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Gametogenesis and larval development in *Sabella spallanzanii* (Polychaeta: Sabellidae) from the Mediterranean Sea

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Abstract *Sabella spallanzanii* (Gmelin) is one of the best known and widely distributed Mediterranean polychaetes, but available data on its general biology has been inferred mostly from populations recently introduced to Australia. In the present paper, data on gametogenesis and larval development of a Mediterranean population from the Gulf of Taranto (Ionian Sea, Italy) are reported. Histological and electron microscopical analysis of gametogenesis showed that oogenesis, a long process beginning soon after spawning in February, is of extraovarian type. By contrast spermatogenesis is a very fast process lasting from September to December, when the coelomic cavity is completely packed with mature gametes and almost devoid of coelomocytes. During the period of spawning, some experiments of in vitro fertilisation were performed, always without success, while some of the collected females naturally spawned fertilised eggs (100%). Particularly interesting was the discovery of a small percentage of eggs which appeared fertilised but unspawned within the coelomic cavity of female. Investigation of the *S. spallanzanii* life cycle, from the naturally spawned fertilised eggs to embryonic and juvenile stages, was performed by daily microscopical observations and by SEM. Despite the presence of large eggs giving rise to a lecithotrophic larva, a long pelagic phase was observed, the longest found among Sabellidae. The larval development pattern is also described. Settlement began after about 2 weeks; metamorphosis occurred 10 d after settlement, when mucus was secreted abundantly and an external tube was formed. The long larval pelagic period and the development pattern, suggesting a high potential for dispersal, support both the introduction and invasive behaviour of the species in Australian waters.

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Introduction

A large body of literature on the reproduction of sabellid polychaetes is available (McEuen et al. 1983; Rouse and Fitzhugh 1994; Bick 1996; Giangrande 1997; Hsieh 1997; Rouse and Gambi 1998a, b; Gambi et al. 2000) so, at present, the Sabellidae is one of the better known polychaete families. Sabellidae show a large array of strategies from broadcasting of gametes to brooding within the tube. A peculiar extratubular brooding mode, with larvae retained inside the tentacular crown is also present. Brooding linked to small body size was inferred to be the plesiomorphic condition for the family, so that external fertilisation and swimming larvae, as well as the ect-aquasperm structure of the mature sperm, should be considered as secondarily derived (Jamieson and Rouse 1989; Rouse and Fitzhugh 1994). However, the small-sized species are much better studied, while few data on large-sized species are available.

The present paper deals with reproduction of one of the most "popular" Mediterranean polychaetes, the fan worm *Sabella spallanzanii* (Gmelin), which is one of the largest sabellids. This species was recently introduced into Australian waters where it is considered a potential "pest" (Clapin and Evans 1995). For this reason, a large amount of data on its biology is available from Australian populations (Clapin and Evans 1995; Clapin 1996; Curry et al. 2000), although the mechanism of its introduction is still unknown (Andrew and Ward 1997).

In contrast, our knowledge of *Sabella spallanzanii* biology relative to Mediterranean populations is scanty. This species was considered to be a gonochoric (Dales 1961) broadcasting spawner, ejecting strings of mucus containing eggs or sperm into the water from the tube (Gravier 1923). The only paper concerning the life cycle

and reproduction of *S. spallanzanii* was published by Giangrande and Petraroli (1994) for a population from the Gulf of Taranto (Ionian Sea, Italy). In that paper the authors reported a rapid growth rate, a spawning occurrence during winter, the presence of probable protandric hermaphroditism, extraovarian oogenesis leading to egg diameters of 250 μm and spermatozoa morphology typical of species with external fertilisation. Data were collected over only 1 year of study, during which the authors failed to observe larval stages.

Recent studies on genetic relationships in different populations of *Sabella spallanzanii* are also available (Andrew and Ward 1997; Patti and Gambi 1998). These authors demonstrated the presence of a "founder effect" in the Australian populations, on the basis of lower genetic diversity with respect to the European populations. The Mediterranean populations seem to be fairly relegated to different Mediterranean subbasins (Patti and Gambi 1998), leading to a relatively low genetic exchange (gene flow) (Patti and Gambi in preparation). However, these genetic studies were not able to determine whether the introduction of *S. spallanzanii* into Australian waters was due to a larval pool transported in ballast waters or to ship-fouling adult specimens. In this respect, knowledge of larval development and the mechanisms of dispersion is of paramount importance to understanding the dynamics of the introduction and of gene flow among populations of this invasive species.

The data presented here complete the previous observations on the Ionian Sea population: we report on histological and electron microscopical analysis during an annual gametogenetic cycle, and provide the first description of larval development, settlement and post-settlement events.

Materials and methods

Specimens of *Sabella spallanzanii* (Gmelin) were hand collected monthly, by SCUBA diving, along an artificial dock wall in the "Mar Grande" of Taranto (Gulf of Taranto, Ionian Sea, Italy). In the laboratory, the worms were placed in aquaria and maintained at temperatures ranging from 11 to 29 °C, according to those recorded in situ during the sampling period.

Sexual maturation was followed and initially assessed by microscopic examination of the coelomic cavity products withdrawn from individuals collected throughout 1 year. Gametogenesis was followed in both sexes from March 1998 to February 1999, also by histological analysis. A few abdominal segments were cut and immediately fixed in Bouin's solution for standard histology and later washed with phosphate buffer (pH 7.4), dehydrated in graded alcohol solutions, treated with xilene and embedded in paraffin wax. The wax blocks were cut into 5- to 9-μm sections on a rotary microtome and stained with haematoxylin-eosin.

Electron microscope observations on gametogenetic stages were also performed. Aliquots of coelomic fluid drawn from sexually mature individuals were fixed for electron microscopy with 2.5% glutaraldehyde in 0.2 *M* cacodylate buffer and washed in 0.1 *M* cacodylate buffer. After treatment with 2% uranyl acetate, samples were dehydrated in a graded ethanol series, transferred to propylene oxide and later to both epoxy resin and propylene oxide (1:1) and finally embedded in epoxy resin. For transmission electron microscopy (TEM), thin sections (70 nm) were cut on an ultramicrotome and stained with lead citrate.

In order to investigate the pattern of fertilisation, eggs and sperm were obtained by withdrawing coelomic fluid from individuals with mature gametes in their cavities. The attempts to stimulate in vitro fertilisation were carried out in February, following two different procedures: (1) two or three drops of the coelomic fluid with mature spermatozoa were placed in glass dishes with mature eggs, which had been collected in the same way; (2) spermatozoa were directly injected into the space between the external tube and the body of a female.

Furthermore, visual observations were made of individuals naturally spawning in the aquarium. Fertilised eggs and subsequent developmental stages were reared at 14 °C in jars containing sea water which was replaced weekly. Developmental stages were observed daily by optical microscopy.

Specimens belonging to different developmental stages were fixed for scanning electron microscopy (SEM) in 2.5% glutaraldehyde, dehydrated in a graded alcohol series, gold coated after critical point drying and examined with a Phillips 505 scanning microscope.

Results

The sexes of *Sabella spallanzanii* are separate, and a sex ratio 1:1 was observed. Both males and females lack discrete ovaries or testes. Gametes, derived from peritoneal cells, were found in the coelomic cavity where free coelomocytes were also present. These latter cells, detectable in large quantities only in sexually immature individuals, appear to decrease when germinal products mature in the coelom. Maturation of oocytes (turquoise coloured) and spermatozoa (white coloured) involves a change in the coelomic content colour, usually ranging from bright orange to brown, which modifies to green and tan, respectively. Minimal size of sexually mature individuals was about 150 mm body length (crown excluded).

Gametogenesis

The present data complement the 1-year data on gametogenesis from the same population studied only by dissections of fresh samples by Giangrande and Petraroli (1994) during 1991/92. Oocyte development was found to be a long process beginning soon after spawning (February). Females collected in March still had some unspawned eggs that were probably reabsorbed. Coelomocytes were already well represented, and spherical bodies, not observed again during the rest of the year and probably identifiable as forming coelomocyte "centres", were also detectable in transverse sections (Fig. 1a). In the following 3 months sex was unrecognisable since the only cells in the coelom were a high number of coelomocytes (Fig. 1b).

In July "clusters" of cells, probably representing previtellogenic oocytes entering the coelom, were in evidence (Fig. 1c), while in August single early oocytes, measuring about 50 μm, were already undergoing the vitellogenesis process (Fig. 1d). Vitellogenesis throughout August and September was characterised by asynchronous growth, so that in October and November the immature oocytes showed various cell diameters.

