Annual cycle of stored spermatozoa within the ovaries of *Helicolenus dactylopterus dactylopterus* (Teleostei, Scorpaenidae)

S. VILA*, M. MUNOZ, M. SABAT AND M. CASADEVALL

Department of Environmental Sciences, Universitat de Girona, Campus de Montilivi, 17071 Girona, Spain

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The aim of this work was to analyse the ultrastructure of storage crypts and stored spermatozoa, and to describe changes during the annual reproductive cycle of the bluemouth *Helicolenus dactylopterus dactylopterus*, which has internal fertilization and a zygoparous mode of reproduction. Spermatozoa had elongated heads and long midpieces, two characteristics which are thought to be fairly advanced and correlated with internal fertilization, as is the case of the bluemouth. A remarkable spermatozoon feature was the retention of a significant quantity of cytoplasm around the head, a condition that appeared to be related to nourishment during the long storage period, up to 10 months in the intraovarian crystal structures of the female. Male sex cells’ protection against the female immune system was ensured by junctional complexes between the crypt cells composed of tight junctions and desmosomes.

Key words: annual cycle; *Helicolenus dactylopterus*; sperm storage; spermatozoa; ultrastructure.

INTRODUCTION

Animals show a great variety of reproductive strategies, ranging from simple oviparity, with the release of gametes to the environment and external fertilization, to internal fertilization and placental viviparity.

Commonly, internal fertilization involves the fusion between an egg and a spermatozoon almost immediately after insemination. In other cases, sperm cells are stored in the ovary for an extended period of time before fertilization.

Storage of sperm in both male and female vertebrates is an integral component of the productive process in many species. In males, spermatozoa produced in the testis mature, are nourished and are maintained in a viable state in the male genital ducts. Various male accessory glands, as well as the male genital ducts, contribute specific components to the seminal fluid and to the maintenance and maturity of the spermatozoa. In females, spermatozoa

*Author to whom correspondence should be addressed. Tel.: +34 972 418498; fax: +34 972 418150; email: silvia.vila@udg.es
are sequestered in specialized storage organs or reservoirs, such as the oviducal gland in elasmobranchs (Hamlett et al., 2002), spermathecae in amphibia (Sever, 2002), sperm storage tubules in birds (Holm & Ridderstrale, 2002) and the isthmic reservoir in mammals. In most vertebrate groups where females store spermatozoa, there are glands or macroscopic vesicles assigned to this task, but in bony fishes the occurrence of specialized structures to store sperm is poorly documented (Hamlett, 2002).

Reproductive modes in teleosts are particularly interesting because they present an enormous diversity of reproductive patterns ranging from unspecialized oviparity to highly specialized viviparity, including different stages of lecithotrophic and matrotrophic viviparity and gradations between them (Wourms et al., 1988). Within this group some, but not many, species are found that are able to store sperm inside their ovaries for extended and variable periods of time.

In almost all the teleost species that store sperm within the ovaries, there are no specialized, differentiated storage structures. In some cases, as in Alcichthys alicornis (Herzenstein) (Koya et al., 1997), spermatozoa remain floating freely within the ovarian lumen for relatively short storage periods of time. In other cases, exemplified by Cymatogaster aggregata Gibbons (Gardiner, 1978), spermatozoa are stored for several months with their heads embedded in the cells composing the ovarian epithelium. As is typical in poeciliids, female platyfish Xiphophorus maculatus (Günther) are also capable of storing viable sperm for several months, due to specific epithelial cells that line the gonoduct (Potter & Kramer, 2000). Other documented species, however, present slightly more specialized storage mechanisms, such as Sebastodes paucispinis Ayres (Moser et al., 1977) where the male gametes can be surrounded by the ovigerous lamellae epithelium, and the bluemouth Helicolenus dactylopterus dactylopterus (De la Roche) in which there are relatively specialized structures that allow spermatozoa to be stored within the ovary for as long as 10 months (Muñoz et al., 1999).

