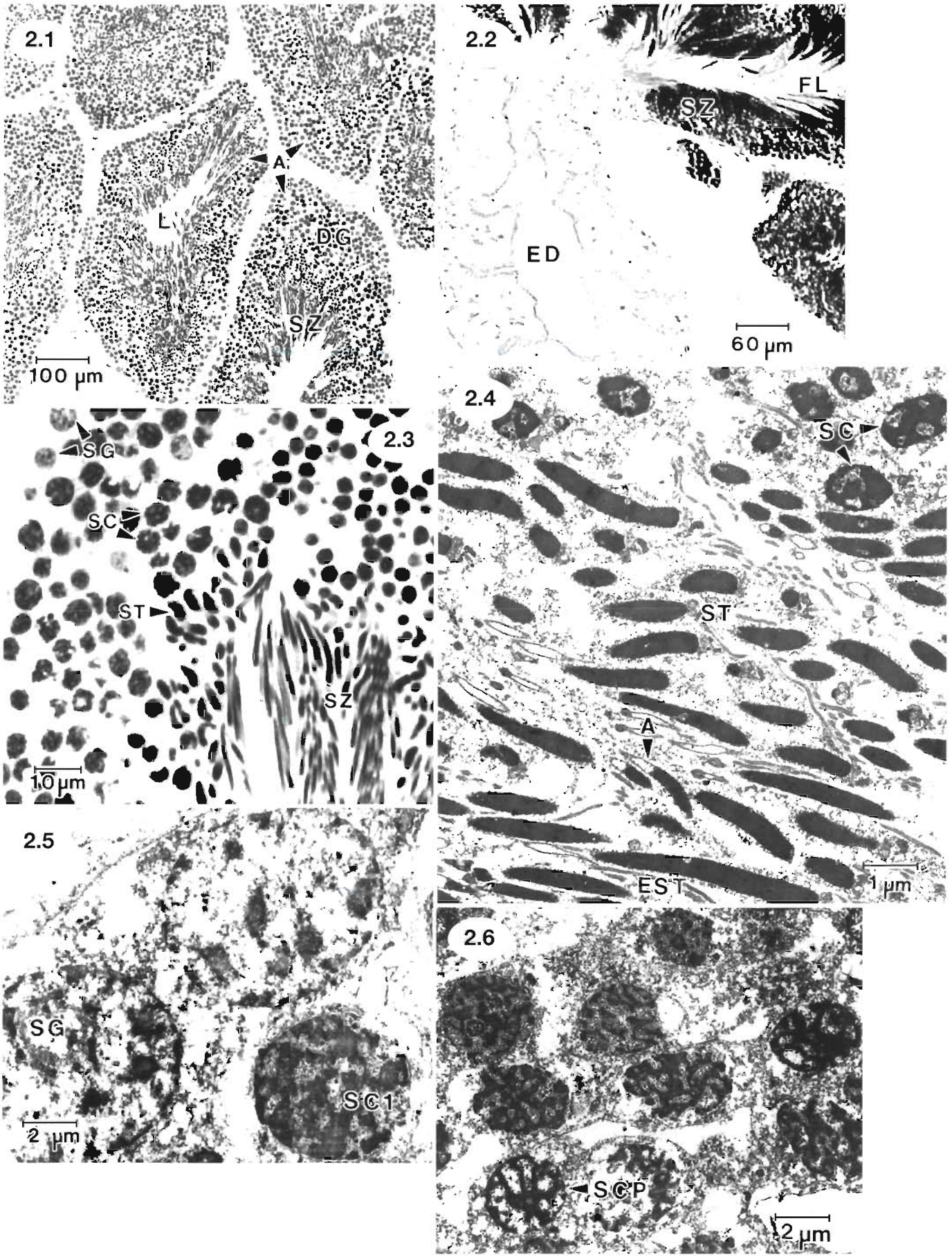
Based on measurements of histological sections, the flagellae of mature spermatozoa were approx. 100 µm in length. None of the TEM sections yielded the cross-sectional views necessary for observation of microtubule arrangement within the flagellum. Similarly, the state of fixation precluded any definitive statements concerning the presence or absence of bacteria in the male gametes.