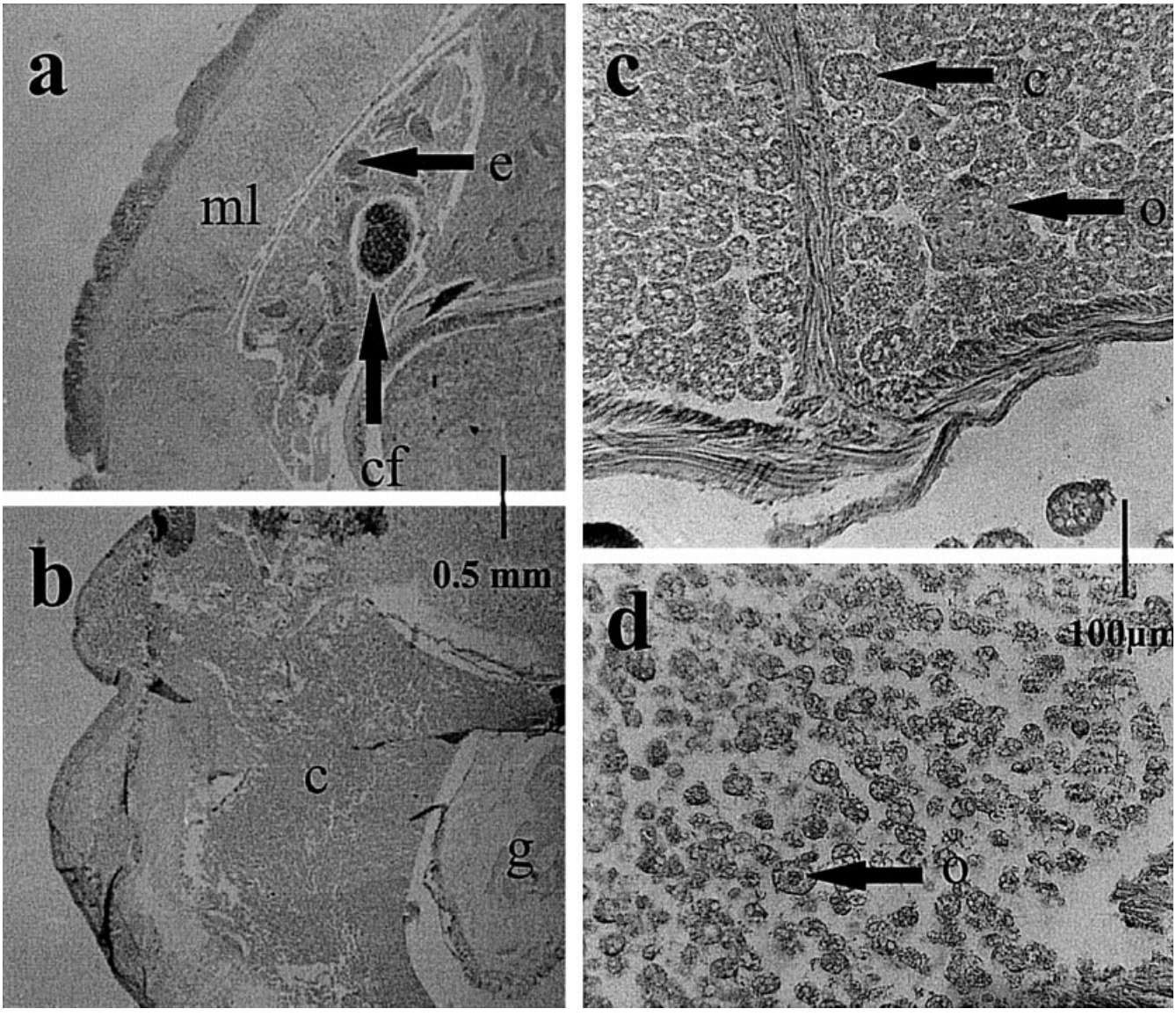

Fig. 1 *Sabella spallanzanii*. Transverse sections: **a** female with resorbing eggs and developing coelomocytes (March); **b** individual sexually unidentifiable with coelom packed with coelomocytes (April); **c** higher magnification of a female showing an oocyte cluster (July); and **d** of a female showing a developing oocyte (August) (*c* coelomocyte; *cf* center of developing coelomocites; *e* mature egg; *g* gut; *ml* muscolar layer; *o* oocyte)

However, in the following month the coelomic cavity was packed with mature oocytes, all of a similar size, while coelomocyte numbers decreased drastically (Fig. 2a). The number and size of the eggs detected in freshly dissected individuals, or in histological sections, remained stable until February when egg spawning occurred. Oogenesis with regards to increasing egg size is summarised in Fig. 3.

In comparison with oocyte development, spermatogenesis was a rapid process. Early spermatids were recognisable only in September as small assemblages dispersed in the coelom, but in October and November a high number of free spermatozoa could be detected, together with spermatids. Development of the male gametes proceeded until December, when the coelomic cavity was completely packed with mature spermatozoa and almost devoid of coelomocytes (Fig. 2b).

Ultrastructural analysis confirmed that in August oocytes were already in a vitellogenic stage, showing some yolk granules in the cytoplasm, and a well-developed rough endoplasmatic reticulum. The appearance of microvilli on the surface of oocytes was also observed (Fig. 4a). In the following vitellogenic stage (September), the maximum development of very elongated microvilli was observed (Fig. 4b). Pits and vesicles along the surface of the eggs were never observed. In December, eggs had matured completely, and their diameter had reached 250 µm. At maturity, drastic changes involving the egg membrane were observed. Cortical granules appeared under the cellular membrane, which became thick and lighter in colour; in addition the morphology of the microvilli changed to a shorter and wider form (Fig. 4c).