The bluemouth is a benthic species that usually inhabits the sea bottom between 200 and 1000 m of depth (Whitehead et al., 1986). The distribution pattern of this species is very complex. Eschmeyer (1969) identified two Atlantic sub-species, H. d. dactylopterus and Helicolenus dactylopterus lahillei Norman. The first is made up of four separate populations (north-east Atlantic and Mediterranean Sea, Gulf of Guinea, South Africa and north-west Atlantic), and the second is located off the Argentine and Uruguayan coasts. It is a widespread species, living on the slope of the continental shelf in these areas. Although it has not traditionally been a species with great commercial interest, nowadays this interest is growing with the search for new fishing resources as the traditional ones are progressively exhausted. As a result, deep-living species have been exploited more in many countries.

The peculiar reproductive characteristics of H. d. dactylopterus make it very interesting from a reproductive point of view. The ovaries of H. d. dactylopterus are of the cystovarian type (Hoar, 1969). They are saccular with an approximately circular transverse section and have muscular-connective stroma crossing them longitudinally from which sprout some ovigerous lamellae that are suspended within the ovarian lumen by highly vascularized, fibromuscular
trunks. On the surface of the trunks are oocytes in different stages of development, growing to maturity as they move away from the central stroma (Muñoz et al., 1999).

When 38 gonads were used to describe the different stages of maturity of this species, from March to November the ovaries were at the previtellogenesis phase (Muñoz et al., 1999). During this period, the presence of oogonia and oocytes in various stages of development could be detected. In December, the largest oocytes entered into the vitellogenic process, and in January and February mature oocytes could be observed. During these months, stored spermatozoa could fertilize ripe eggs. The post-spawn period was in March, coinciding with that of the males. These males entered into the spermatogonial proliferation phase in April, and in May and June their testicular cysts contained germinal cells in all stages of development. In July and October the lobular lumens were full of spermatozoa, but the phase of functional maturity was from November to February (Muñoz et al., 1999).

Previous work by Muñoz et al. (1999, 2000) discovered intraovarian sperm storage that can last as long as 10 months, an extremely long period for a teleost. Light and electron microscopy observations have corroborated the existence of rounded crypt-like structures that store the sperm at the base of the lamellae. Nevertheless, details of the mechanisms that permit spermatozoa to remain viable within the ovary are completely unknown. In this study, the mechanisms that allow sperm to be maintained within crypts during such a long period of time in the ovary were analysed. The annual changes in the structure and the ultrastructure of storage crypts and spermatozoa throughout the female’s ripening cycle were examined using light and electron microscopes.

**MATERIAL AND METHODS**

The 26 females of *H. d. dactylopterus* with standard lengths (LS) between 152 and 257 mm and the 10 males with LS between 253 and 290 mm used in this study were chosen from captures using the fishing line method made monthly over a 1 year period in Palamós (Costa Brava, north-west Mediterranean Sea). Every month fishermen caught five to 10 bluemouths and preserved them in a container of 10% formalin solution. Upon return to port, the samples were collected immediately from the fishermen. Samples of ovaries from specimens caught throughout the year were analysed to observe annual cyclic changes in the crypts. Testicular samples of all male specimens were also obtained in order to study their ultrastructure.

After fixation in a 10% formalin solution, they were weighed and stored in 70% ethyl alcohol. Subsequently, the gonads were embedded in glycol methacrylate, and then sections c. 5 μm thick were cut. Toluidine blue was used as a general stain.

For ultrastructural analysis, fishermen kept the specimens alive in a drum of sea water. Ovary and testis samples were obtained immediately after sacrificing the animals, by a blow to the head, in order to have fresh gonads. A large number of transverse sections were made of different parts of the male and female gonads. The samples (<1 mm³ in volume) were fixed in a mixture of glutaraldehyde (2.5%) and paraformaldehyde (2%) in a 0.1 M cacodylate buffer. After fixing for 2 h at 4°C, they were washed with a 0.1 M cacodylate buffer. Post-fixation was conducted in 1% osmium, also in a cacodylate buffer, at 4°C for 1 h. The samples were washed several times, dehydrated through an alcohol series and finally embedded in Spurr’s resin. Sections of c. 40–50 nm were made with a Reichert ultramicrotome, stained with uranyl acetate.
and lead citrate, and examined with a Zeiss EM-910 transmission electron microscope (TEM).

