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# Reproduction in a Brackish-Water Mytilid: Gametogenesis and Embryonic Development

by

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*Abstract.* The fine structure of gametogenesis, ultrastructure of the gametes, and embryonic development at a salinity of 9‰ and at three temperatures (15, 20, and 24°C) of the brackish-water mussel *Brachidontes virgiliae* (Barnard) are described. The spermatozoon is similar to that found in other Pteriomorphia, with a mid-piece of five or six mitochondria, a round electron-dense nucleus, and a hollow conical acrosome. Implications of spermatozoon structure in determining the taxonomic position of this species are discussed. Three stages of oogenesis were recognized: previtellogenic, and early and late vitellogenic. Cortical granules appear to be the source of the vitelline membrane material. The embryonic development of *B. virgiliae* is rapid at 24°C, with spat formation within 24 h of fertilization. Development slows by a factor of 1.5 at 20°C and 2.3 at 15°C. About 18% of developing ova fail to reach the spat stage at 20 and 24°C, increasing to 34% at 15°C. The embryonic development of the animal is discussed in the light of its ecology in southern Cape estuaries and coastal lakes.

## INTRODUCTION

*Brachidontes virgiliae* (Barnard, 1964) is a mytilid bivalve mollusk found in South African waters from the Great Brak River to Mozambique (BARNARD, 1964). The mussel was first identified and named *Musculus virgiliae* by BARNARD in 1964, the name being confirmed by DAVIES (1980). More recently KILBURN & RIPPEY (1982) claim that the animal belongs to the genus *Brachidontes,* although they point out that this is by no means certain. Despite the uncertainty, however, we have elected to use the more recent classification and name.

Apart from descriptive work, there is limited information on the biology and ecology of the species. A few unpublished works deal with its distribution, feeding, reproduction, and spatfall (MCLAREN, 1977; PLUMSTEAD, 1976; SHARP, 1977; COETZEE, 1978). DAVIES (1980) noted that *Brachidontes virgiliae* inhabits the upper reaches of estuaries, favoring low, fluctuating salinities (<15‰) in areas where the substratum is relatively free of silt. Although a small bivalve (maximum shell length 30 mm), *B. virgiliae* is an extremely important component of the invertebrate standing stocks of many coastal lakes along the southern and eastern seaboard of South Africa (BOLTT, 1973; ALLANSON, 1981; DAVIES, 1982, 1984). A recent decline in standing stocks of the littoral macrophyte *Potamogeton pectinatus* Linnaeus in these coastal systems and a concomitant collapse of the *B. virgiliae* populations has had profound effects upon the food chains, with particularly severe consequences for the ichthyofauna (WHITFIELD, 1982, 1984). Clearly further ecological studies on this bivalve are called for, especially with regard to its association with *P. pectinatus* in these systems.

In this study we describe, using microscopic techniques, the fine structure of gametogenesis, the structure of the spermatozoon (which may aid the classification of this species), and the embryonic development of the organism.

## MATERIALS AND METHODS

### Gametogenesis

Specimens were collected during January and February 1984 from boulders at the head of the Kowie estuary (33°36′S, 26°54′E; Figure 1). Portions of testes and ovaries, which are found mainly in the mantle lobes, were excised from the animals and prepared for electron microscopy. Tissues were fixed in 2.5% phosphate buffered (pH 7.2) glutaraldehyde at 4°C and left overnight. After washing with phosphate buffer (pH 7.2) small pieces of tissue were post-fixed in 1.0% osmium tetroxide for 90 min, dehydrated, and embedded in Taab 812 resin via propylene oxide. Thin sections were cut with a glass knife, stained with uranyl acetate (30 min) and lead citrate (3 min), and examined with a JEOL 100 CXII microscope.

### In Vitro Embryonic Development

In vitro fertilization experiments were carried out during the summer of 1978. Viable eggs and spermatozoa were obtained from animals kept in continuous culture and "wild" stocks from Swartvlei (34°0′S, 22°46′E) and the head of the Kowie estuary (Figure 1).

Swartvlei populations of *Brachidontes virgiliae* were normally sexually active at shell lengths from 5 to 6 mm, at which size the gonads could clearly be seen through the thin shell when viewed under transmitted light. The gonads within the mantle wall are typically branched structures: female, brown to russet in color (the species is dioecious), and male gonads, creamy white. Swartvlei stocks maintained in continuous culture systems at a salinity of 4‰ provided gametes at shell lengths between 6 and 10 mm, while those maintained at 9‰ provided viable gametes at shell lengths of 7 mm and larger. Stocks from the Kowie estuary provided viable gametes at shell lengths greater than 10 mm and showed no signs of sexual activity below this size. Because of the thickness of the shell, the sex and state of gonad development of these individuals could only be determined by opening the shell valves. Specimens from the estuarine population were frequently used for in vitro fertilization experiments, but shell lengths between 15 and 27 mm gave the most consistent results in terms of successful embryonic development. Animals from either source, which were less than 4.5 mm shell length, were invariably of indeterminate sex, although gonad development was visible in a few cases (mainly Swartvlei "wild" stocks).

In vitro fertilization experiments and observations of embryonic development were carried out in petri dishes containing filtered water maintained at a salinity of 9‰ and at temperatures of 15 ± 2, 20 ± 2, and 24 ± 2°C. Gametes were obtained by removing the right valve and rupturing the exposed mantle with a fine needle. Eggs and spermatozoa released in this way were rapidly caught up by the ciliary currents of the gills and carried along the feeding grooves to the labial palps. Here they collected and were either periodically gathered by the tip of the foot and pushed ventrally from the cavity or were picked up by currents along the inner mantle wall and discharged through the posterodorsal exhalant siphon. Gametes were transferred to cavity slides or glass wells using micropipettes, mixed, and subsequently monitored using a Wild stereomicroscope. Development was photographed using an Olympus Vanox automatic exposure system.

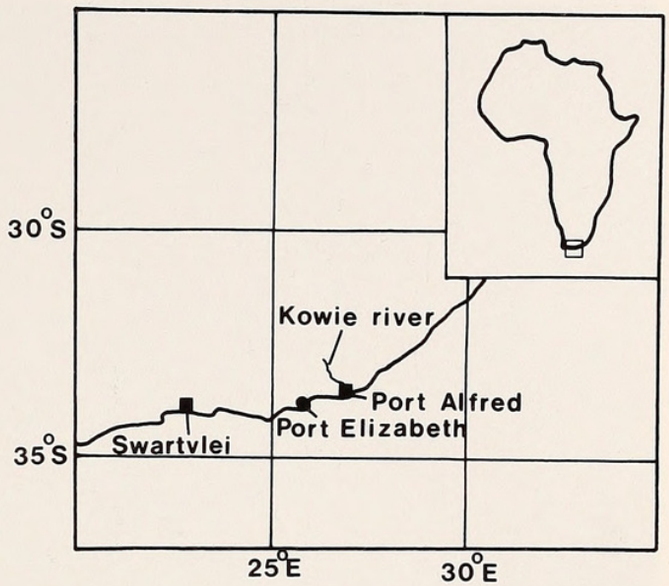



Figure 1

Map showing the positions of Swartvlei and Kowie estuaries on the coast of southern Africa, from which specimens of *Brachidontes virgiliae* were collected for studies of gametogenesis and embryonic development.