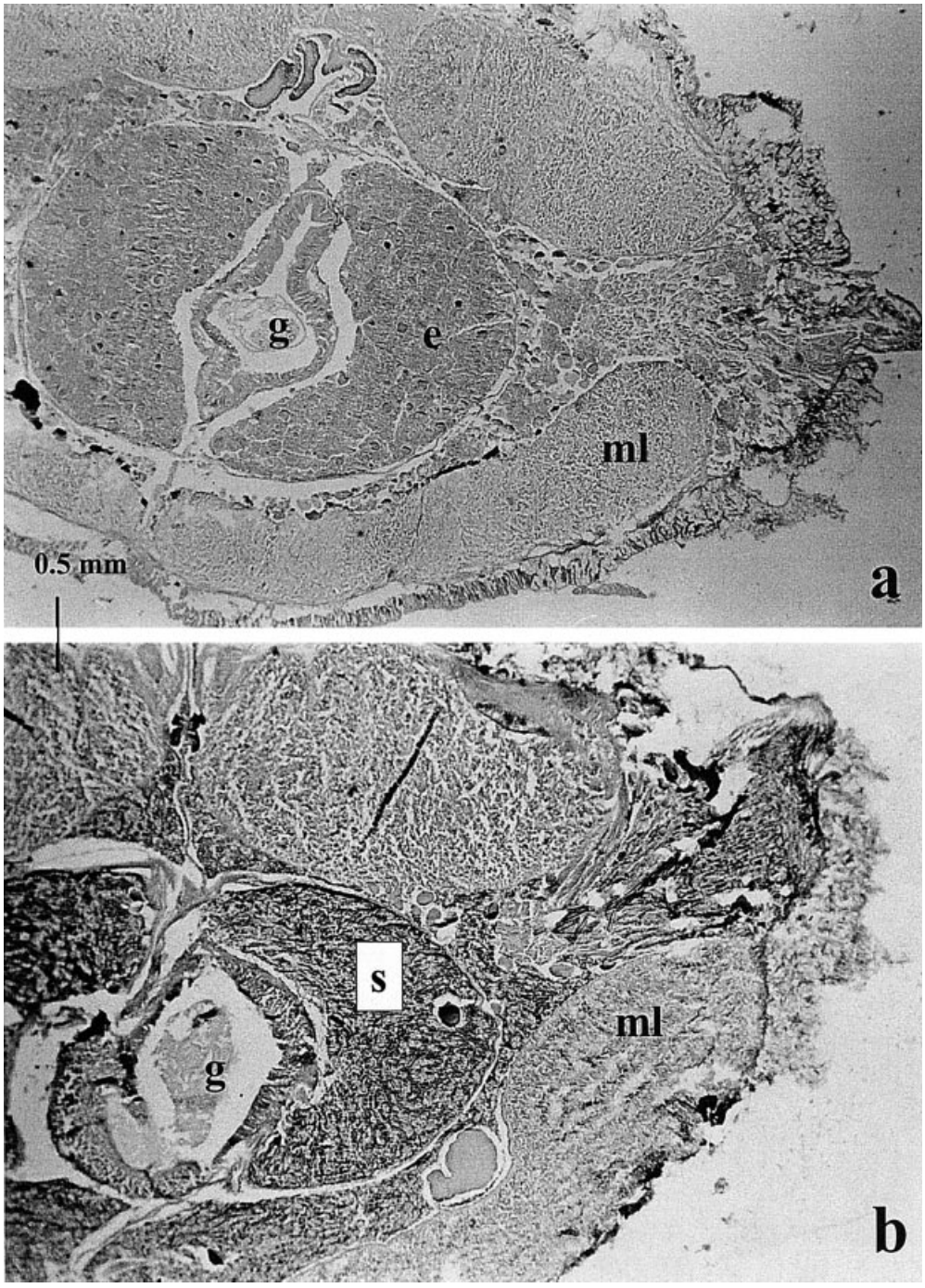

Fig. 2 *Sabella spallanzanii*. Transverse sections: **a** female with mature eggs (December); and **b** male with mature spermatozoa (December) (*e* mature egg; *g* gut; *ml* muscolar layer; *s* spermatozoa)

The electron microscopic investigation conducted on coelomic contents drawn from male individuals in October showed tetrads of both early and late spermatid stages, the latter containing a spherical and slighter condensed nucleus, and a simple acrosomal structure (Fig. 5a, b, c). In December, although late spermatids were also found, spermatozoa predominated, while in January and February only mature spermatozoa were detected (Fig. 5d, e, f). In mature sperm the spherical head comprises an acrosome showing a rather hemispherical outline. The acrosome structure consists of multiple invaginations forming a complex subacrosomal space over a flattened or slightly invaginated nucleus. In the large middle piece, between the base of the nucleus and the flagellum, the axoneme is surrounded by four spherical mitochondria. A transverse section of the tail showed a typical microtubular 9 + 2 pattern.

Egg fertilisation

Mature eggs were brown, by reflected light; they were discoidal in shape with a diameter of approximately 250 μm. First attempts to realise in vitro fertilisation by

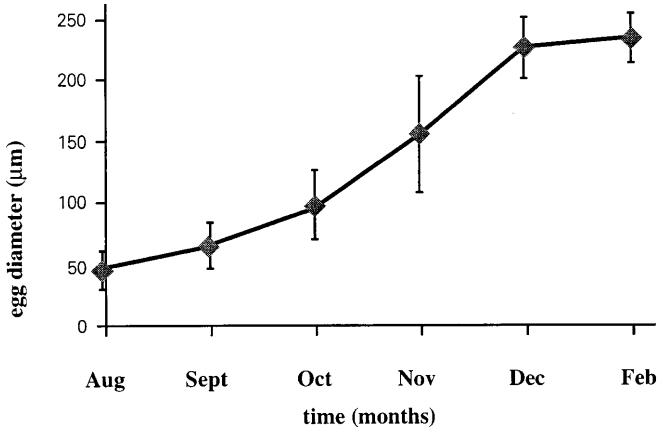

Fig. 3 *Sabella spallanzanii*. Oocyte growth. Each data point represents mean ± standard deviation (n = 100)

employing eggs and sperms diluted in sea water failed, since the eggs became wrinkled and collapsed as soon as they were placed in the sea water. When undiluted sperm were put into a glass dish containing eggs and coelomic fluid drawn from a female, changes in egg morphology were observed, with the appearance of a membrane which could be interpreted as a sign of fertilisation. However, the percentage of these "fertilised eggs" was very low (about 2%). Moreover, coelomic fluid drawn from several females also revealed the presence of a small percentage of eggs enveloped by this kind of membrane. In order to assess the existence of internal fertilisation, undiluted sperm was injected into the space between the body wall and internal tube of females, whose coelomic content had previously been examined and determined to be free of eggs with fertilisation membranes. These females were isolated from the aquarium in single tanks and reexamined after about 1 h. At that time the coelomic fluid showed the presence of eggs appearing to be fertilised, even though, once again, in small percentages.

At the end of January 1999, worms naturally spawned in the laboratory provided fertilised eggs (100% fertilisation) embedded in a string of mucus (Fig. 6a, b). The examination of female coelomic contents soon after they had spawned revealed the occurrence of a high number of still unfertilised eggs in the body cavity. After fertilisation the germinal vescicle broke down and egg contents became more homogeneous, enclosed in a large membrane. A change in egg shape from discoidal to spherical also took place, so that fertilised eggs appeared smaller compared to unfertilised ones (Fig. 6c, d). In accordance with Currie et al. (2000), gametes were expelled through paired lateral coelomoducts extending from the coelom of each chaetiger and opening at the neuropodia of the next posterior segment. Gametes were then propelled toward the tube opening along the ventral fecal groove by cilia. The transfer of fertilised eggs along the fecal groove was visually observed.

Larval development

Naturally spawned fertilized eggs were placed in glass-jars at 14 °C where they rapidly sank to the bottom. After 24 h both early embryos (Fig. 6b) and already free-swimming trochophore stages (Fig. 7a) could be observed. Both developmental stages, still enveloped within a membrane, were almost hemispherical and filled half of the cavity inside the egg membrane. At the trochophore stage, cilia of the developing prototroch were observed to protrude through the membrane (Fig. 9A). After 36 h, when the egg membrane disappeared, all individuals were at the trochophore stage having a distinct pair of eye spots (Figs. 7b, c, 9B). Only the prototroch was present at this stage, a telotroch was not present at any stage, and an apical tuft was absent. The trochophore stage continued until the fourth day (Fig. 7d), when the hyposphere elongated and segmentation started to appear with the formation of the first chaetiger. At this stage a poorly developed neurotroch appeared. Five-day-old larvae showed two chaetigers (Fig. 8a, b). Except for further body elongation, this pelagic stage remained unchanged for at least 2 weeks. During this period mortality in the water column was almost null, even though a few larvae attached to the bottom of the jars by mucus and then died. The following developmental stage presented high modification of the hyposphere and the formation of the third chaetiger and a well-differentiated pigydium (Figs. 8c, d, 9C, D). The prostomium showed a pair of extensions that represented precursors of the branchial filaments. At this stage settlement began, although the cilia of the prototroch continued to beat. The larvae were gregarious, and recently settled specimens often were found in clusters. Metamorphosis, marked by the disappearance of the prototroch and the appearance of an anal opening, occurred 10 d after settlement (Fig. 10a, b), when a mucus tube was secreted, forming an envelop through which the juveniles moved using the chaetae (Fig. 10c, d).

This pattern was followed by most of the individuals, even though a few remained in the water column at the two-chaetiger stage for a longer time. However, after 21 d, all pelagic stages had settled out of the water column. After settlement, the mortality rate increased, reaching up to 90% at metamorphosis. Mortality was particularly high when larvae settled directly on the glass. Since jar corners, sand grain and gravels were observed to be especially attractive to competent larvae, a small amount of shell debris was put in the jars to offer crevices and small holes to facilitate settlement.