DISCUSSION

Representativity

The results of the present study rest upon data from only 1 individual of each sex. The recovery of addi-

Fig. 2. *Acharax alinae*. General male gonadal structure and gametogenic cell types. (2.1) Transverse section through acini (A) of a maturing gonad. Developing gametes (DG) are situated at the periphery, while mature spermatozoa (SZ) progressively fill the lumen (L). 5 µm histological section, Masson's trichrome stain. (2.2) Junction of acinus and evacuating duct (ED). The acinus is partially filled with spermatozoa (SZ), with flagellae (FL) directed toward the lumen. 5 µm histological section, Masson's trichrome stain. (2.3) Overview and histological appearance of gametogenic cells. SG, spermatogonia; SC, spermatocytes; ST, spermatids; SZ, spermatozoa. 5 µm section, Masson's trichrome stain. (2.4) Transmission electron micrograph of gametogenic cells, showing spermatocytes (SC) with condensed, electron-dense nuclei and patches of electron-lucent nucleoplasm, early spermatids (ST), and elongated spermatids (EST). Note the appearance of the acrosome (A) in the elongating spermatid. (2.5) Transmission electron micrograph detail of the spermatogonia (SG) and primary spermatocytes (SC1). Note nuclear condensation in the spermatocyte. (2.6) Transmission electron micrograph of primary spermatocyte in pachytene stage of first meiotic prophase (SCP)