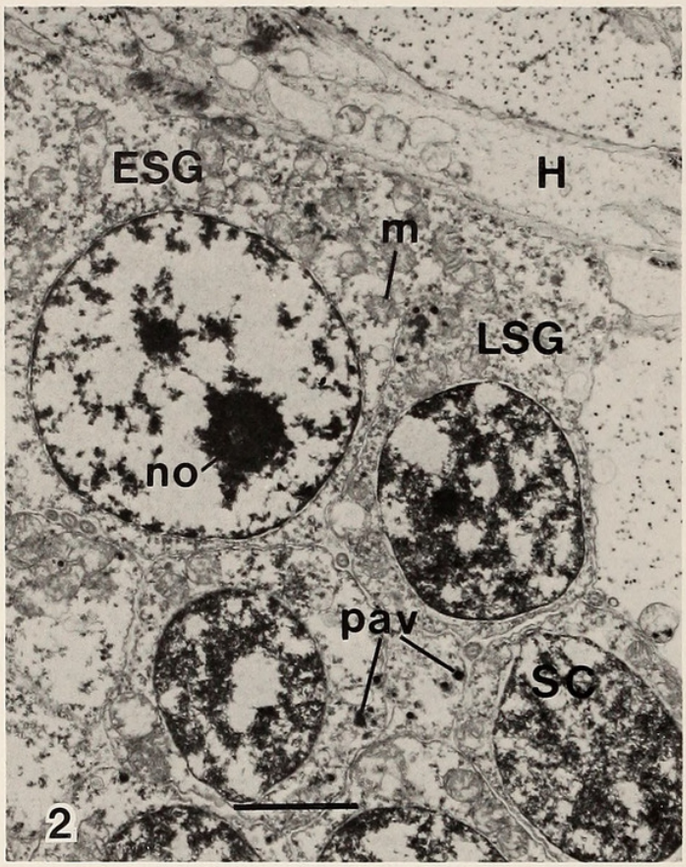
## RESULTS

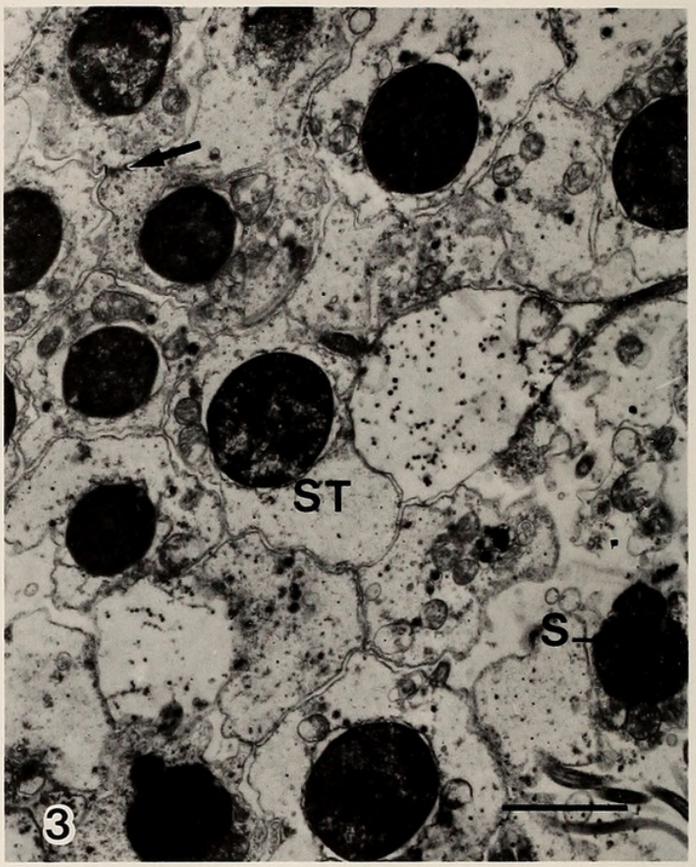
### Spermatogenesis

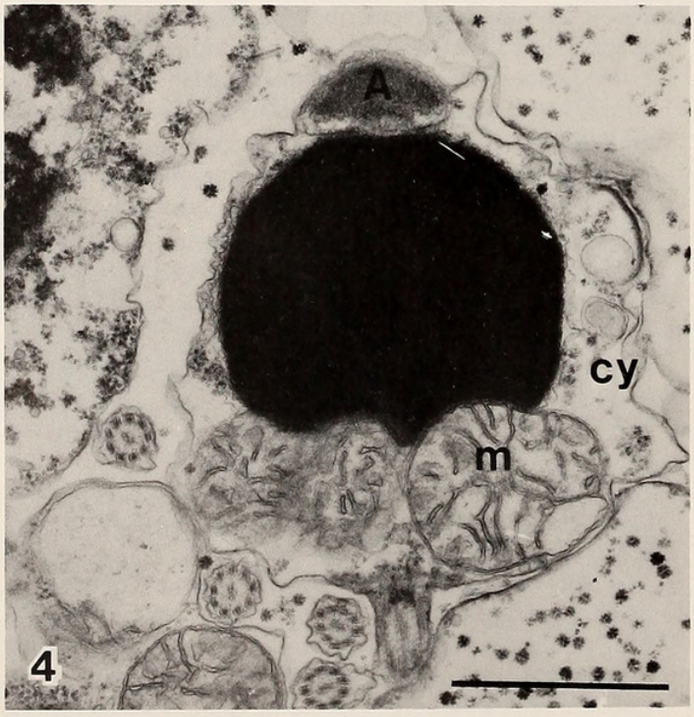
Most stages of spermatogenesis may be observed throughout the year. Early spermatogonia lie close to the haemocoelic space that surrounds each germinal follicle and are characterized by a large spherical or ellipsoidal nucleus (4 × 5 μm) with a prominent electron-dense nucleolus (0.7 μm diameter) (Figure 2). The nucleus contains small clumps of electron-dense chromatin which are often associated with the inner nuclear membrane. The cytoplasm contains numerous small mitochondria (0.3 μm diameter), free ribosomes, and rough endoplasmic reticulum. Late spermatogonia have a smaller nucleus (ca. 3 μm diameter) with a more granular electron-dense nucleoplasm (Figure 2).

Two stages of spermatocyte development, presumed to be primary and secondary, can be found in the walls of the germinal follicle of some specimens. The nucleus of the spermatocyte is similar in size and shape to that of the late spermatogonium; however, the nucleolus is no longer prominent and the chromatin is in the form of a patchwork (Figure 2). The cytoplasm contains a similar complement of organelles to the spermatogonia, as well as Golgi bodies and a few dense, osmiophilic granules—the proacrosomal vesicles (Figure 2)—which are formed by the Golgi bodies. Although similar in size and organelle complement to the primary spermatocyte, the secondary spermatocyte is characterized by less osmiophilic chromatin.

In the early stages of spermatid development the nucleus is spherical and occupies the center of the cell (Figure 3). The cytoplasm contains numerous mitochondria, rough endoplasmic reticulum, Golgi bodies, and proacrosomal vesicles. Intercellular bridges connect the spermatids. As they mature, spermatids are displaced towards the center of the germinal follicle, cytoplasm is lost by sloughing (although cells remain joined by bridges), and the nuclear contents begin to condense (Figure 3). Proacrosomal vesicles migrate to the presumptive anterior of the cell where they coalesce to form a single electron-dense vesicle (Figure




Explanation of Figures 2 to 4

Figure 2. Section through a germinal follicle wall in the testis of *Brachidontes virgiliae*. ESG, early spermatogonium; H, haemocoelic space; LSG, late spermatogonium; m, mitochondrion; no, nucleolus; pav, proacrosomal vesicles; SC, spermatocyte. Scale bar = 2 μm.

Figure 3. Portion of a germinal follicle showing spermatids (ST) and a mature spermatozoon (S). Note the intercellular bridge (arrow) linking two spermatids. Scale bar = 2 μm.

Figure 4. Longitudinal section through the middle of a late spermatid showing structure of the acrosome (A) after the proacrosomal vesicles have coalesced. Note the excess cytoplasm (cy) still surrounding the nucleus and the proximity of the mitochondria (m) to the nucleus. Scale bar = 1 μm.

4), while mitochondria become less numerous but increase in size.