To verify the existence of cytoplasm bags in almost all the spermatozoa during most of the year, and to be sure that it was not an artefact provoked by osmotic problems, ovarian and testicular samples were fixed with hypotonic and hypertonic fixatives. The hypertonic fixative (1532 mOsm) was made of a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, and the hypotonic fixative (448 mOsm) was made of 2.5% glutaraldehyde in 0.1 M cacodylate buffer.

RESULTS

Spermatozoa storage structures are located near the connective-muscular rachis that crosses the gonad centrally and longitudinally. Ultrastructural studies reveal that sperm is stored within crypt-like structures at the base of the ovarian lamellae, which are often near blood vessels [Fig. 1(a)–(c)].

The crypt or specialized storage structure in *H. d. dactylopterus* is made up of a cryptal epithelium surrounding and closing the stored spermatozoa and is connected to the ovarian lumen by a duct. Hence, the cryptal epithelium represents a zonal specialization of the ovigerous lamellae epithelium.

Male gonads contain ripe spermatozoa from November to February (Muñoz et al., 1999), a time during which they can inseminate more than one female. During the late storage period, when females oocytes are in an advanced phase of maturation, several groups of joined spermatozoa, probably from a new insemination event, are observed floating freely within the interlamellar space [Fig. 1(d)]. Nonetheless, the majority of sperm are found inside the crypts.

The number and distribution of crypts seems to show slight variation according to the phase of the reproductive cycle. Thus, during the ovarian storage period, when all the existing crypts contain an enormous quantity of spermatozoa, they are highly vascularized and seem to be more numerous. Vascularization of the crypts appears to increase with the approach of the spawning season, concentrated in the months of January and February, and when female oocytes are totally ripe. During the postspawning period, when some oogonia nests were observed within the ovarian germinal epithelium, some crypts were partially empty of spermatozoa and full of an amorphous material. Optical microscopy observations suggest the existence of empty crypts, but when those observations were compared with those obtained with an electron microscope, it was determined that they were not totally empty. In fact, a gradual decrease in the number of spermatozoa in some crypts occurs as the spawning period approached. Thus, at the end of the year (coinciding with the end of the storage period and the ripening of almost all the oocytes) partially full crypts were found [Fig. 1(e)] and during the first months of the year (when fertilization, the laying of eggs and the beginning of another ovarian cycle occur) there were crypts in which there was only amorphous material [Fig. 1(f)], and which had notably lesser vascularization.