The first three chaetigers developed adult type thoracic notochaetae (Fig. 8d). Inferior thoracic notochaetae appeared first, followed by superior ones and the neuropodial uncini. Companion chaetae were never observed at this stage. Juveniles remained at the three-chaetiger stage until the branchial crown developed to a food-gathering stage and the anus opened. The fourth chaetiger appeared only 20 d later. During this time the branchial crown rudiments were enlarged by division and bifurcated

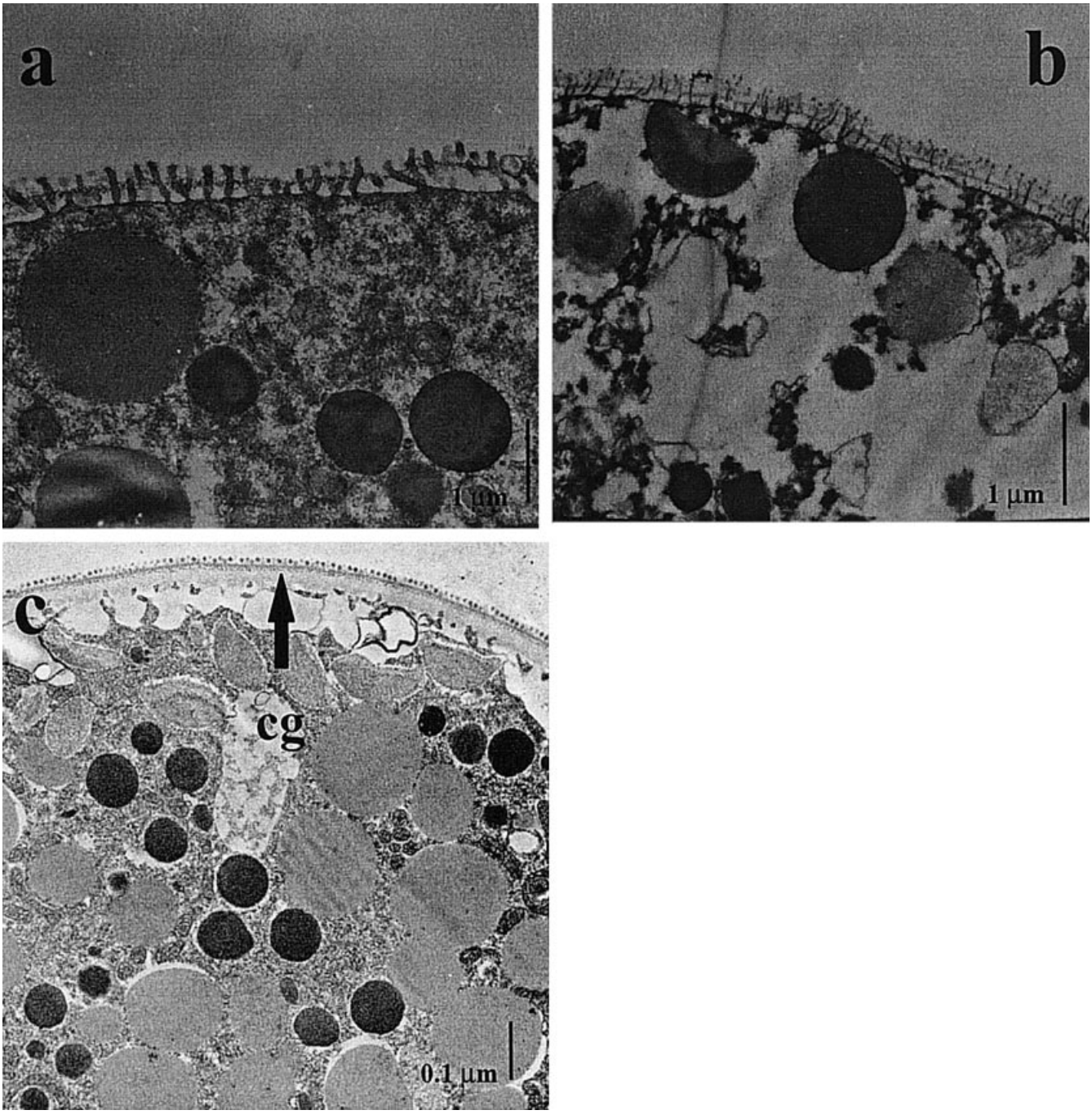

Fig. 4 *Sabella spallanzanii*. Oogenesis: **a** section through early vitellogenetic oocyte (August) showing yolk granules in the cytoplasm, rough endoplasmatic reticulum and developing microvilli on plasmalemma; **b** section through developing oocyte (September) showing the elongation of microvilli and **c** section through mature oocyte (December). Layer of cortical granules (*cg*), involved in forming the fertilisation membrane that prevents polyspermy, is also detectable under cellular membrane

distally. All these projections gave rise to lateral filaments (pinnules). Additional radioles were ventrally formed and the dorsalmost pair of filaments remained the longest and the best developed during ontogeny. As the branchial crown was forming, the anterior end of the prostomium became pronounced and the eye spots migrated posteriorly (Fig. 10c, d). The chaetigers formed subsequently were still of thoracic type to the third chaetiger. Tube lengths reached 1 mm only 1 month later.

Discussion

Present data confirm most of the previous observations by Giangrande and Petraroli (1994) on the same population and by Currie et al. (2000) on an Australian population (Port Phillip Bay). Minimal size for reproduction is reached at about 150 mm; reproduction is

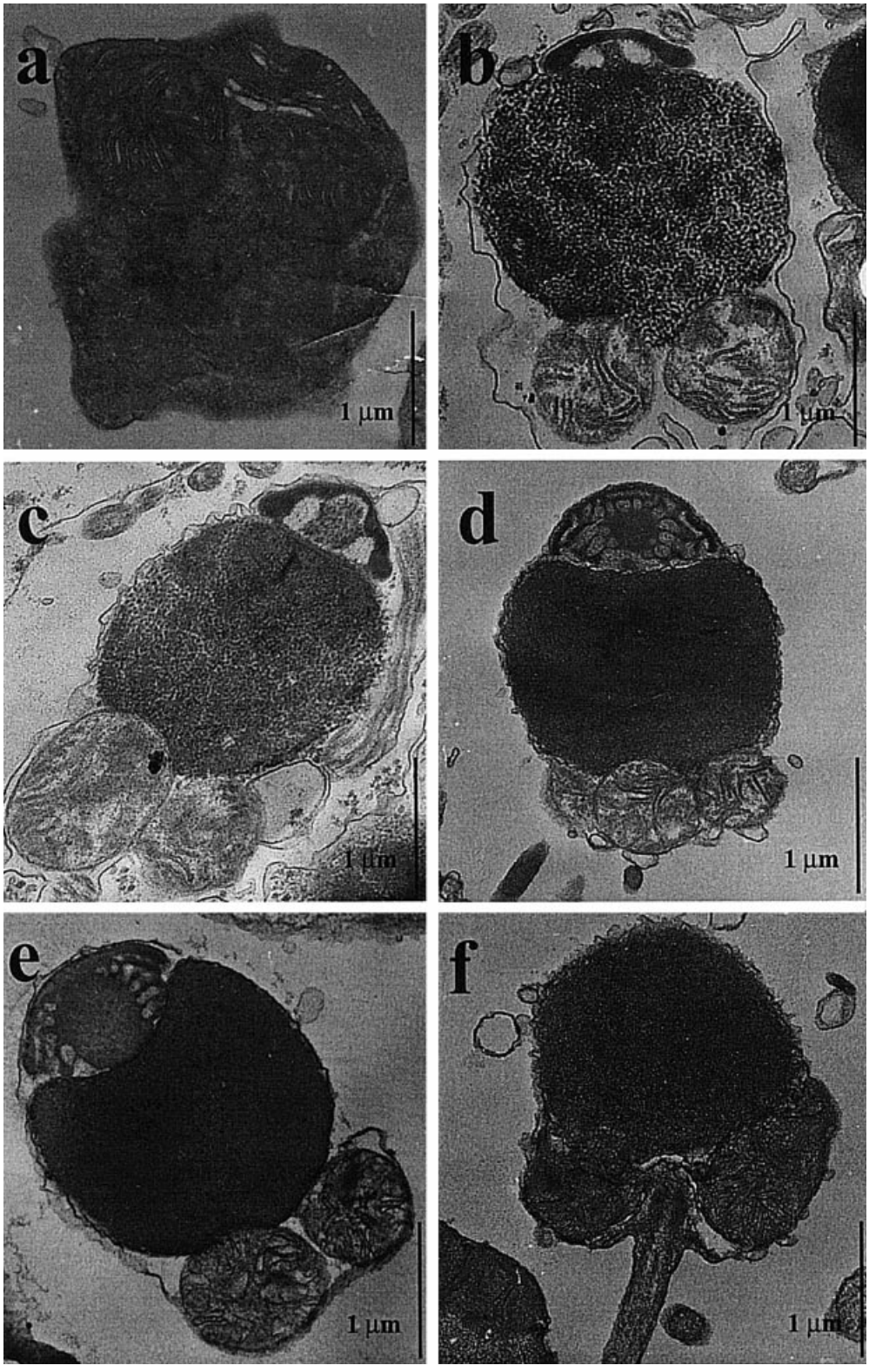

Fig. 5 *Sabella spallanzanii*. Spermiogenesis: **a** early spermatid with condensing nucleus and rounded mitochondria (October); **b, c** spermatid stages with spherically condensing nucleus, and early acrosome showing the cap-like structure encapsulating the core (October); and **d, e, f** different views of mature spermatozoa (December to January) showing the final structure of the acrosoma with tubules radiating from the central core to the peripheral ring of the core

synchronised among individuals and the entire process of oogenesis takes about 9 months. Spawning occurs when sea water temperature ranges from 11 to 14 °C in both the Ionian Sea (February) and Port Phillip Bay (August) under similar environmental conditions. Mediterranean and Australian populations are in fact located at comparable latitudes and exposed to similar seasonal changes. In the Mediterranean Sea spawning was observed over a period of about 1 month, from late January to the end of February. Several spawning episodes by the same female may be hypothesised since ripe oocytes were found within the coelom of females that had