As development progresses, the mitochondria come to occupy the end of the cell opposite the acrosome, forming the sperm mid-piece, and there is a steady loss of cytoplasm by sloughing, a decrease in nuclear size, and a condensation of nuclear material. The acrosomal vesicle assumes an oval shape with the short axis in the anteroposterior plane of

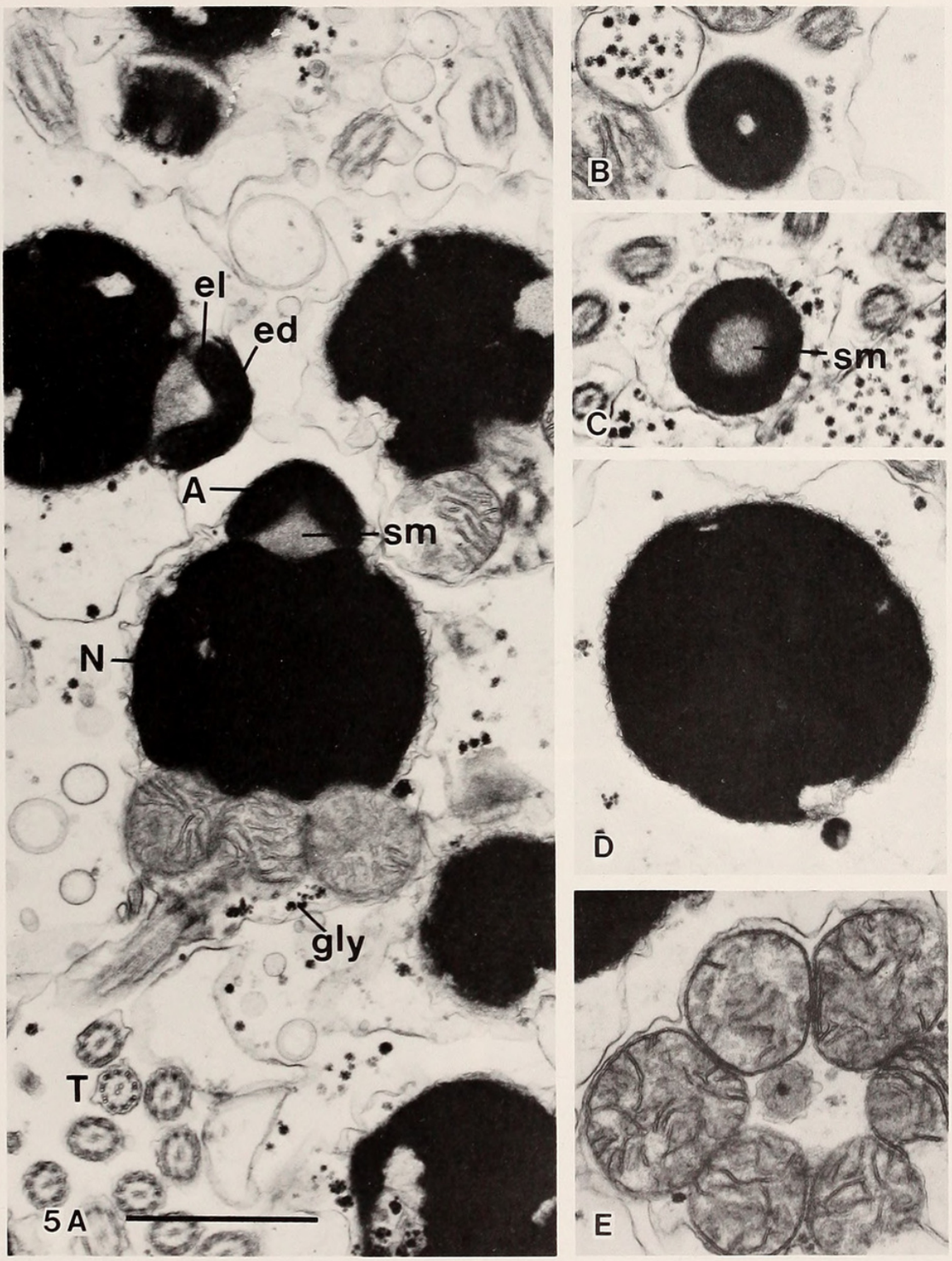
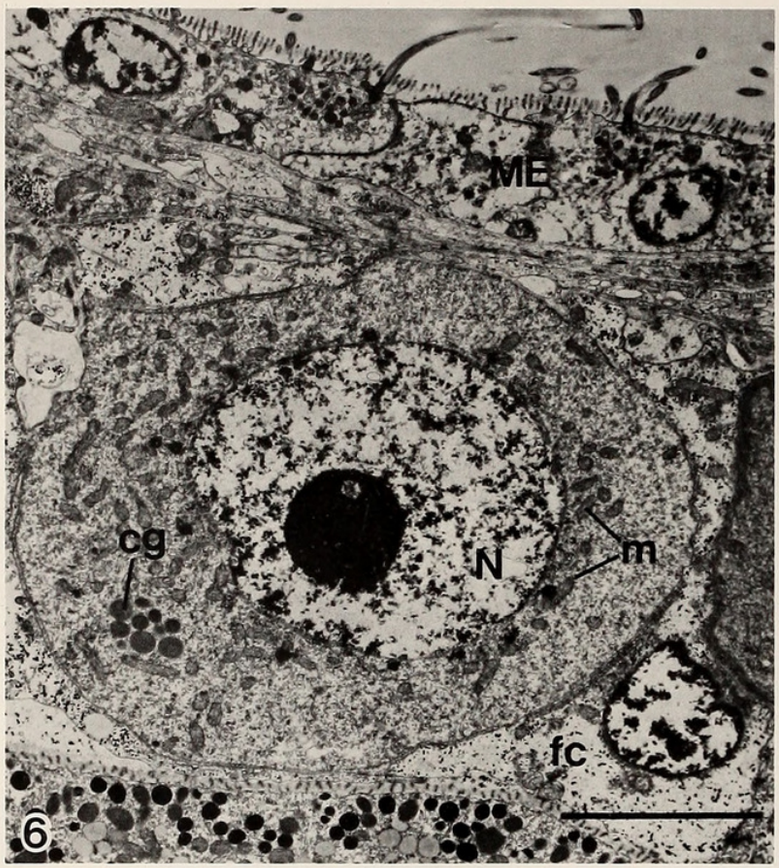



Figure 5

Longitudinal (A) and transverse (B–E) sections through a spermatozoon. B and C are sections through the acrosome; D through the nucleus, and E, the mid-piece. Key: A, acrosome; ed, electron-dense region of acrosome; el, electron-lucent region of acrosome; gly, glycogen; N, nucleus; sm, subacrosomal material; T, tail. Scale bar = 1 $\mu$m.

the spermatid (Figure 4). This is followed by invagination of the adnuclear surface and elongation to form the characteristic hollow conical-shaped acrosome (Figure 5).