The crypt epithelium is made up of a simple cubical epithelium that rests on a basal lamina, and is surrounded by a layer of smooth muscular fibres [Fig. 2(a)]. This epithelium seems to originate as a specialization of the lamellar epithelium because the cellular morphology of both is very similar. The nuclei of epithelial cells have oval or spherical shapes with prominent indentations during the spawning period. Their morphology, however, changes after the
Fig. 1. (a). Optical microscope view that shows the ovarian internal structure (la, ovigerous lamellae; lu, ovarian lumen; o, oocytes; r, ovarian rachis). Bar = 100 μm. (b) Spherical sperm storage crypt located at the lamellae base (bv, blood vessel; cr, crypt; lab, lamellae base; ol, oogonia; r, ovarian rachis). Bar = 50 μm. (c) General view of a crypt where the cryptal sheath can be observed (bl, basal lamina; cc, cryptal cells; mf, muscular fibres; sz, spermatozoa). Scale bar = 20 μm. (d) Group of spermatozoa floating freely within the interlamellar lumen of the ovary (b, cytoplasmic bag; lu, ovarian lumen). Bar = 5 μm. (e) Partially empty crypt at the final stage of the storage period (am, amorphous material; cc, cryptal cells; h, heads of spermatozoa; f, flagella of spermatozoa). Bar = 10 μm. (f) A crypt in which only some amorphous material remains at the end of the storage period (am, amorphous material; cc, cryptal cells; sz, spermatozoa). Bar = 10 μm.
Fig. 2. (a). Ultrastructure of the cryptal epithelium (bl, basal lamina; cc, cryptal cells; mf, muscular fibres; n, nucleus; sz, spermatozoa). Note the round-elliptical shapes of the nuclei of the cryptal cells. Perinuclear myofilaments of the cryptal cells. Bar = 10 μm. (b) Cytoplasmic constituents indicate an enhanced secretory activity of the cryptal cells (er, endoplasmic reticulum; ga, Golgi apparatus; m, mitochondria; pm, plasma membrane; v, vesicles). Lysosomes. Bar = 10 μm. (c) Desmosomes between two cryptal cells (cc, cryptal cells; d, desmosome; m, mitochondria). Bar = 5 μm. (d) Desmosome separation process between two cryptal cells in the postspawning period (cc, cryptal cells; d, desmosomes; pm, plasma membrane). Bar = 2 μm. (e) An intraepithelial phagocyte next to a crypt (cc, cryptal cells; ph, phagocyte; ps, pseudopodes of the phagocyte; n, nucleus; f, flagella of spermatozoa). Bar = 10 μm. (f) Initial phase of the degeneration of one spermatozoon (dsz, degenerative spermatozoon; h, heads; f, flagella). Bar = 5 μm. The inset shows a higher magnification of a spermatozoon suffering a degenerating process within the crypt. Bar = 2 μm.
laying event: they are smaller and have numerous deep folds, the nuclei are slightly electron-dense and surrounded by cytoplasm rich in perinuclear myofilaments and in the cytoplasm, as can be seen in Fig. 2(b), there is a Golgi apparatus, a large number of secretory vesicles, lysosomes and residual bodies, all of which indicate the secretory characteristics of the epithelium during the time the oocytes are ripening. Specifically, the number of lysosomes, secretory vesicles and vacuoles seems to increase as the spawning period approaches and oocytes complete their maturation.

The epithelial cells surrounding the crypts are firmly joined together by tight intercellular junctions and an enormous quantity of desmosomes [Fig. 2(c)], indicating that there is a compartmentalization of the ovarian cavity. After the laying event, a decrease in the number of desmosomal and tight junctions as well as the breaking of some of them was observed, as can be seen in Fig. 2(d).

Cytological comparisons of the cryptal cells and the Sertoli cells of the testis has been made in order to determine if they have cytological similarities. Light and electron microscope observations show that both cells are morphologically similar and their nucleus is rounded and situated centrally in the cell. Regarding the nucleolus, Sertoli cells have an evident peripheral nucleolus, which is not so evident in the crypt cells nucleus.

As spermatogenic cysts grow and mature, Sertoli cells differentiate and upon spermiation they contain abundant smooth and rough endoplasmic reticulum, lysosomes and lipid droplets. TEM images show that crypt cells also have a cytoplasm rich in smooth and rough endoplasmic reticulum, and, specifically during the post-spawn period, they contain some electron-dense lysosomes.

The distribution of the spermatozoa within the crypts does not seem to follow any particular organized pattern; they organize themselves minimally, side by side, and appear more tightly packed as the spawning period approaches. There are, nonetheless, discrete groups of spermatozoa within the same crypt in which the individual spermatozoa of each group are always perfectly aligned, so that a clear intragroup organization exists. For this reason, in the immense majority of crypts, a head zone is found next to a tail zone which in turn is found next to another head zone [Figs 1(e) and 2(f)]. During the postspawning period, however, residual spermatozoa inside the crypts are less compacted and do not display any organized pattern.