2.1
A
L
DG
SZ
100 µm
2.2
FL
SZ
ED
60 µm
2.3
SG
SC
ST
SZ
10 µm
2.4
SC
ST
A
EST
1 µm
2.5
SG
SC1
2 µm
2.6
SCP
2 µm

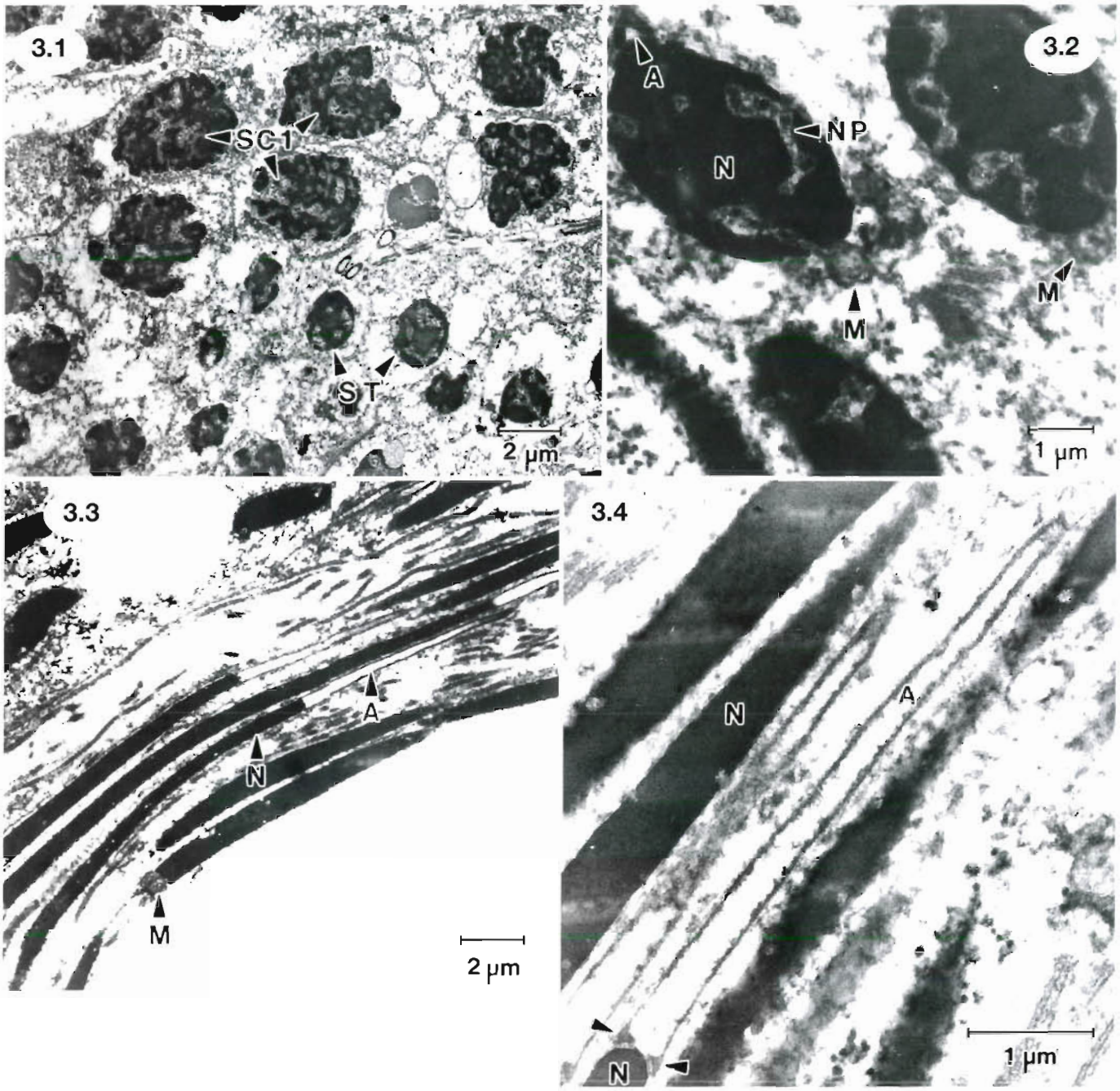


Fig. 3. *Acharax alinae*. Male gametogenic cell types. (3.1) Transition from primary spermatocytes (SC1) to early spermatids (ST). Note decrease in size and round aspect of these early spermatids. (3.2) Beginning of spermiogenesis, with mitochondrial collar (M) at basal pole of spermatid, and formation of acrosome (A) at apical pole. Nucleus (N) highly condensed, with some patches of nucleoplasm (NP). (3.3) Mature spermatozoa, showing great elongation and curvature. (3.4) Detail of the junction between nucleus (N) and acrosome (A). Note typical pyramidal structures (arrowheads)

tional specimens is unlikely, even in the event of a second expedition to the same sampling site, since *Acharax alinae* is endobenthic and not readily visible. While the probability that these are atypical specimens is low, it cannot be totally disregarded. We are unaware, however, of any previous study which reported atypically large gametes in individuals from samples, and this has also been our own experience in work on a large number of samples from several different species.

General anatomical organization

The anatomical organization of the bivalve gonad, including the coastal cryptodont *Solemya reidi*, consists of acini and anastomatosing gonoducts (Franc 1960, De Jong-Brink et al. 1983, Gustafson et al. 1987). The tubular form of the female *Acharax alinae* acini corresponds to the ellipsoid shape of the maturing oocytes, and is thus related to the packaging of the extraordinarily large gametes in the gonad of this species.

The bivalve gonad normally contains reserve tissue between the acini; the proportion of this tissue shows a reciprocal relationship to gamete development (Coe 1943, Lubet et al. 1976, Beninger 1987). The lack of reserve tissue in *Acharax alinae* suggests either that in this individual all such reserves were depleted, or that metabolite transfer to the developing oocytes is accomplished exclusively via other mechanisms (e.g. circulating haemocytes—see Le Pennec et al. 1991). The complete lack of reserve tissue contrasts with the situation found in the male gonad of 3 species of the hydrothermal vent genus *Bathymodiolus* (Le Pennec & Beninger 1997), where inter-acinal reserve tissue was found in various amounts depending on sampling date.

Female gametes

The presence of approximately equal numbers of female gametes in all stages of development indicates that spawning is continuous or at least repeatedly partial in this species. A similar observation was made by Berg (1985) for the hydrothermal vent vesicomyid clam *Calyptogena magnifica*, and by Gustafson et al. (1987) for the littoral reducing-sediment clam *Solemya reidi*. Too little is known of reproductive dynamics in deep-sea reducing environment bivalves to permit generalizations, however, since evidence for seasonality was found in 2 *Calyptogena* species from Monterey Canyon (Lisin et al. 1997).

The large size of the female gametes is remarkable. The first cells of the germ line, the oogonia, are as large or larger (80 µm) than the mature oocytes of most bivalves (Table 1). Assuming a roughly elliptical shape within the elongated gonad acini, the equivalent circular TEM micrograph diameter of fixed, mature oocytes can be calculated using the formula