Mature spermatozoa, each comprising head, mid-piece, and tail, occupy a more central position within the follicle. The head is a 1.8-$\mu$m long structure with a round to oval electron-dense nucleus (ca. 1.5 $\mu$m diameter) with a characteristic anterior fossa (Figure 5A) and an acrosome 0.5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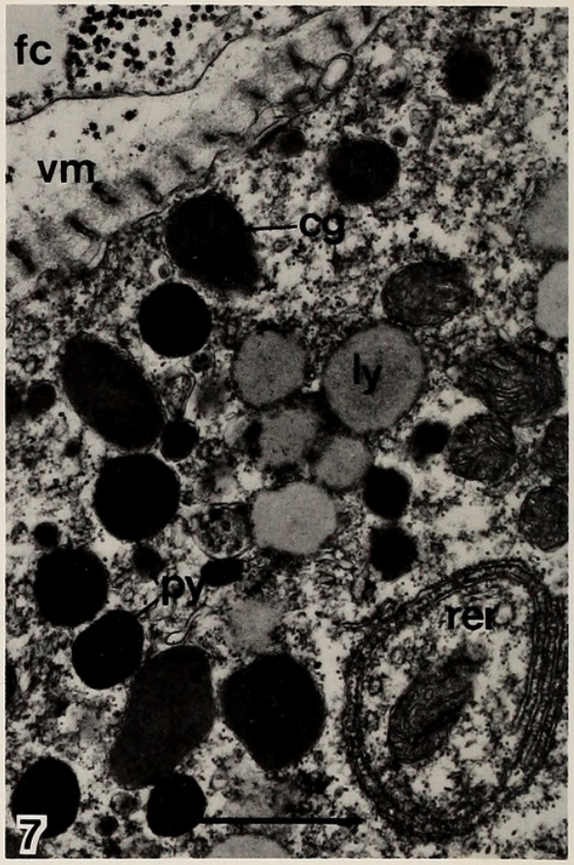
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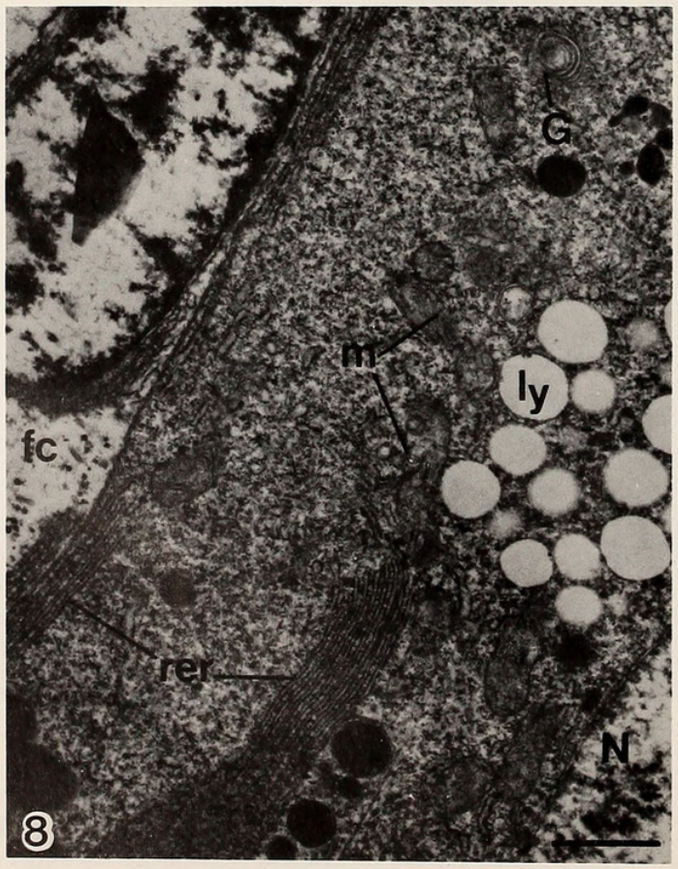
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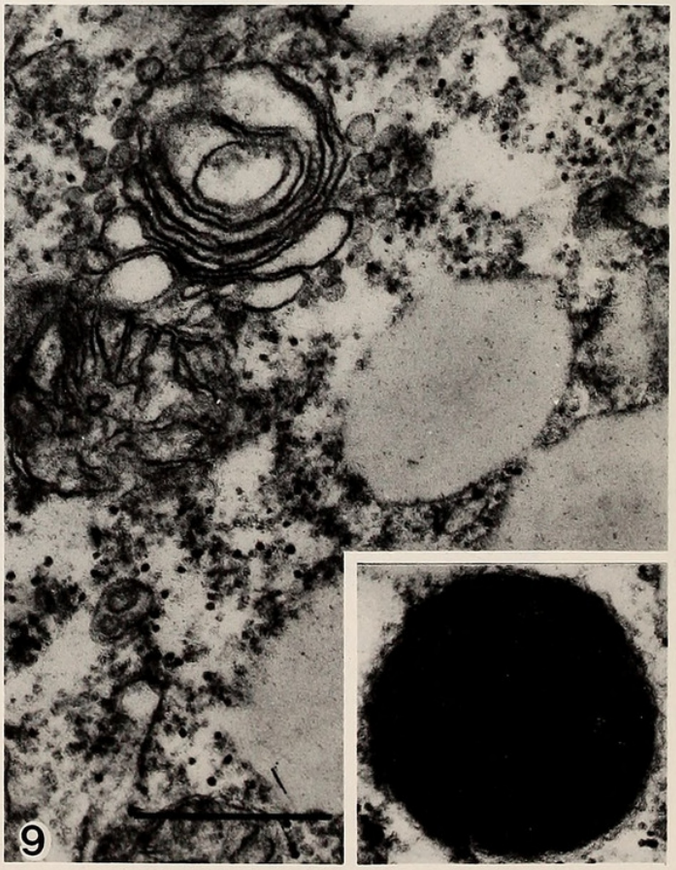
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μm long. The hollow, conical acrosome has a uniformly thick wall and contains both an outer electron-dense and inner electron-lucent substance (Figure 5A). Beneath the acrosome is subacrosomal material that has a granular appearance (Figures 5A, C).

The mid-piece contains six (rarely five) spherical mitochondria (Figure 5E) which lie tightly against the nucleus. Glycogen granules lie between the mitochondria while in the center of the ring are the proximal and distal centrioles (Figures 5A, E). The tail, which originates from the distal centriole, has the typical 9+2 arrangement of microtubules (Figure 5A).

## Oocyte Maturation

Three stages of oocyte maturation (previtellogenic, early vitellogenic, and late vitellogenic) can be recognized in the ovary of *Brachidontes virgiliae*. The previtellogenic oocyte is about 17 μm in diameter with a large (9 μm diameter) nucleus and a prominent nucleolus (Figure 6). The cell membrane has no microvilli and the vitelline membrane has not yet been formed. The cytoplasm is highly granular and contains numerous spherical and rod-shaped mitochondria, many of which are in a perinuclear position (Figure 6), some strands of rough endoplasmic reticulum, and a few cortical granules. The previtellogenic oocyte is surrounded by several follicular cells which are characteristically irregular in shape with large nuclei (Figure 6). The cytoplasm of the follicular cells is not as electron-dense as that of the oocyte and contains many glycogen granules, some mitochondria, and rough endoplasmic reticulum (Figures 6–8).

During the two vitellogenic stages there is production of lipid and protein yolk bodies and probably the continued production of cortical granules. Lipid yolk bodies, which are commonly found in association with mitochondria (Figure 8), are spherical and of variable size. They are not membrane bound, occur in clumps, and are relatively electron-lucent (Figures 7–9). Protein yolk bodies occur in two forms: in one, the contents appear to comprise numerous small vesicles, with areas of variable electron density (Figure 9, inset), while in the other, the contents are uniformly electron-dense (Figure 7). Both types of protein yolk body are spherical and membrane bound (Figures 7, 9). The cortical granules are of variable shape (often ovoid), occur singly, are membrane bound, and are of intermediate electron density (Figure 7).

During early vitellogenesis the cell membrane develops microvilli and the vitelline membrane is laid down (Figure 10). Associated with this deposition of the vitelline membrane is an arrangement of some of the cortical granules so as to be in contact with the oocyte cell membrane (Figure 10). Early vitellogenesis is further characterized by an increase in nuclear diameter to about 25 μm, with the nucleus becoming increasingly irregular in shape and the nuclear membrane more porous (Figures 10, 11). In addition to protein and lipid yolk bodies and cortical granules, the cytoplasm of the early vitellogenic oocyte contains numerous mitochondria, complex arrays of rough endoplasmic reticulum, and few Golgi bodies with associated vesicles (Figures 8, 9). In the early vitellogenic oocyte the cortical granules, protein, and lipid yolk bodies occur in the ratio 1:2:3. In the cytoplasm of the late vitellogenic or full-size oocyte (Figure 12), the cortical granules, protein, and lipid yolk bodies are abundant and occur in the ratio 1:4:5. At this stage the vitelline membrane is fully formed, and there has been no change in the complement of oocyte organelles. The follicular cells of the full-size oocyte are restricted to those parts of the oocyte that are in proximity to the germinal epithelium of the gonad.

## In Vitro Embryonic Development

**Development at 9‰ and 24 ± 2°C:** Eggs are spherical, granular and russet-brown in color, with a mean diameter of 38.3 μm (SE = ±3.6 μm, $n$ = 300) (Figure 13A). Embryonic development is rapid and the results for 23 separate in vitro fertilization experiments carried out at 9‰ and 24 ± 2°C are summarized in Table 1.