The existence of intraepithelial phagocytes in the neighbourhood of the crypts [Fig. 2(e)], especially evident at the end of the spawning period, denotes the phagocytic activity that takes place against the residual spermatozoa or what remains of them [Fig. 2(f)]. Some phagocytes can be found sporadically during the spawning period, surrounding the crypts but slightly distant from the epithelium. The phagocytic cells have a very electron-dense cytoplasm and the feature that identifies them most readily is the existence of pseudopods, very characteristic of this type of cell [see Fig. 2(e)]. Their nucleus is irregular in form and markedly electron-dense; with TEM it is seen as a very black tone surrounded by cytoplasm that is also very dark. The nucleus contains characteristic highly electron-dense heterochromatin masses, discontinuously arranged and joined to the nuclear envelope. The cytoplasm is rich in ribosome, cisternae of the rough endoplasmic reticulum, vesicles and mitochondria.
Fig. 3. (a). General structure of the spermatozoa (b, cytoplasmic bag; h, head; m, mitochondria; mp, middle-piece; f, spermatozoon flagella). Bar = 5 μm. (b) Cytoplasmic bags surrounding spermatozoa’s heads within the crypt (b, cytoplasmic bag; m, mitochondria). Bar = 10 μm. (c) Spermatozoon mid-piece at the early storage period where many rounded mitochondria can be seen (f, flagellum; m, mitochondria). Bar = 2 μm. (d) Spermatozoon mid-piece at the late stage of storage period with several flattened mitochondria (f, flagellum; m, mitochondria; v, vacuoles). →, the two lateral fins of the spermatozoon’s flagella. Bar = 5 μm. (e) Spermatozoon during late storage just before spawning where the decrease in number of mitochondria, and the near absence of cytoplasm bag can be clearly observed (m, mitochondria; v, vacuoles). →, the position of the cytoplasm bag at the early storage stage. Bar = 5 μm.
Spermatozoa have the typical morphology of the majority of teleosteans, with three clearly differentiated zones: head, midpiece and tail or flagellum [Fig. 3(a), (e)]. The nucleus contains a homogenous, condensed chromatin, without any specific accumulations. A distinguishing feature of sperm is the presence of a prominent ‘cytoplasmic bag’ to one side of the nucleus of the majority of the male sex cells. Using hypotonic, hypertonic and a regular fixative protocol, the existence of this bag was verified and not an artefact provoked by osmotic problems. This cytoplasmic bag is found in spermatozoa that remain inside the crypts [Fig. 3(b)] as well as in those that are freely floating within the ovarian lumen [Fig. 1(d)]. It should also be noted that the January sperm samples, obtained by pressing directly on the testicles of captured specimens, also displayed a prominent cytoplasmic bag surrounding the nucleus. With both fixatives spermatozoa preserve their cytoplasmic bags. TEM observations revealed that ripe spermatozoa wrapped within the cysts do not show the large cytoplasmic bags observed in those in the ovarian crypts, so it is probable that sperm cells acquire them during their passage through the spermatic duct and are inseminated into the ovarian cavity with the cytoplasmic bags around their heads.

TEM observations throughout the reproductive cycle of *H. d. dactylopterus* reveal that as the spawning period approaches, the amount of material within the cytoplasmic bag decreases until it nearly disappears in the months of February and March, when the oocytes are reaching full maturity. The stored sperm cells inside the ovary hardly present a hydrodynamic morphology because of the enormous quantity of cytoplasm retained around their heads during almost the entire storage period. As the spawning period approaches, spermatozoa have less and less cytoplasm and are more hydrodynamic. The midpiece is moderately long, c. 2 μm, and is formed by four mitochondrial layers, each of which is integrated with four to 10 mitochondria. These mitochondria decrease considerably both in number and size [compare Fig. 3(c) and (d)] to the point where they are hardly detected in several spermatozoa [Fig. 3(e)]. In this figure, numerous vacuoles can also be observed in the midpiece.

The spermatozoon flagellum displays a classical axoneme structure with two central microtubules and nine peripheral doublets. The flagellar membrane usually has one or two lateral extensions that look like two small wings of variable lengths [Fig. 3(d)].

**DISCUSSION**

One particular feature of *H. d. dactylopterus* reproduction is sperm storage that takes place, during long periods of time, inside the intraovarian, cryptal structures (Muñoz *et al.*, 1999). The cytological differentiation of epithelial cells to form these structures can be explained by the long period of time the spermatozoa reside within the ovary; in other sperm storage species in which the storage period is shorter, like *A. alcicornis* (Koya *et al.*, 1997) or *C. aggregata* (Gardiner, 1978), no specialized storage structures exist.

