Histol Histopathol (2007) 22: 79-83

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Fine structure of spermatozoa in the gilthead sea bream (*Sparus aurata* Linnaeus, 1758) (Perciformes, Sparidae)

G. Maricchiolo[1], L. Genovese[1], R. Laurà[2], V. Micale[1] and U. Muglia[2]

[1]Institute of Coastal Marine Environment, National Research Council (CNR), Messina, Italy and

[2]Department of Morphology, Biochemistry, Physiology and Animal Productions, Morphology Unit, Faculty of Veterinary Medicine, University of Messina - Polo Universitario Annunziata, Messina, Italy

Summary. Scanning and transmission electron microscopy were used to investigate the fine structure of the sperm of the sparid fish *Sparus aurata* L. The mature spermatozoon of gilthead sea bream belongs, like that of the other sparid fish, to a "type I" as defined by Mattei (1970). It has a spherical head which lacks an acrosome, a short, irregularly-shaped midpiece and a long cylindrical tail. The nucleus reveals a deep invagination (nuclear fossa) in which the centriolar complex is located. The two centrioles are approximately perpendicular to each other and show a conventional "9+0" pattern. The proximal centriole is associated with a cross-striated cylindrical body lying inside a peculiar satellite nuclear notch which appears as a narrow invagination of the nuclear fossa. The distal centriole is attached to the nuclear envelope by means of a lateral plate and radial fibres made of an electron-dense material. The short midpiece houses one mitochondrion. The flagellum is inserted perpendicularly into the base of the nucleus and contains the conventional 9+2 axoneme.

Key words: Sperm ultrastructure, Fish, *Sparus aurata,* Sparidae

Introduction

Research on fish spermatozoa has revealed a great variety in both morphology and ultrastructure, which has been useful in establishing phylogenetic relationships among species (Jamieson, 1991). Fish sperm may vary from aflagellate to biflagellate and show an enormous range of shapes, sizes and structures; the number and location of organelles may also vary (Baccetti et al., 1984, Baccetti 1986; Jones and Butler, 1988). The structure of fish sperm may vary more or less widely even within families. For example, an ultrastructural study of the spermatozoa of seven Cyprinidae revealed that each was characterized by a specific organization of sperm organelles within a general pattern common to the whole family (Baccetti et al., 1984).

Within the family Sparidae, the spermatozoon ultrastructure has been examined in twelve species (Mattei, 1970; Gwo et al., 1993; Gwo, 1995; Lahnsteiner and Patzner, 1995; Taddei et al., 1998; Maricchiolo et al., 2004; Gwo et al., 2004, 2005), which all have a uniflagellate anacrosomal aquasperm, as is typically found in external fertilizing fish (Jamieson, 1991), and share some ultrastructural features, such as location of the centriolar complex and insertion of the flagellum with respect to the nucleus. However, they differ in a number of characteristics, such as the number of mitochondria, presence of sidefins and organization of cell organelles.

Submicroscopical features of sperm cells may be of evolutionary significance and could be used as additional characters for taxonomic classifications. Moreover, a knowledge of sperm structure may also prove useful for evaluation of possible cell damage consequent to either cryopreservation procedures (Leveroni Calvi et al., 1993, 1994; Lahnsteiner et al., 1996) or exposure to contaminants (Van Look and Kime, 2003). For this reason, we decided to investigate the sperm ultrastructure of gilthead seabream *Sparus aurata*, with the purpose of increasing the current knowledge of sperm morphology in the family Sparidae.

Materials and methods

Semen samples from adult male *S. aurata* (total body length = 30.2±1.6 cm, body weight = 650±15 g, n=10) held in captivity at the facilities of the Istituto Sperimentale Talassografico of Messina (Sicily – Italy) were collected at the peak of spawning season

Offprint requests to: G. Maricchiolo, Institute of Coastal Marine Environment, National Research Council (CNR), Spianata S. Raineri 86, 98122 Messina, Italy. e-mail: giulia.maricchiolo@iamc.cnr.it

(November), two weeks after the beginning of spermiation. Fish were anesthetized with MS-222 (0.1g/l), urine was extruded by gently squeezing the fish near the genital pore, faeces were carefully discarded, and the genital area dried. Milt was stripped from running males by gentle abdominal massage and collected in glass tubes.