Within 5 min of mixing gametes, polar body extrusion is evident (Figure 13A; Table 1). The first division to produce a micro- and a megamere takes place within 25 min of fertilization (Figure 13A) and is followed by a second division within 45 min. Normally this takes place by division of the megamere (Figure 13A), but occasionally micromere divisions can be observed. The 8-cell stage is reached 115 min after fertilization (Table 1) and is followed by very rapid dexiotropic cleavage to form a blastula

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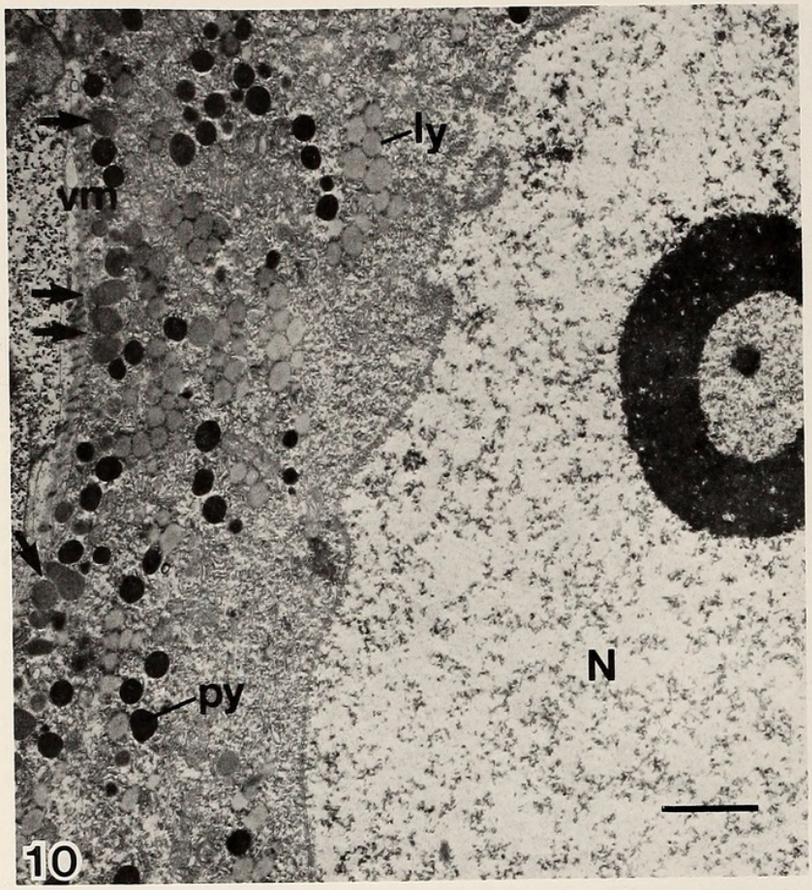
Explanation of Figures 6 to 9

Figure 6. Section through a previtellogenic oocyte showing its peripheral position in the gonad. Note that the oocyte, with its nucleus (N) and prominent nucleolus is surrounded by follicular cells (fc). cg, cortical granule; m, mitochondria; ME, mantle epithelium. Scale bar = 5 μm.

Figure 7. Section through part of a vitellogenic oocyte showing lipid yolk bodies (ly), protein yolk bodies (py), and cortical granules (cg). Note also the structure of the vitelline membrane (vm) and the glycogen in the follicular cell (fc). rer, rough endoplasmic reticulum. Scale bar = 1 μm.

Figure 8. Part of an early vitellogenic oocyte showing parallel arrays of rough endoplasmic reticulum (rer) and the close association of mitochondria (m) and lipid yolk bodies (ly). fc, follicle cell; G, Golgi body; N, nucleus. Scale bar = 1 μm.

Figure 9. Higher magnification of Golgi body and associated vesicles. Inset: protein yolk body from early vitellogenic oocyte. Scale for figure and inset the same, bar = 0.5 μm.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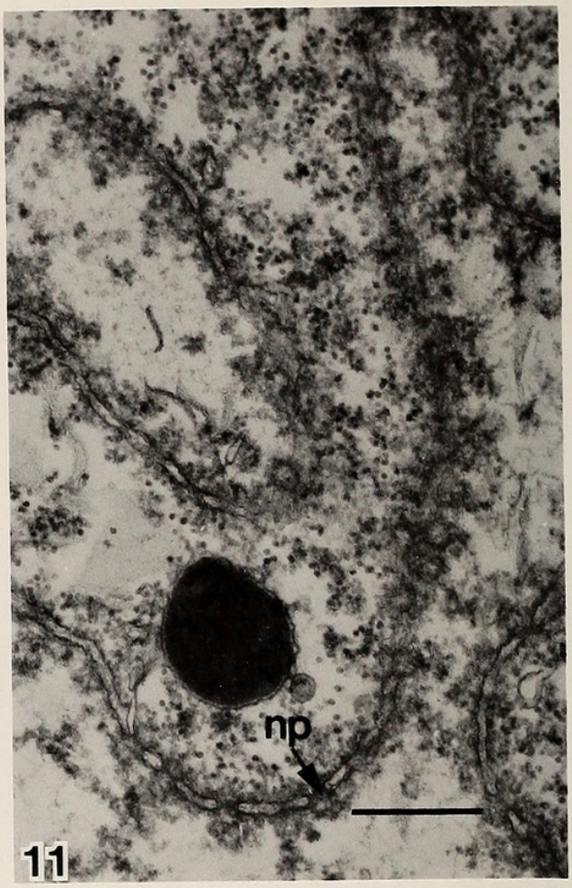
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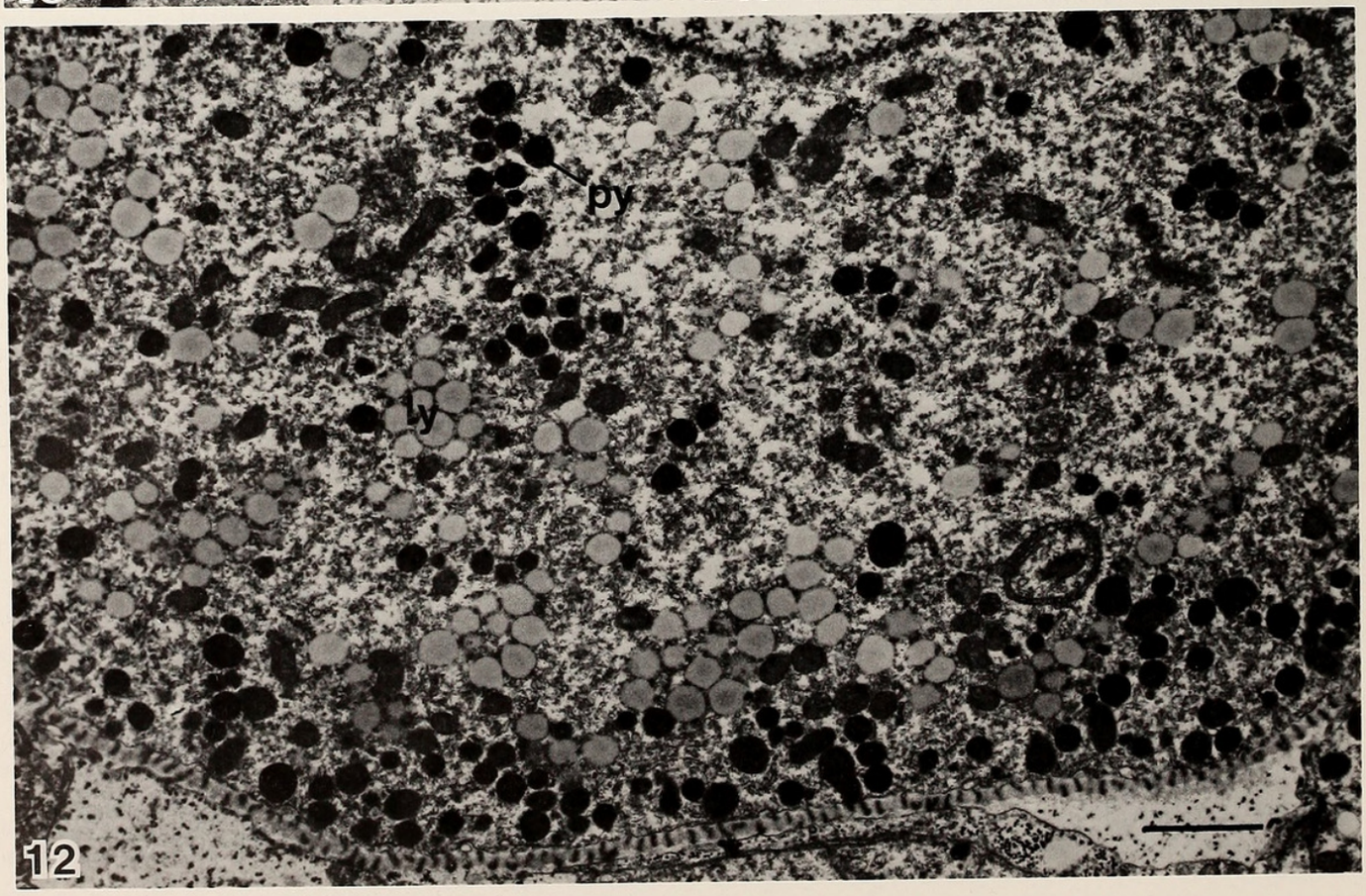
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(Figure 13B) some 4 h post-fertilization. Gastrulation (epibolic) occurs between 4 and 5 h (Figure 13C) and the morula begins to perform spiral swimming movements, as short ectodermal cirri begin to develop (Figure 14A).