The spermatozoa of *H. d. dactylopterus* are type I anacrosomal aquasperm as defined by Jamieson & Leung (1991). In general, spherical-headed spermatozoa with short midpieces are thought to be evolutionarily primitive
and correlated with external fertilization, and morphologically specialized sperm heads (often elongated) with long midpieces are thought to be more advanced and correlated with internal fertilization, as is the case of the blue-mouth. Elongation of the sperm nucleus may correlate with sperm transport and storage in internally fertilizing species (Jamieson, 1987). The streamlined nucleus may aid the passage of spermatozoa through narrow portions and viscous fluids of the female reproductive tract (Gardiner, 1978) or aid the formation of sperm bundles (Atwood & Chia, 1974). Elongation of the sperm nucleus may facilitate the side-by-side alignment of spermatozoa moving through the testicular (and ovarian) ducts (Burns et al., 1995).

Insemination of the female probably takes place during one or several phases of the reproductive cycle of this species and a large number of male sex cells are released into the ovarian lumen (Muñoz, 2000). An indication of this probable multiple copulation is the fact that free spermatozoa have been found inside ovaries that also contain crypts full of spermatozoa. Thus, the spermatozoa within the crypts and those floating freely within the ovarian lumen may come from different males.

Both the distribution of spermatozoa inside the testis detected by Muñoz et al. (2002a, b), and some seminal secretions probably very rich in polysaccharides and proteins, similar to the ones studied by Downing & Burns (1995) in other teleosteans, ease the entrance into the ovary of spermatozoa in groups.

It is evident that long sperm storage requires some characteristic features, such as those related to nutrition and defence from the female's immune system, to ensure the maintenance of the living sperm until the moment of fertilization. With respect to immune protection, it must be pointed out that a large number of tight junctions and desmosomes are located between the cryptal cells, setting up an efficient barrier around the crypt that protects spermatozoa residing within it. Specifically, one of the more important functions of these junctions is the protection of sperm from the female immune system. Hence, some phagocytic cells have been observed near the crypts, mainly at the end of the storage period. These phagocytic cells have spermatozoa in their cytoplasm, evidence of their function in eliminating the non-functional spermatozoa after the breakage of some junctional cell complexes.

This fact has been documented in a previous study on H. d. dactylopterus (Muñoz et al., 2000), as well as in other species with intravarian sperm storage, such as A. alcicornis in which traces of peroxidase confirm a breakdown of these junctional complexes after the spawning period (Koya et al., 1997). Similar observations have been made in C. aggregata (Gardiner, 1978), in X. maculatus (Potter & Kramer, 2000), as well as in oviparous teleosts such as Fundulus heteroclitus heteroclitus (L.) (Brummett et al., 1982) and Syngnathus scovelli (Evermann & Kendall) (Begovac & Wallace, 1987).

The observable decrease in the number of desmosomal and tight junctions after the spawning period, as well as the observed breakdown or separation of some desmosomes, would facilitate the entrance of phagocytic cells into the interior of the crypts. In this way, the increase of phagocytes during the postspawning period, basically in March and April, would serve to eliminate the remnants of non-functional spermatozoa or residual material that might remain within the crypts.
On the other hand, blood vessels located near the crypts become especially obvious at the end of the storage period, a fact that could be related to the production of different substances by the female. During the entire storage period, cryptal cells show considerable secretory activity. Secretory vesicles remain near the plasma membrane of the cell until an extracellular signal causes the release of their contents (Alberts et al., 1996). In fact, mucopolysaccharide granules, released by the cryptal epithelium into a cavity retaining spermatozoa, have been documented in this species with optical microscopy (Muñoz et al., 2002b). Moreover, the presence of significant amounts of rough endoplasmic reticulum, a well-developed Golgi apparatus, secretion vesicles and free ribosomes are evident characteristics of protein synthesis.