$$d = \sqrt{a \times b}$$

where d is the circular diameter and a, b are the lengths of the major and minor elliptical axes. The resulting diameter for *Acharax alinae* oocytes up to 900×400 µm is 600 µm. A literature survey comprising 62 bivalve species, belonging to 30 families, 8 orders, and 5 of the 6 subclasses, shows that this diameter is 6.5 standard deviations from the overall mean oocyte diameter and 11 standard deviations from the mean of deep-sea bivalves from conventional habitats (Table 1). The largest size reported to date for littoral bivalve oocytes is *Musculus discors* at 300 × 220 µm, or a spherical diameter of 257 µm (Franzén 1983), while the largest diameter reported for any previous species was that of the hydrothermal vent clam *Calyptogena magnifica* (482 µm; Berg 1985). Many of the oocyte diameters reported in Table 1 correspond to measures of spawned eggs, which were not subject to dehydration/embedding shrinkage. The deep folds observed in the cell membranes of *A. alinae* oocytes probably result from the elliptical distortion of oocytes which become spherical upon spawning. Assuming a dehydration/embedding shrinkage coefficient of 10% (Bozzola & Russell 1992), the estimated diameter of spawned *A. alinae* eggs would be approximately 660 µm, dwarfing all other bivalve oocytes of which we are aware, including the contemporary littoral cryptodonts *Solemya reidi* and *S. velum* (Table 2).

With the exception of their large size and elongation, the female gametes of *Acharax alinae* presented the succession of gamete histological characteristics typical of oogenesis in bivalves from much more recent taxa: oogonia, previtellogenic oocytes, pedunculated oocytes, early and late vitellogenic oocytes, mature oocytes (Sastry 1979, Pipe 1987, Dorange & Le Pennec 1989a, Beninger & Le Pennec 1991, Eckelbarger & Davis 1996).

Male gametes

In contrast to the oogonia, the spermatogonia size (diameter 7 µm) was similar to that reported for other bivalves (Sastry 1979, Dorange & Le Pennec 1989b). The histological and ultrastructural profile of these cells was also similar to these data. The subsequent decrease in size of the primary spermatocytes and the spermatids is also quite typical of marine bivalves, as is their ultrastructural profile (Sastry 1979, Dorange & Le Pennec 1989b). With the exception of the extreme elongation of the spermatid and spermatozoon, the attributes and stages of gametogenesis in *Acharax alinae* are similar to those of more contemporary bivalve taxa, indicative of conservation throughout the evolution of this class.

The length of the mature spermatozoon in *Acharax alinae* is unsurpassed in the entire bivalve class. A literature survey of 44 species from 17 families, 6 orders and 3 of the 4 subclasses other than the Cryptodonta shows the mean length and standard deviation of the head+midpiece to be 5.1 ± 3.7 µm, while the

Table 1. Maximum reported diameters for bivalve oocytes. S.C.: subclass; O.: order; F.: family. *Acharax alinae* was not included in calculations of mean ± SD values