Between 5 and 10 h after fertilization, the morula undergoes rapid organ differentiation, commencing with the development of velar cirri and velar lobes (Table 1), and by 10 h obvious veliger larvae (Figure 14B) are actively swimming, with more directed movements than the simple spiral swimming of earlier stages. After 20 h, embryonic development has progressed to the "early spat" stage, with the formation of the larval shell and regression of the velar lobes (Figure 14C). At this time, larvae respond to disturbance by cessation of swimming and sinking through the water column. A few hours later (ca. 24 h post-fertilization), the characteristic bilaterally flattened spat is obvious (Figure 14D), with a thickened shell and a pronounced hinge ligament. Adductor muscles are functional at this stage and, as before, the spat exhibit "avoidance" reactions to disturbance by closing the valves, cessation of swimming, and sinking to the bottom of the culture vessel. The rudimentary foot is ciliated and much of the swimming movements of spat are directed by this structure. The developing gut is visible through the transparent shell.

Additional observations indicate that spat remain active for at least a further 48 h (up to 72 h post-fertilization) after which they tend to reduce swimming activities and settle on the bottom of the culture vessel. Approximately 18% of fertilized ova fail to develop to the spat stage and of these, most fail to reach the 8-cell stage, while the remainder suffer deformity during transition from blastula to the early veliger larva.

**Development at 9‰ and 15 ± 2 or 20 ± 2°C:** In vitro embryonic development experiments at a salinity of 9‰ and at temperatures of 15 ± 2 and 20 ± 2°C were carried out during January 1979. The averaged results of four experiments at 15°C and seven at 20°C are listed in Table 2. Briefly, temperature reduction to 15°C slows development by a factor of 2.3 in terms of the time taken to reach the spat stage (up to 55 h post-fertilization). More important, perhaps, is the proportion of fertilized ova that fail to reach the spat stage—approximately 34%. In this case, most developmental failures occur in the very early stages (first and second cleavage). Embryonic development at 20°C slows by 12 h compared to that at 24°C (Table 2), while the failure rate is similar (ca. 16%).

At both 15 and 20°C, spat remain active for over 72 h post-fertilization and show no sign of settling. The longevity of spat at these temperatures is, however, unknown because of culture maintenance problems. Spat usually begin to show signs of osmotic stress after 70 h even after careful attempts to reduce water loss from culture vessels.

## DISCUSSION

The structure of the spermatozoon of *Brachidontes virgiliae* is similar to that described for other bivalves of the subclass Pteriomorphia (POPHAM, 1979). The spermatozoa of such bivalves have a mid-piece of 4–6 mitochondria and a head comprising a round electron-dense nucleus capped by a conical acrosome. It has been suggested that, when evaluated correctly, the ultrastructure of spermatozoa can be used for taxonomic purposes or as an aid to the identification of invertebrates (POPHAM, 1979; BACCETTI, 1979; ADIYODI & ADIYODI, 1983; FRANZÉN, 1983). Recent work on two closely related species of *Mytilus, M. edulis* (Linnaeus) and *M. galloprovincialis* Lamarck, has shown this to be so (HODGSON & BERNARD, 1986), for although these mussels are difficult to separate on shell characteristics, they are easily separated using spermatozoon morphology.

In the case of *Brachidontes virgiliae,* identification is still in doubt (KILBURN & RIPPEY, 1982); it was originally classified as *Musculus virgiliae* by BARNARD (1964). In an attempt to shed some light on its position, we have compared the spermatozoon of *B. virgiliae* with that of *M. discors,* which was described by FRANZÉN (1983). Figure 15 shows that the two spermatozoa are very different, suggesting that the two bivalves do not belong to the same genus. The difference, however, may be more closely linked to the mode of fertilization employed by each species. FRANZÉN (1983), for example, has noted that some invertebrates having a modified reproductive biology have an elongate nucleus. *Musculus discors* has direct development, with simple brood protection (THORSON, 1935). *Brachidontes virgiliae,* on the other hand, employs external fertilization, and like all other bivalves with external fertilization, it has a primitive spermatozoon (FRANZÉN, 1983). Clearly a comparative investigation of other species of *Brachidontes* and *Musculus,* supported by electrophoresis, is required before the problem of the correct identification of *B. virgiliae* can be solved.

The observations presented here on spermatogenesis mirror the findings of LONGO & DORNFELD (1967) for *Mytilus edulis* and BERNARD & HODGSON (1985) for *Perna perna* (Linnaeus). The greatest changes in cell morphology

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Explanation of Figures 10 to 12

Figure 10. Section through an oocyte at the early vitellogenic stage showing the enlarged nucleus (N) with irregular border. Note the alignment of the cortical granules (arrows) next to the vitelline membrane (vm). Scale bar = 1 μm.

Figure 11. Section through an oocyte at the vitellogenic stage showing the irregular nuclear membrane, with numerous nuclear pores (np). Scale bar = 0.5 μm.

Figure 12. Section through part of the full-size oocyte showing the accumulation of lipid (ly) and protein (py) yolk bodies in the ooplasm. Scale bar = 2 μm.