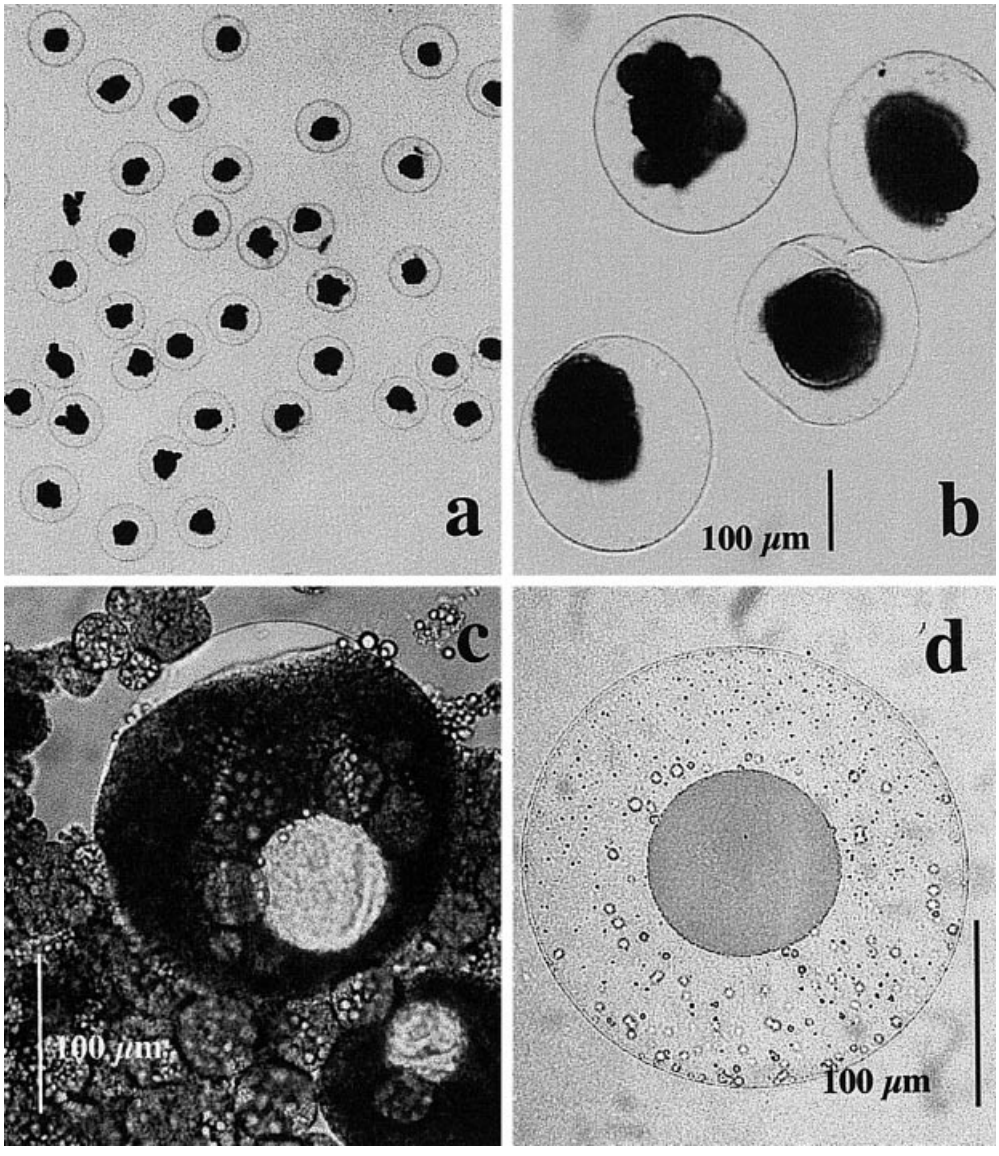

Fig. 6 *Sabella spallanzanii*. Eggs: **a** naturally spawned eggs showing 100% fertilisation; **b** details of embryos at different stages of development; **c** unfertilised egg drawn from coelomic cavity and **d** fertilised egg showing a large fertilisation membrane

already spawned. Both sexes were equally represented, in accordance with Currie et al. (2000) and in contrast to the previously hypothesised protandric hermaphroditism of the species (Giangrande and Petraroli 1994).

Gametogenesis

The pattern of gametogenesis did not show great differences when compared to the previously studied cycle in 1991/92 (Giangrande and Petraroli 1994), even though histological analysis indicates that oogenesis started sooner than previously hypothesised. Moreover, in August, yolk granules were already present within oocytes, leading us to suppose the presence of a brief previtellogenetic phase. Vitellogenesis was completed by January. As already observed by Dales (1961), coelomocytes, probably representing available reserves for developing oocytes, almost disappear when oocytes are mature. Extraovarian oogenesis was present in *Sabella spallanzanii* and lasted for a long period, as commonly encountered in discrete semelparous breeders in which yolk autosynthesis could be involved. An indication of yolk autosynthesis may be the absence of pits and vesicles along the surface of the eggs and the presence of developed microvilli in early oocyte plasmalemma, through which low molecular weight precursors enter the oocyte. However, as pointed out by Eckelbarger (1983), yolk formation is never completely autosynthetic. Spermatogenesis was shorter than oogenesis, starting in September. In October spermatocytes were observed and mature spermatozoa were detectable from November to February. This is not in accordance with findings for the Australian population where two distinct periods of spermatogenesis occur (Currie et al. 2000). As seen in most sabellids, spermatids were observed in tetrads, a feature considered plesiomorphic (Rouse and Fithzugh 1994).