Classification	Maximum reported oocyte diameter (µm)	Source
S.C. Cryptodonta		
O. Solemyioida		
F. Solemyidae		
Solemya reidi	271	Gustafson & Reid (1986)
Solemya velum	200	Gustafson & Reid (1986)
Acharax alinae	**600**	**Present study**
S.C. Palaeotaxodonta		
O. Nuculoida		
F. Nuculidae		
Nucula sulcata	160	Franzén (1983)
Nucula nucleus	180	Franzén (1983)
S.C. Pteriomorphia		
O. Arcoida		
F. Glycymeridae		
Glycymeris glycymeris	170	Lucas (1965)
O. Mytiloida		
F. Mytilidae		
Bathymodiolus sp.	60	Le Pennec et al. (1984)[a]
Modiolus barbatus	49	Cahour (1968)
Modiolus modiolus	100	Franzén (1983)
Musculus discors	257	Franzén (1983)
Mytilus edulis	70	Franzén (1983)
F. Pinnidae		
Pinna nobilis	80	De Gaulejac et al. (1995)
O. Pterioida		
F. Pectinidae		
Aequipecten irradians	63	Sastry (1968)
Argopecten gibbus	60	Costello et al. (1973)
Chlamys distorta	70	Le Pennec (1978)
Chlamys opercularis	70	Amirthalingam (1928)
Chlamys tehuelcha	45	Christiansen & Oliver (1971)
Chlamys varia	70	Le Pennec (1978)
Equichlamys bifrons	120	Dix (1976)
Pecten maximus	90	Mason (1958)
Pecten meridionalis	71	Dix & Jardin (1975)
Placopecten magellanicus	90	Naidu (1970)
F. Anomiidae		
Anomia ephippium	49	Cahour (1968)
F. Ostreidae		
Crassostrea virginica	45	Daniels et al. (1971)
S.C. Palaeoheterodonta		
O. Unionida		
F. Unionidae		
Cucumerunio novaehollandiae	40	Jones et al. (1986)
S.C. Heterodonta		
O. Veneroida		
F. Lucinidae		
Codakia orbicularis	108	Alatalo et al. (1984)
F. Cyrenoididae		
Cyrenoida floridana	133	Kat (1982)
F. Ungulinidae		
Diplodon chilensis	150	Peredo & Parada (1984)
F. Astartidae		
Astarte borealis	200	Thorsen (1946)[c]
Astarte elliptia	200	Thorsen (1946)[c]
Astarte montagui	200	Thorsen (1946)[c]
Astarte sulcata	220	Franzén (1983)[c]
F. Cardiidae		
Cardium exiguum	64	Thorsen (1946)
Cardium fasciatum	80	Thorsen (1946)
Cerastoderma edule	50	Thorsen (1946)
F. Mactridae		
Spisula solidissima	40	Jones (1981)
Spisula subtruncata	64	Cahour (1968)
F. Solenidae		
Ensis siliqua	70	Thorsen (1946)
F. Tellinidae		
Macoma baltica	80	Thorsen (1946)
Macoma calcarea	170	Thorsen (1946)[c]
F. Scrobiculariidae		
Scrobicularia plana	58	Lucas (1965); very large spermatozoa, small oocyte
F. Arcticidae		
Arctica islandica	80	Jones (1981)
F. Vesicomyidae		
Calyptogena magnifica	482	Berg (1985)[a]
F. Corbiculidae		
Corbicula sp.	45	Kennedy & Van Huekelem (1985)
F. Veneridae		
Venerupis aurea	73	Cahour (1968)
Tapes decussatus	90	Gharagozlou-Van Ginnekin & Pochon-Masson (1971)
Venerupis pullastra	97	Cahour (1968)
Venus gallina	80	Thorsen (1946)
Venus ovata	50	Thorsen (1946)
Venus striatula	60	Lucas (1965)
Turtonia minuta	145	Franzén (1983)
F. Pristiglomidae		
Microgloma yongei	120	Sanders & Allen (1973)[b]
Pristigloma alba	115	Sanders & Allen (1973)[b]
Pristigloma nitens	190	Sanders & Allen (1973)[b]
F. Psammobiidae		
Gari fervensis	160	Thorsen (1946)[c]
F. Siliculidae		
Silicula mcalisteri	90	Allen & Sanders (1973)[b]
F. Dreissenidae		
Dreissena polymorpha	60	Franzén (1983)
O. Myoida		
F. Corbulidae		
Corbula gibba	60	Thorsen (1946)
F. Hiatellidae		
Hiatella arctica	80	Thorsen (1946)
F. Pholadidae		
Zirfaea crispata	40	Thorsen (1946)
Pholas dactylus	49	Cahour (1968)
F. Teredinidae		
Teredo navalis	60	Franzén (1983)

Overall mean = 108.1 ± 75.9 µm
Deep-sea other mean = 128.8 ± 42.9 µm
Continental shelf, non-pelagic mean = 198 ± 17.9 µm

[a]Deep-sea vents or seeps
[b]Deep-sea other
[c]Continental shelf, non-pelagic oocytes

mean flagellum length is 34 ± 17.1 µm. The largest dimensions reported to date are those of *Scrobicularia plana*, with a head+midpiece length of 23.6 µm and a flagellum of 48 µm (Sousa et al. 1989). These dimensions are dwarfed by those of *A. alinae*, with a head+midpiece length of 28 µm and a flagellum length of 100 µm. The dimensions for *A. alinae* are therefore 6.2 and 3.9 standard deviations from their respective means. Spermatozoon elongation has been shown to be correlated with large oocyte size, which itself is correlated with lecithotrophic larval development [Franzén 1983; although *S. plana* appears to be an

Table 2. Maximum reported lengths (µm) for bivalve spermatozoa. *Acharax alinae* was not included in calculations of mean ± SD values