Samples were fixed in 0.1M cacodylate buffer (pH 7.5) containing 4.5% paraformaldehyde, 2.2% glutaraldehyde and 5% sucrose for 2 h in ice bath, postfixed in 1% osmium tetroxide in 0.1M cacodylate buffer with 5% sucrose for 1 h in ice bath and centrifugated at 900 g for 10 min. The sperm samples were then processed for transmission (TEM) or scanning (SEM) electron microscopy. For TEM, sperm pellets were encapsulated in agar (Glauert, 1975), dehydrated in an ethanol series and embedded in Araldite. Ultrathin sections were cut using an ultramicrotome (Ultracut-E, Reichert-Jung) stained with uranyl acetate and lead citrate, and were examined under a Jeol Jem 100SX transmission electron microscope. For SEM, sperm pellets were glued on poly-L-lysine-coated coverslips (Scala and Pasquinelli, 1987). After dehydration through an ascending ethanol series, samples were critical-point dried using liquid argon (Balzers CPD 030), coated with 20 nm gold-palladium in an SCD050 sputter coater (BAL-TEC) and examined under a Cambridge Stereoscan 240 SEM operating at 20 kV.

The following morphometrical parametres of at least 50 randomly chosen spermatozoa were measured: head length, head width, midpiece length and flagellum length. All measurements were carried out on light micrographs of semen smears stained with crystal violet and rose Bengal, using a LEICA IM1000 software.

Results

The mature spermatozoon of gilthead seabream is a uniflagellated cell, differentiated into an acrosome-less head, a short midpiece and a long cylindrical tail (Fig. 1a,b). The rounded head measured 1.87±0.19 μm (n=50) and contained a nucleus with condensed granular chromatin. At the base of the nucleus, the nuclear envelope invaginated, forming a depression called the nuclear fossa. The centriolar complex was formed by the proximal and distal centriole that were located inside the nuclear fossa. The two centrioles were approximately perpendicular to each other, although not in the same axis, and showed a conventional "9+0" microtubular triplet pattern. The proximal centriole was associated with a cross-striated cylindrical body, surrounded by an electron-dense material, lying inside a peculiar satellite nuclear notch, which appeared as a narrow invagination of the nuclear fossa. The two centrioles were linked to each other, as well as to the nuclear envelope, by an electron-dense material organized into diverse structures. A disk of electron-dense material was located between the two centrioles (Fig. 1c). Moreover, the lateral surface of the distal centriole was attached to the nuclear envelope by means of a lateral plate and radial fibres made of an electron-dense material (Fig. 1d).

The midpiece was short and irregularly-shaped (Fig. 1b). One mitochondrion could be seen in the midpiece region, containing an electron-dense matrix and irregularly arranged tubular cristae. At the midpiece level, the axoneme was separated from the plasma membrane by a narrow cytoplasmatic canal which was formed by an invagination of the membrane itself. Vesicles and inclusions were irregularly distributed inside the cytoplasm (Fig. 1e).

The axoneme had a typical eukaryotic organization, consisting of nine outer doublet microtubules and a central pair of singlet microtubules (9+2 pattern) (Fig. 1f). The doublets were connected to each other by microfilaments and radial spokes. The flagellum, which had a cylindrical shape throughout its length, measured 53.27±5.57 mm (n=50) and was inserted perpendicularly to the base of the nucleus.

Discussion

The gilthead sea bream possesses the typical *uniflagellate anacrosomal aquasperm* which is characteristic of many external fertilizing fish (Jamieson, 1991). This sperm type is characterized by a spherical or ovoid nucleus, a short midpiece with a few spherical mitochondria and a flagellar tail with the usual 9+2 microtubular pattern. Although very simple in morphology, the *anacrosomal aquasperm* of the Teleostei can adopt a wide range of structural variations that may bear phylogenetic implications and prove valuable in taxonomy (Baccetti et al., 1984; Jamieson, 1991; Mattei, 1991). Such diversities clearly persist at lower taxonomic categories, as has been shown for the order Perciformes, where the general structure of the spermatozoon is not always uniform at the suborder or even the family level (Mattei, 1991). Mattei (1970) recognized two types of *simple anacrosomal aquasperm*. Type I aquasperm has the flagellum and centrioles inserted directly into the nucleus, resulting in a nucleus which surrounds the centrioles. In Type II aquasperm, the flagellum remains parallel to the base of nucleus and although a depression (fossa) is usually found at this point, the centrioles remain outside of it (Mattei, 1970, 1988). Since the latter configuration is the most widely distributed among the perciforms, although not exclusive to them (Jamieson, 1991; Mattei, 1991; Lahnsteiner et al., 1994), it is usually termed the perciform-type sperm.