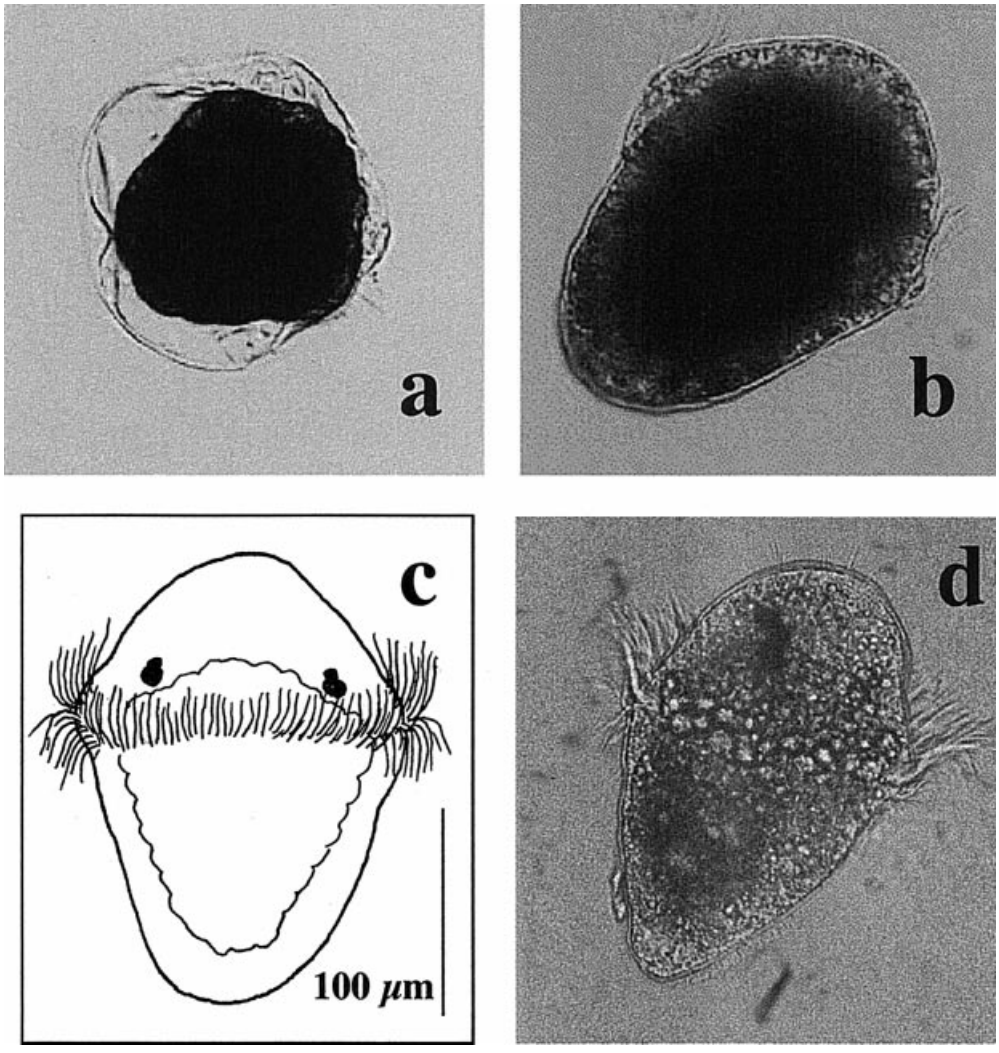

Fig. 7 *Sabella spallanzanii*. Larval development: **a** early swimming trochophore still enveloped within fertilisation membrane (24 h); **b, c** trochophore, 36 h old; and **d** trochophore, 48 h old

Sperm morphology, egg size and fertilisation biology

The ect-aquasperm structure found in *Sabella spallanzanii*, with rounded nucleus and with acrosome and mitochondria, is generally associated with external fertilisation and free-spawning (Jamieson and Rouse 1989; Rouse and Fitzhugh 1994). These authors considered the sabellid ect-aquasperm a re-evolution of the ent-aquasperm type, since sperm storage, fertilisation in the tube and brooding have been hypothesised as the ancestral condition for Sabellidae. Within this family, sperm similar to that of *S. spallanzanii* is commonly found in broadcast spawners such as *Potamilla reniformis* (Chugthai 1986), *Branchiomma luctuosum* (Sordino and Gambi 1992; Giangrande and Gambi 1998) and *Perkinsiana littoralis* (Gambi et al. 2000). However, this morphology has also been documented for the extratubular brooding species *Perkinsiana riwo* (Rouse 1996). The acrosome of mature spermatozoa in *S. spallanzanii* shows a different structure from the simple rounded cap-like vesicle proposed as primitive (Jamieson and Rouse 1989), with the perforatorium showing numerous membrane invaginations over an invaginated nucleus. This acrosomal structure has also been found in other examined sabellids supposed to be broadcast-spawners.

Among polychaetes, the morphologies of ent-aquasperm and intro-sperm often reflect considerable variation, with elongation of the nucleus, mid-piece or both. These modifications could be related to sperm storage in spermathecae, or to the facilitation of sperm movement in internally fertilising species (Westheide 1984; Rouse 1992a, b). The variety of sperm morphologies, including modifications of the acrosome as found in other species such as *Perkinsiana antarctica* (Gambi and Patti 1998; Gambi et al. 2000), could represent adaptations to swimming by sperm to reach eggs encased in mucous sheets and brooded within the branchial crown.

However, as pointed out by Jamieson and Rouse (1989), to define the spermatozoa type without information on fertilisation biology is only speculative. The ect-aquasperm is often correlated with other reproductive traits such as small eggs and external fertilisation, because the very small size of ect-aquasperm allows the production of a large number of sperm as an

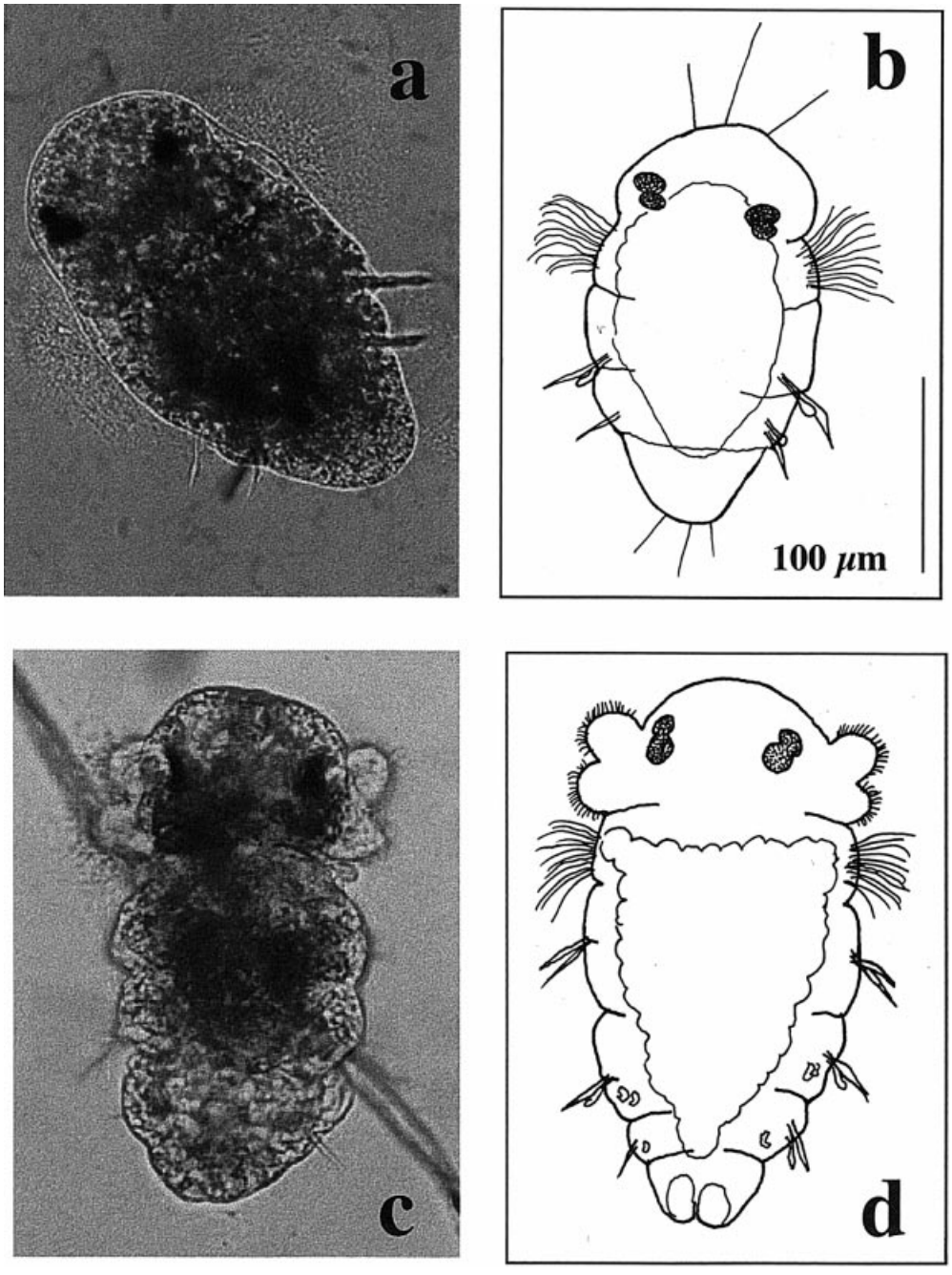

Fig. 8 *Sabella spallanzanii*. Larval development: **a, b** two-chaetiger stage, 5 d old; and **c, d** just settled three-chaetiger stage, 18 d old

adaptation to the hazards of broadcast spawning. Entaquasperm, adapted to in situ fertilisation, similarly require a large number of sperm but fewer eggs than in external fertilisation, and this seems to be accompanied by a tendency to lecithotrophy. A few cases of the introsperm type are known within class Polychaeta but none among the Sabellidae. Finally, up to now, no species associated with internal fertilisation has been found with structures similar to those of externally fertilising sperm (Jamieson and Rouse 1989).

In most of the studied sabellids no observations on fertilisation biology have been published. The best studied species are of relatively small size, retain eggs or have a short developmental period (Giangrande 1997). In these forms, larvae directly observed inside the tube or the branchial crown, and often the presence of spermathecae, led us to assume the occurrence of in situ fertilisation; the sperm morphology supported this view.