Classification	Maximum total length	Maximum length of head+midpiece	Maximum length of flagellum	Source
S.C. Cryptodonta				
O. Solemyioida				
F. Solemyidae				
Acharax alinae	**128**	**28**	**100**	**Present study**
S.C. Palaeotaxodonta				
O. Nuculoida				
F. Nuculidae				
Nucula hartvigiana		7.5		Popham & Marshall (1977)
Nucula nucleus		8		Franzén (1983)
Nucula nudens		8		Popham & Marshall (1977)
Nucula sulcata		9.2		Franzén (1983)
S.C. Pteriomorphia				
O. Arcoida				
F. Arcidae				
Barbiata foliata		3		Reunov & Hodgson (1994)
Barbiata obliquata		3		Reunov & Hodgson (1994)
F. Glycymerididae				
Glycymeris glycymeris		7.2		Lucas (1965)
O. Mytiloida				
F. Mytilidae				
Arcuatula capensis		2.3		Reunov & Hodgson (1994)
Bathymodiolus elongatus		4		Le Pennec & Beninger (1997)[a]
Bathymodiolus puteoserpentis		3.8		Le Pennec & Beninger (1997)[a]
Bathymodiolus thermophilus		3.6		Le Pennec & Beninger (1997)[a]
Brachiodontes semistriatus		4.5		Reunov & Hodgson (1994)
Modiolus barbatus		4		Cahour (1968)
Modiolus modiolus		2		Franzén (1983)
Choromytilus meridionalis	50	4.2	45.8	Hodgson & Bernard (1986)
Mytilus galloprovincialis	50	6.4	43.6	Hodgson & Bernard (1986)
Aulacomya ater	55	7	48	Hodgson & Bernard (1986)
Musculus discors		8.3		Franzén (1983)
Mytilus edulis		2.5		Franzén (1983)
O. Pterioida				
F. Pteriidae				
Pinctada albina	23.5	2.5	21	Tranter (1959)
F. Pectinidae				
Chlamys distorta	47	2	45	Lucas (1965)
Chlamys varia		2		Reddiah (1962)
Pecten maximus		2		Dorange & Le Pennec (1989b)
Placopecten magellanicus		1.5		Naidu (1970)
F. Anomiidae				
Anomia ephippium		3		Cahour (1968)
F. Ostreidae				
Crassostrea virginica		2		Franzén (1983)
S.C. Heterodonta				
O. Veneroida				
F. Astartidae				
Astarte sulcata		10.5		Franzén (1983)
F. Cardiidae				
Cerastoderma edule		6		Cahour (1968)
F. Mactridae				
Spisula subtruncata		4		Cahour (1968)
F. Scrobiculariidae				
Scrobicularia plana	71.6	23.6	48	Sousa et al. (1989); very large spermatozoa, small oocyte
F. Dreissenidae				
Dreissena bugonsis		4.7		Denson & Wang (1994)
Dreissena polymorpha		2.8		Franzén (1983)
F. Vesicomyidae				
Calyptogena magnifica	11.5	3	8.5	Le Pennec unpubl.[a]
Calyptogena pacifica	15.5	3.5	12	Le Pennec unpubl.[a]
F. Corbiculidae				
Corbicula sp.		2.5		Kennedy & Van Heukelem (1985)
F. Veneridae				
Tivela polita		3.2		Reunov & Hodgson (1994)
Venerupis aurea		5		Cahour (1968)
Venerupis corrugata		3.7		Franzén (1983)
Venerupis pullastra		5		Cahour (1968)
Venerupis rhomboides		3.8		Franzén (1983)
Tapes decussatus		7.9		Franzén (1983)
Venus striatula		7		Franzén (1983)
Turtonia minuta		10.5		Franzén (1983)
O. Myoida				
F. Pholadidae				
Pholas dactylus		5		Cahour (1968)

Overall mean: head+midpiece = 5.1 ± 3.7 µm, flagellum = 34 ± 17.1 µm

[a]Deep-sea vents or seeps

exception to this rule (Table 2)]. The unusually large size of the spermatozoon in *A. alinae* is thus probably a co-evolutionary consequence of the extremely large oocyte size.

Phylogenetic considerations

The structure of the spermatozoon of *Acharax alinae* does not resemble any of the basic types described for the other bivalve subclasses (Popham 1979, Hodgson & Bernard 1986), reinforcing the status of the Cryptodonta as a distinct subclass. Further studies on other Solemyidae, including the use of silver staining (Sousa et al. 1995), might provide additional insights.