The cytological similarities between Sertoli cells and the crypt cells indicate that they should share some functions as both are crucial for the development and survival of sperm cells. An important Sertoli cell function during spermiation is phagocytosis of residual bodies or cytoplasmic remnants of spermatids (Grier, 1993).

The Sertoli cells provide the microenvironment and cytoarchitectural support for the developing spermatogenic cells and directly regulates the reproductive endocrinology of the male. This is in part accomplished through the secretion of a wide variety of proteins. These secretory products include transport proteins to provide nutrient support to the germ cells, extracellular matrix and cell adhesion molecules to promote appropriate cell–cell interactions. These Sertoli cell secreted factors can be categorized as nutritional factors (e.g. transport proteins) that support the nutrient requirements of the germ cells, environmental factors (e.g. extracellular matrix) that influence the physical content and extracellular environment between cells, and regulatory factors (e.g. growth factors) (Skinner, 1991). In this way, crypt cells also may secrete, by means of exocytosis, nutritional factors in order to nourish stored sperm cells, as it has been observed in electron microscope micrographs. More intensive investigations will determine what kind of proteins are secreted by crypt cells and if they also secrete some hormones to the stored sperm cells as the existence in their cytoplasm of an important amount of smooth and rough endoplasmic reticulum denotes.

The spermatozoa inside the crypts, as well as those floating freely within the ovarian lumen, possess a sizeable cytoplasmic bag around their heads which decreases in volume as the spawning period approaches. In addition, sperm that are directly extracted from the testes also have a large amount of cytoplasm around their nucleus, indicating that they enter into the ovary in this state. Once inside, this cytoplasm would serve as a reservoir of nutrients during the long storage period.

Spermatozoa retaining remains of the cytoplasm bag around the head and midpiece seems to be a specific characteristic of this species, because this phenomenon has not been previously described in any species.

A possible explanation would be that the spermatozoa were not totally mature and were spermatids at a final phase of maturation, i.e. very close to spermiogenesis. This hypothesis would be reasonable if the spermatozoa remained inside the testes, and would be interpreted as a case of semicystic spermatogenesis, i.e. the release of spermatids due to a premature breakage
of the cyst bounded by the Sertoli cell. This kind of spermatogenesis, despite being unusual, has been described in several species related to the bluemouth, such as other scorpaenids (Muñoz et al., 2002c; Sábat, 2002). It shows the simultaneous existence in the seminal fluid of both types of germinal cells, spermatoozoa and spermatids, the last completing their spermatogenesis as free cells within the fluid of the ducts. In H. d. dactylopterus, the totally condensed nuclear chromatin of these germinal cells and the existence of a unique type of cells in the efferent and spermatic ducts suggest that they are ripe spermatoozoa waiting for the insemination event.

It is also not possible that the spermatozoa of H. d. dactylopterus are paraspermatozoa, as recently described in the cottoid fish, Blepsias cirrhosus (Pallas), which is also a scorpaeniform, (Hayakawa & Munehara, 2004). The primary reason is that there were cytoplasmic bags in practically all of the spermatozoa observed and, thus, the studied species would have hardly any euspermatozoa. On the other hand, paraspermatozoa described in the above-mentioned cottoid species and in some invertebrates such as Lepidoptera, Heteroptera and Gastropoda (Sivinski, 1984; Jamieson, 1987) are specifically characterized by a nucleus that develops abnormally, which is not the case here.

Stored spermatozoa remain protected against the female’s immune system by a large number of intercellular junctions in the cryptal epithelium. Their nourishment could be achieved in two ways: through their own energy reserves contained in cytoplasmic bags around their heads, or by means of a nutritive contribution from the cryptal epithelium that store the spermatozoa. The fact that the total amount of cytoplasm and mitochondria they contain decreases as the spawning period approaches suggests that the purpose of this cytoplasmic bag is related to the maintenance of the viability of spermatozoa during the long period of time in which they are stored, waiting for the moment in which the female has mature eggs to be fertilized.

Finally, at the moment of fertilization, spermatozoa of the bluemouth display almost no remnants of cytoplasm [see Fig. 3(e)], a morphology that permits entry through the egg micropyle and subsequent fertilization.

References