No fertilisation event has ever been documented among larger sabellids, which are all presumed to be broadcasters. However, spawning of gametes was observed in some species, suggesting the occurrence of fertilisation in the water column (Berrill 1977; Dean et al. 1987; Yun and Kikuchi 1991a, b). Moreover, in the few cases where in vitro fertilisation was attempted (Okuda 1946; Dean et al. 1987), these were only successful in *Megalomma vesiculosum* and in *Sabella pavonina* (Wilson 1936). However, this could have been

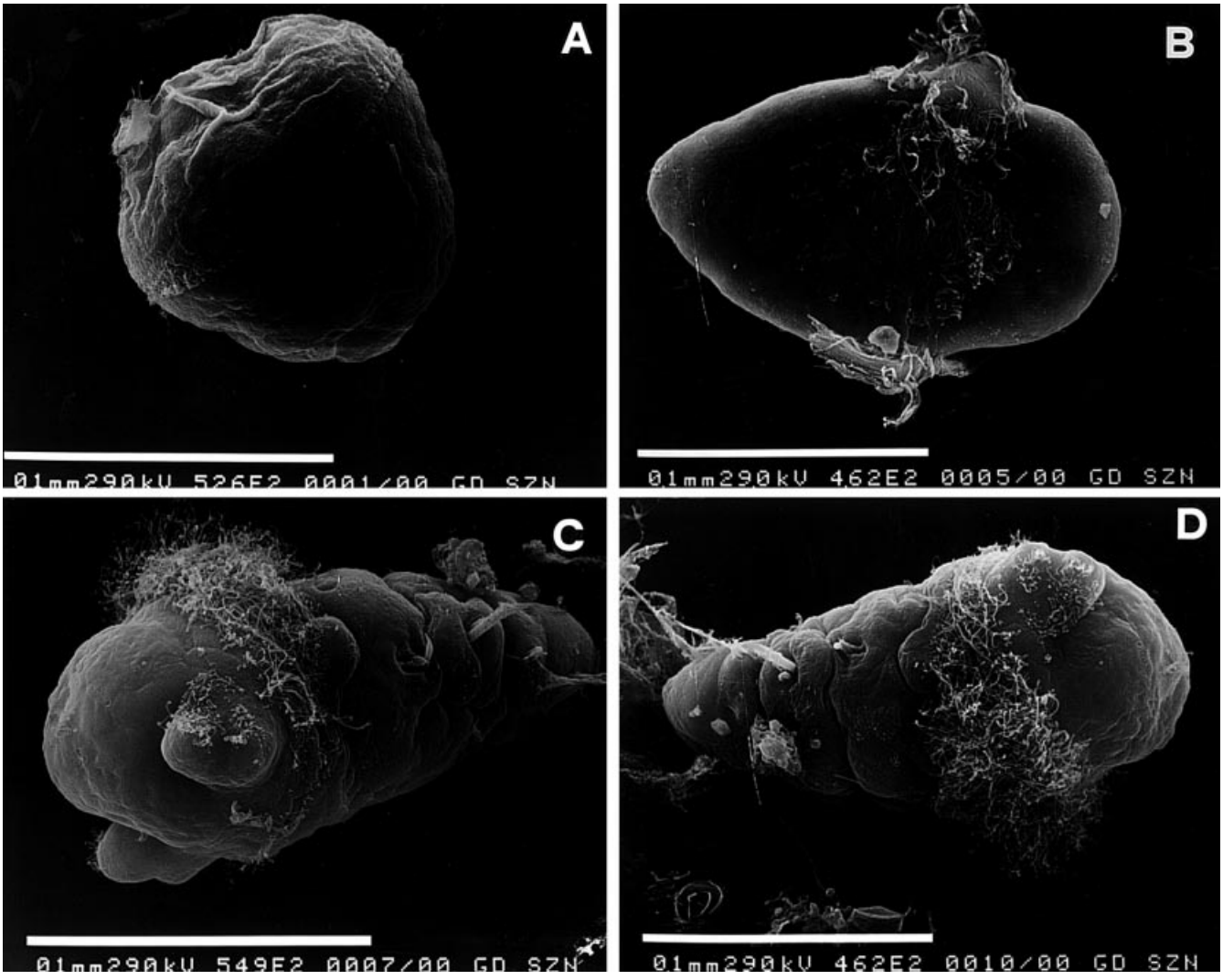

Fig. 9 *Sabella spallanzanii*. SEM micrographs of larval development: **A** early swimming trochophore, 24 h old; **B** trochophore, 36 h old; **C, D** just settled three-chaetiger stage, 18 d old

due to a lack of knowledge about the meiotic stage, i.e. the time at which the sperm may enter the egg. This stage is variable among species (Cross 1984), a fact which could have limited the success of experiments. The only exception is *Laonome albicingillum* (Hsieh 1997), for which up to 94% fertilisation success was obtained by in vitro experiments. In this species self-fertilisation was also found, and authors have observed already fertilised eggs emerging from the branchial crown, stressing the high variability encountered in sabellid reproductive strategies.

Present observations on *Sabella spallanzanii* suggest that fertilisation does not occur in the water column, it could take place either internally or in situ. During natural spawning events, only fertilised eggs were found enveloped in the mucus. Moreover, eggs observed moving along the fecal groove were already fertilised, so that fertilisation could be supposed to occur internally or within the tube just after egg release. As far as the first hypothesis is concerned, although the presence of apparently fertilised eggs within the coelom of *S. spallanzanii* could be an artefact, due to the manipulation leading to pre-maturation (Cross 1984), these eggs were only observed when spermatozoa were injected into the female tube. Further study is needed to assess whether the spermatozoa really enter the coelom, especially since the morphology of *S. spallanzanii* does not seem consistent with the occurrence of fertilisation inside the coelomic cavity. The second hypothesised fertilisation strategy appears, therefore, more likely; even though in contrast to what was observed for this species, in situ fertilisation is usually correlated with the retention of eggs, direct development and, often, modified spermatozoa.

This kind of fertilisation strategy, not involving egg dispersal in the water column, ensures a high success of fertilisation. Its presence in *Sabella spallanzanii* could be explained by the relatively small number of eggs produced by a female (50 000 in larger individuals; Currie et al. 2000), coupled with the risk of high larval mortality due to the length of pelagic development, the longest recorded among Sabellidae.