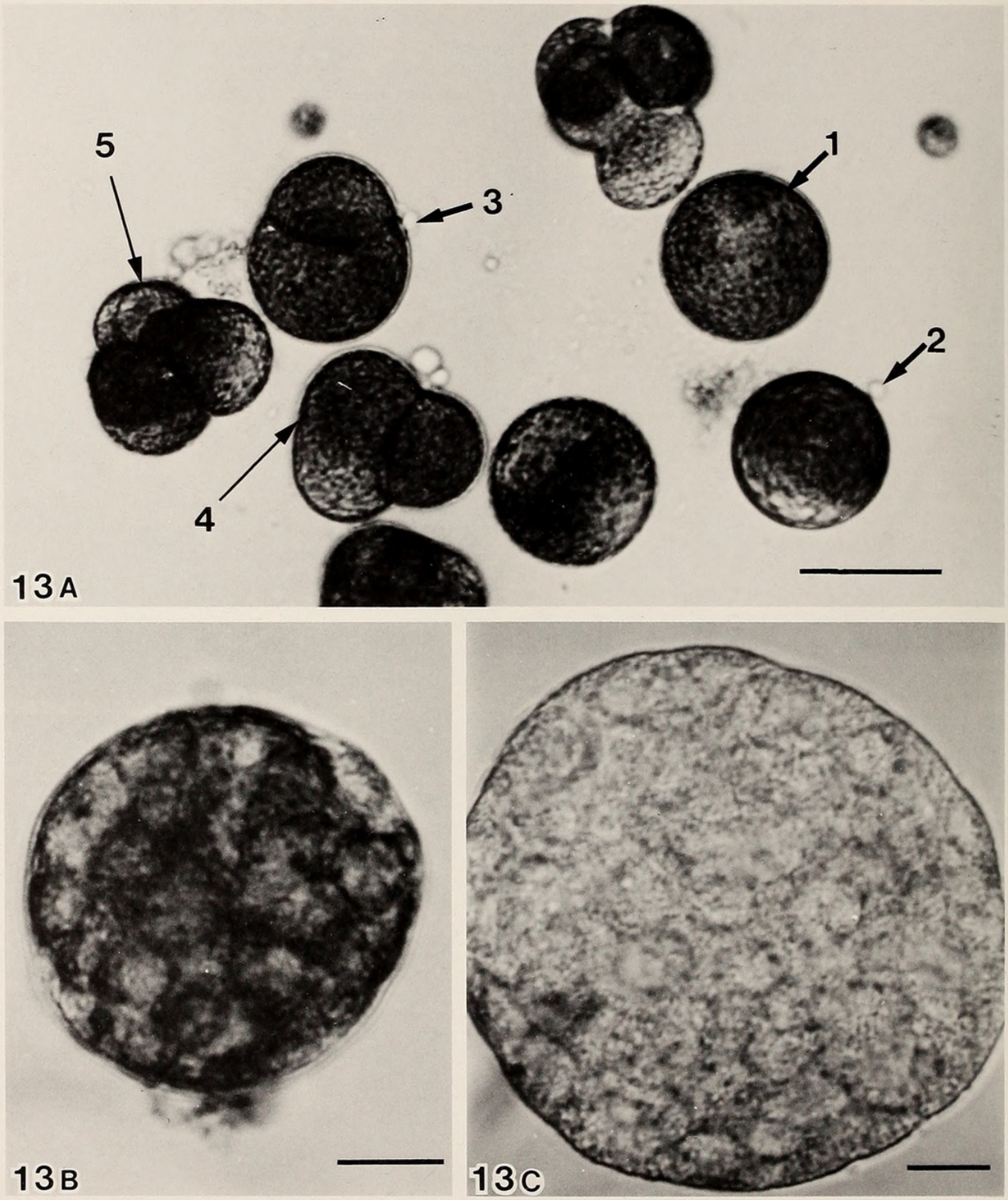


Figure 13

Photomicrographs of embryonic development of *Brachidontes virgiliae* at 24°C and 9‰. A. The newly fertilized egg (1) has a granular cytoplasm and vitelline membrane. Five minutes after fertilization the polar body (2) has been extruded, and first cleavage to produce distinct micro- and megamere (3) occurred within 30 min. (4) shows the first megamere about to cleave, and (5) a 4-cell embryo. Scale bar = 25 μm. B. Early blastula, 4 h post-fertilization. Scale bar = 10 μm. Early gastrula, approximately 4.5 h after fertilization. Scale bar = 5 μm.

occur at the spermatid stage, with nuclear chromatin condensation, mitochondrial fusion to form the mid-piece, and acrosome formation.

Two significant processes occur during oocyte maturation: the production and accumulation of yolk, and the deposition of the vitelline membrane. It is generally accepted that in mollusks, lipid yolk bodies are formed in association with mitochondria (Beams & Sekhon, 1966; Norrevang, 1968; De Jong-Brink *et al.*, 1983) and the close physical association between lipid yolk bodies and mitochondria in *Brachidontes virgiliae* supports this view. Several sources have been proposed for the protein yolk bodies (see Norrevang, 1968, for review). Humphreys (1962) has described bodies intermediate between mitochondria and protein yolk platelets in the oocytes of *Mytilus edulis*; such structures were not, however, seen in *B. virgiliae*. Beams & Sekhon (1966), Anderson (1969), and Taylor & Anderson (1969) have suggested that rough endoplasmic reticulum produces a precursor that is modified by the Golgi bodies and released as small vesicles, which later unite to form the definitive protein yolk body. Based on the abundance of rough endoplasmic reticulum, the presence of Golgi bodies, and the appearance of the two types of protein yolk body, we would suggest that these bodies in *B. virgiliae* are formed via a similar route, and further, that the protein yolk body in which small vesicles can be seen, is an intermediate stage.

The vitelline membrane, which is laid down in early vitellogenesis, is a product of the oocyte (De Jong-Brink *et al.*, 1983), and in *Mytilus edulis*, the cortical granules may be the source of this material (Humphreys, 1967). The arrangement of cortical granules, in contact with or very close to the cell membrane in *Brachidontes virgiliae* (present study) and *M. galloprovincialis* (Bernard & Hodgson, unpublished data), supports the suggestion of Humphreys (1967).

The follicular cells of *Brachidontes virgiliae* are arranged in a manner characteristic of the bivalves (De Jong-Brink *et al.*, 1983), and these authors have reviewed the functional roles suggested for follicle cells in the Mollusca. The cytoplasm of the follicle cells of *B. virgiliae* has large amounts of glycogen, rough endoplasmic reticulum, and mitochondria. The rough endoplasmic reticulum lies close to the cell membrane of the developing oocyte, suggesting that the follicle cells may play a significant role in the maturation of the oocyte. However, the exact function of the follicle cells of *B. virgiliae* remains to be elucidated.

The working temperatures and salinity used in the in vitro embryonic development studies were selected on the basis of data generated by Howard-Williams (1976, 1978) for Swartvlei. During the mouth-open phase of the lake, surface and mid-column salinities varied between 9 and 15‰, with the lowest end of the range occurring for 8 of 17 months of study, and the highest for 3. Temperatures varied between 14 (June–October, winter–spring) and 24°C (December–March, summer) during the same 17-month study period. Development of the mussel is very rapid at 24°C (fertilization to spat within 24 h) and is still relatively rapid at 15°C, while the difference in development time between 20 and 24°C (ca. 10 h) is, perhaps, surprising given the temperature variation overlap during the experiments.

Table 1

Summary of the in vitro early development of *Brachidontes virgiliae* at 24°C and a salinity of 9‰.

| Time* | Development stage | Figure |
|---|---|---|
| 5 min | Polar body extrusion | 13A |
| 25 min | First cleavage, micro- and megamere production | 13A |
| 70 min | 3-cell stage developing | 13A |
| 80 min | 4-cell stage | 13A |
| 115 min | 8-cell stage; pronounced dexiotropic cleavage | |
| 130 min | 32-cell stage | |
| 4 h | Early blastula free of the vitelline membrane | 13B |
| 5 h | Blastopore closing after epibolic gastrulation. Morula performing limited spiral movements; ectodermal cilia short. | 13C |
| 10 h | Development of velar cirri | |
| 13–15 h | Developing velum; digestive system developing, directed swimming. | 14B |
| 20 h | Velar resorption commences; shell and hinge forming; characteristic bilateral symmetry. | 14C |
| 23–24 h | Free-swimming spat; shell well developed, adductor muscles forming; ciliated foot; avoidance response to disturbance. | 14D |

* Average development time from fertilization.

Coetzee (1978) has recorded high densities of "lamellibranch veliger larvae" in Swartvlei (*Brachidontes virgiliae* is the only possible source) between spring and early summer (October–December, with a peak of >40,000 $m^{-3}$ at 6 m depth) and in autumn (April–May, between 37,000 and 39,000 $m^{-3}$). Spatfall on the submerged plant *Potamogeton pectinatus* reached >2.5 million individuals $m^{-2}$ of lake bed in November 1978 and >1.25 million individuals $m^{-2}$ in April (autumn) 1978 (Davies, unpublished data), confirming the main reproductive periods of *B. virgiliae* within the system. The double peak of spatfall is difficult to explain in terms of the ecology of the animal, particularly as the autumnal peak occurs as temperatures are falling within the system, and as the *Potamogeton* community is beginning to senesce (with concomitant loss of the enormous area available for attachment of *B. virgiliae*). Mortality must be very large indeed at this time.