The spermatozoon of the primitive protobranch *Nucula* spp. (Subclass Palaeotaxodonta, Order Nuculoida) is elongated but much smaller (7.5 to 11.7 µm); the acrosome is cap-shaped rather than elongated (Popham & Marshall 1977, Franzén 1983). The spermatozoon of *Dreissena bugonsis* (Subclass Heterodonta, Order Veneroida) is slightly elongated and curved, but is also much smaller (head+midpiece = 4.7 µm), and the acrosome is not elongated (Denson & Wang 1994). Furthermore, spermatozoon curvature does not appear to be a distinguishing taxonomic characteristic above the species level, as the spermatozoon of *D. polymorpha* is uncurved (Denson & Wang 1994).

Although the spermatozoon of *Scrobicularia plana* (Subclass Heterodonta, Order Veneroida) has a head+midpiece region which is only 5 µm shorter than that of *Acharax alinae*, as well as an elongated acrosome, its structure is very different, having highly unusual perinuclear mitochondria (Sousa et al. 1989, Healy 1996). It is thus apparent that nuclear and acrosome elongation are also not useful taxonomic criteria, being related to developmental strategy (Franzén 1983) rather than phylogenetic position. More generally, we may conclude that while the analysis of spermatozoon characteristics has proven to be a useful tool in taxonomy and phylogeny, it should be used with great prudence when comparing taxa presenting different developmental strategies.

Hypotheses arising from gamete size

As mentioned above, large oocyte size is traditionally associated with lecithotrophic development, i.e. either a very brief planktonic larval stage or direct development, either benthic or in a brooding female (Sastry 1979, Franzén 1983). While this interpretation holds true for 'conventional' littoral habitats, deep-sea reducing habitats present a very different constellation of parameters to which the associated fauna are adapted to different degrees (Le Pennec et al. 1995), requiring re-assessment of the adaptive value of developmental strategies in these environments (Mullineaux & France 1995). Given that the pericalymma larva of the Solemyidae is a non-feeding stage in which development is essentially direct within an autonomous ciliated test (Gustafson & Reid 1988), extensive reserves would enable a prolonged larval stage (direct absoption of dissolved organic matter or bacterial capture by the ciliated test epithelium would probably provide more sustenance for the test than the enclosed developing larva). In all likelihood, the extraordinarily large and vitellus-rich eggs of *Acharax alinae* are thus an adaptation for extended lecithotrophic development in the deep demersal environment. The longevity of the larval stage could be further extended by other mechanisms such as the low temperature of the deep-sea environment (Mullineaux & France 1995). The only available data on larval development in Cryptodonta is for the littoral species *Solemya reidi* (Gustafson & Reid 1988). The pericalymma larva of this species have a short larval period of 5 d in the laboratory. However, like the very short larval lives of the lecithotrophic Pandoridae (Subclass Anomalodesmata), this may be an adaptation to limit dispersal in species requiring highly localized but temporally stable habitats (Allen 1961, Boss & Merrill 1965). In contrast, the unspawned oocyte of *A. alinae* is twice the size of that in *S. reidi*, and thrice that in *S. velum* (Table 2), suggesting a much longer lecithotrophic larval stage. An extended demersal stage would allow either increased dispersal or protracted benthic development. It should be noted that the single prodissoconch of *A. alinae* is also unusually large among the Bivalvia: 1.35 × 0.68 mm (Métivier & von Cosel 1993), demonstrating a very large larval size (see Le Pennec 1978, 1980 and Gustafson & Reid 1986 for prodissoconch sizes in bivalves). Unfortunately, as in other cryptodonts there is no distinction between prodissoconchs I and II, so relative sizes of these structures cannot be used to verify the hypothesis of extended lecithotrophy (Turner et al. 1985).

Extended lecithotrophic development appears characteristic of some ancient lineages, as well as those groups which exploit an unusual habitat (Blacknell & Ansell 1974). This of course raises the interesting and as yet unanswered question of whether bivalves and other metazoans initially evolved in reducing habitats, especially geologically active vents (Corliss et al. 1981, Baross & Hoffman 1985, Nisbet 1985). The existence of relict species such as *Bathypecten vulcani* (Le Pennec et al. 1988), and the chronological correspondence between the extensive degree of geological activity of the earth's crust and the appearance of most of the major invertebrate taxa do indeed support this idea (see Tunnicliffe 1991).

The present study of the gonad and gametes of *Acharax alinae* has elucidated several fundamental aspects of the reproductive biology of this primitive bivalve from deep-sea reducing habitat. The questions and hypotheses it raises are complex and intriguing, with repercussions for the understanding not only of abyssal reducing habitat fauna, but also of contemporary taxa of bivalves in conventional habitats.

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