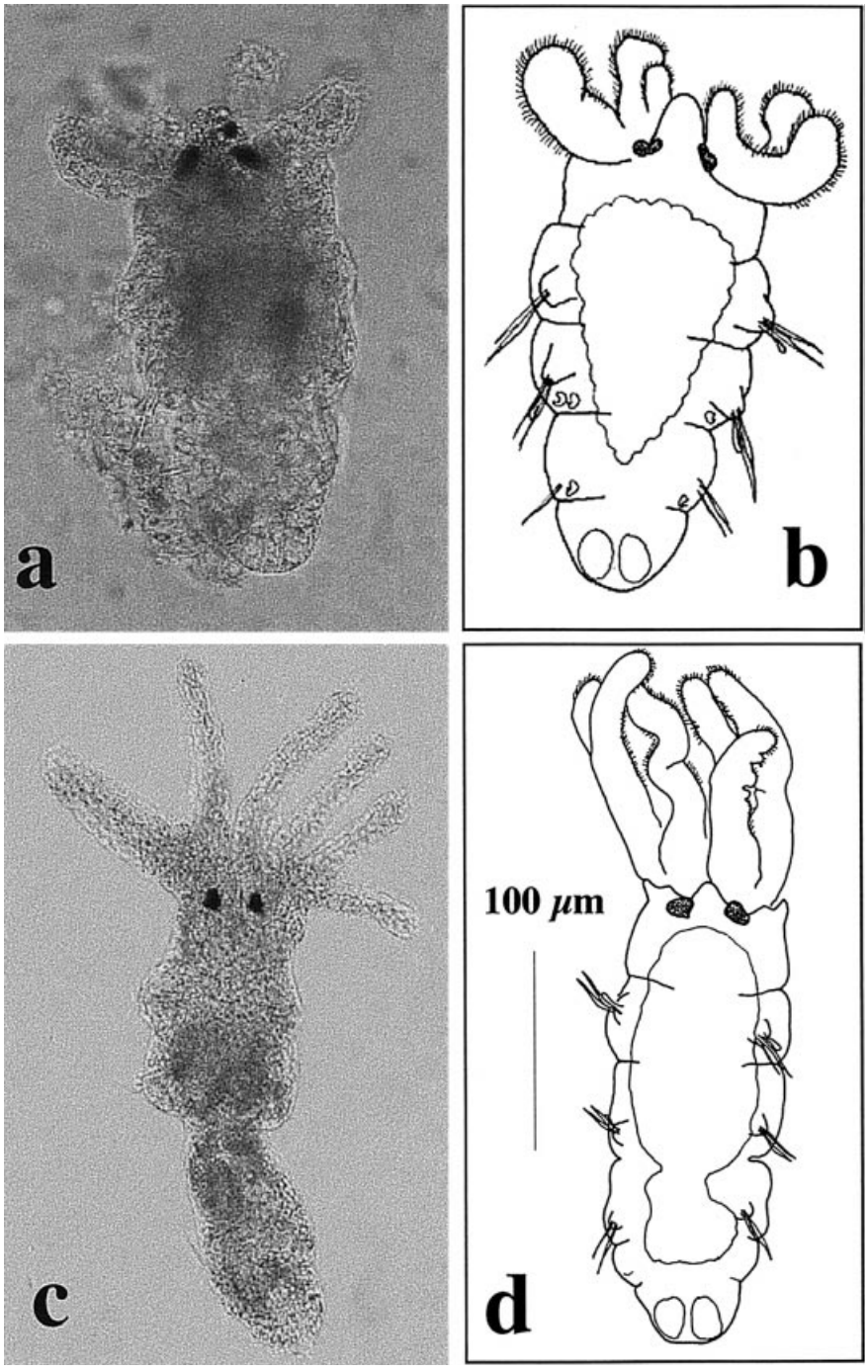

Fig. 10 *Sabella spallanzanii*. Juvenile stages: **a, b** individuals immediately after metamorphosis, 28 d; and **c, d** 38 d-old individual

Despite this long pelagic period, *Sabella spallanzanii* eggs have a size (250 μm) often associated with direct development. Sabellids with similar but faster pelagic development, such as *Megalomma vesiculosum* (Wilson 1936), *Branchiomma nigromaculata* (Berrill 1977) and *Myxicola infundibulum* (Dean et al. 1987), have egg sizes ranging between 120 and 150 μm. It is therefore possible that the large egg size of *S. spallanzanii* is just related to the long pelagic period without feeding. Investigations on the actual energetic content of the eggs of *S. spallanzanii* compared to species producing smaller eggs are, however, required to support this hypothesis.

Even though larval strategies are probably selected based on energetic investment, there is evidence in some polychaetes for the evolution of reproductive traits leading to dispersal control (Bhaud and Duchene 1996). The strategy of *Sabella spallanzanii*, a typical fouling species, could be adapted instead to facilitate dispersal: the production of large eggs to allow a longer period of life without feeding, with a concomitant evolution of some strategies to increase fertilisation success.

These hypothetical considerations on the significance of the fertilisation pattern of *Sabella spallanzanii* point out, once again, the need for further study in this area.

Larval morphology

The three chaetigers are formed sequentially as opposed to simultaneously. Their development is followed by the formation of the thoracic segments and then the abdominal ones. This seems to be the most frequent pattern among sabellids, even though Berrill (1977) found a different pattern in *Branchiomma nigromaculata* and *Pothametus elongatus*. He observed that not all of the thoracic segments were produced initially but that some were formed by conversion of abdominal segments. This author also observed in these species a correlation between segmental transformation and thoracic gut extension. However, the dependence of posterior growth and regeneration from intestinal outgrowth is not clear; Kiortsis and Moraitou (1965) did not observe any cases of transformation of abdominal segments into thoracic ones during regeneration of *S. spallanzanii*. The pattern observed by Berrill is typical of species with small eggs, whilst the other is always present in species having very large eggs. Unfortunately, no indication was given by Dean et al. (1987) on chaetiger formation in *Myxicola infundibulum*, one of the few sabellid species characterised by small egg size and pelagic development. While *Myxicola* sp. from Antarctica, having very large eggs and brooding of embryos, forms all thoracic segments sequentially (Gambi et al. unpublished data). Therefore, our knowledge is still too scanty to assess Berrill's hypothesis, especially taking into consideration that the final number of thoracic chaetigers within the subfamily Sabellinae is variable, ranging from only four in the genus *Pseudobranchiomma* to more than 17 in *Perkinsiana*.

Except for *Fabricinuda trilobata*, *Potamilla torelli* (Rouse and Fitzhugh 1994) and *Amphiglena mediterranea* (Rouse and Gambi 1998b), the larvae of which have poorly developed or no ciliary bands, all the investigated species have a well-developed prototroch and a differentially developed neurotroch, independent of direct or pelagic development. A telotroch is never present, except in *Chone teres* (Okuda 1946), while the only genus to have a poorly developed metatroch may be *Amphicorina* (Table 1), both features probably indicating the primitiveness of these genera.

Dispersal, settlement and metamorphosis

The high mortality rate observed during settlement underlines the importance of substrate features, even in these primary coloniser species. The need for slime films as an attachment mechanism, a well-known phenomenon in marine invertebrates (Scheltema 1974), could have caused the high mortality when larvae directly settled on glass, but the microtopography of the substrate (Anderson and Underwood 1994) also seems to be important during this phase. This was shown by the preferential settlement of larvae around different debris, in the jar corners and in crevices. The long period between settlement and metamorphosis, when juveniles started to feed, still remains unexplained. Probably the absence of suitable substrata led to a delay of settlement, thus also influencing the time at metamorphosis. If so, both the long pelagic phase and time at metamorphosis observed could be biased by the experimental conditions. However, these observations demonstrate that the species has at least the potential for a long pelagic period, delaying its settlement and metamorphosis to overcome adverse and unsuitable external conditions.

The long pelagic period evidenced by *Sabella spallanzanii*, at least in the laboratory, has important implications for the potential of dispersal of this species and may provide some insights into the mechanism of introduction to Australian waters. The "founder effect"

Table 1 Sabellids with well-described larval morphologies

Genus	Strategy	Prototroch	Neurotroch	Metatroch	Telotroch	Literature
Fabricinuda	Intra. brooder Direct dev.	Absent	Absent	Absent	Absent	Rouse and Fitzhugh (1994)
Amphicorina	Intra. brooder Direct dev.	Present	Present	Present	Absent	Rouse and Fitzhugh (1994)
Chone	Extra. brooder Mixed dev.	Present	Present	Absent	Present	Okuda (1946)
Myxicola	Broadcaster? Pelagic dev.	Present	Present	Absent	Absent	Dean et al. (1987)
Sabella	Broadcaster? Pelagic dev.	Present	Present	Absent	Absent	Present paper
Megalomma	Broadcaster? Pelagic dev.	Present	Present	Absent	Absent	Wilson (1936)
Demonax	Extra. brooder Direct dev.	Present	Present	Absent	Absent	McEuen et al. (1983)
Amphiglena	Intra. brooder Direct dev.	Abs./Pres.	Abs./Pres.	Absent	Absent	Rouse and Gambi (1998b)
Potamilla	Intra. brooder Mixed dev.	Absent	Absent	Absent	Absent	Rouse and Fitzhugh (1994)
Perkinsiana	Extra. brooder Direct dev.	Present	Present	Absent	Absent	Gambi et al. (2000); Rouse (1996)

observed in Australian populations (Andrew and Ward 1997) could be explained either by the introduction of a few larval pools that encountered the stressful conditions of the ballast waters, or by ship-fouling specimens. However, the introduction by larval pools is the more probable mechanism, because even if *S. spallanzanii* is a fouling organism of artificial docks and pylons, it is not a typical or common fouler of ship hulls.

The high genetic variability in the populations of *Sabella spallanzanii* from the Mediterranean Sea (Patti and Gambi 1998, and in preparation) suggests the existence of some mechanisms of population isolation and gene flow reduction among populations of different sub-basins. Such mechanisms can be partly related to larval biology (length of pelagic phase, dispersal barriers, etc.), but can also be related to environmental and climatic factors acting independently. As an example, the genetic population structure of the endemic seagrass *Posidonia oceanica* (L.) Delile in a sub-basin of the Mediterranean seems to be more influenced by such external factors, than by plant reproductive features (Procaccini and Mazzella 1998).

In conclusion, the larval development observed for the first time in *Sabella spallanzanii* during this study suggests a high potential for dispersal of the species, which is supported by its highly invasive behaviour in Australian waters. The high genetic variability, coupled with the wide ecological requirements, is consistent with data recorded for other highly opportunistic and ''pest'' species.

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