The standing stocks of *Brachidontes virgiliae* within Swartvlei are enormous (Boltt, 1973; Davies, 1982, 1984). Indeed, they may constitute the highest standing

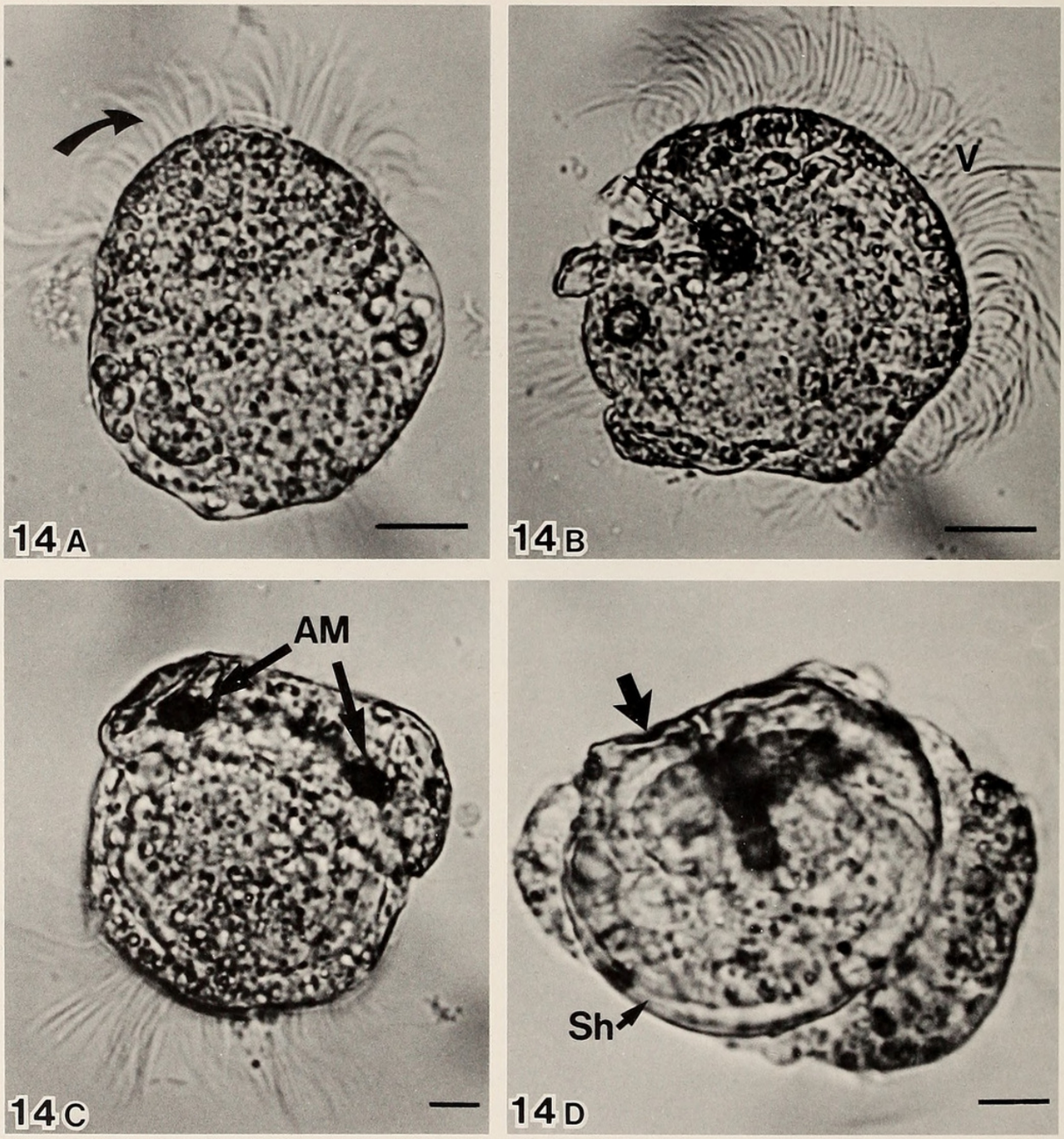


Figure 14

Photomicrographs of development of veliger and spat at 24°C and 9‰. Scale bars = 10 μm. A. Early veliger some 5 h post-fertilization showing development of ectodermal cirri (arrow). B. Veliger at between 5 and 10 h post-fertilization showing development of velar cirri (V) and early organ differentiation (arrow). C. Early spat with pronounced bilateral symmetry showing development of the adductor muscles (AM) and the dorsal aspect of the valves. D. Fully developed spat showing the hinge ligament (arrow), shell (Sh), and developing foot.

stocks of any invertebrate in any aquatic ecosystem anywhere in the world (DAVIES, 1982). Such densities may be a function of the ability of the mytilid to live attached to vertical surfaces (DAVIES, 1982, 1984). In the context of the annual cycle of *Potamogeton* (*e.g.,* HOWARD-WILLIAMS, 1978), the availability of a large area of substratum for attachment is temporally limited. This may account for the small size and early reproductive behavior of *B. virgiliae* populations in Swartvlei, as compared to populations from the estuaries of the eastern Cape. In Swartvlei, the animal is capable of commencing gamete production at 5–6 mm shell length (maximum shell length in Swartvlei = 12 mm) and development to the settling spat stage at ambient temperatures is rapid. By comparison

Table 2

Summary of the in vitro early development of *Brachidontes virgiliae* at 15 ± 2 and 20 ± 2°C and a salinity of 9‰.

| | Development time from fertilization | |
|---|---|---|
| Development stage | 15°C | 20°C |
| Polar body extrusion | 25 min | 8 min |
| First cleavage | 90 min | 40 min |
| 3-cell stage | 3 h 30 min | 100 min |
| 4-cell stage | 5 h | 160 min |
| 8-cell stage | 12 h | 3 h 15 min |
| 32-cell stage | 14 h | 4 h |
| Early blastula | ca. 19 h | 9 h |
| Gastrulation | ca. 23 h | ca. 12 h |
| Early veliger | ca. 31 h | 20 h |
| Mature veliger | ca. 38 h | 24 h |
| Velar resportion | ca. 48 h | ca. 30 h |
| Spat | ca. 56 h | ca. 36 h |

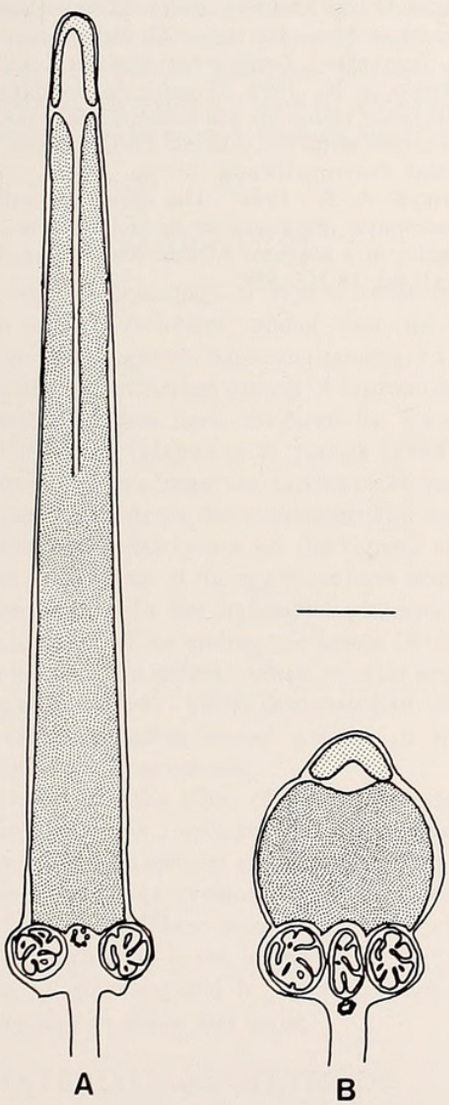


Figure 15

Diagrammatic representation of the structure of the spermatozoon of A, *Musculus discors* (after FRANZÉN, 1983), and B, *Brachidontes* (*Musculus*) *virgiliae* (present study). Scale bar = 1 μm.

populations found at the head of the Kowie estuary, where the substratum for attachment is limited to the protected undersurfaces of relatively large boulders, *B. virgiliae* grows to 30 mm shell length, but does not produce gametes until the shell is approximately 10 mm long. Densities and standing stocks are also very low (DAVIES, 1980, unpublished data). In addition to further studies on the taxonomic position of the species, possibly using the structure of the spermatozoon as a basis, information is also required on its growth, fecundity, and food requirements, together with a comparison between estuarine and coastal lake populations, in order to gain insight into the biology of this remarkable animal.

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