The spermatozoon of *S. aurata* shows a flagellar axis perpendicular to the nuclear base and a location of centrioles internal to the nuclear fossa; these morphological features suggest that this sperm belongs to type I, according to Mattei (1991). This sperm type, which has been reported in only 12 out of the 41 families of the perciforms studied so far (Mattei, 1991), occurs in the six sparid species previously investigated (Mattei, 1970; Gwo et al., 1993; Gwo, 1995; Lahnsteiner and Patzner, 1995; Taddei et al., 1998; Maricchiolo et al.,

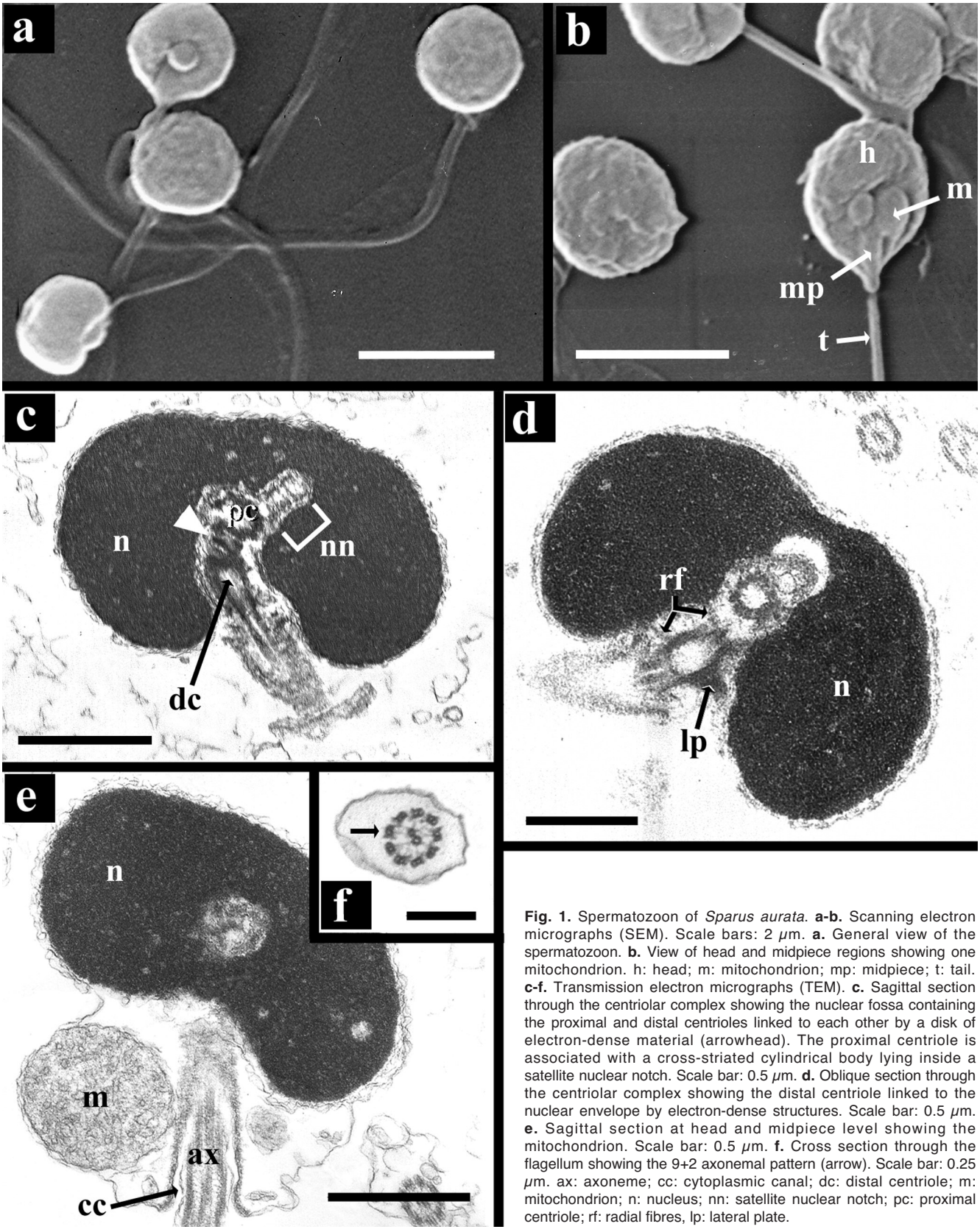

Fig. 1. Spermatozoon of *Sparus aurata*. **a-b.** Scanning electron micrographs (SEM). Scale bars: 2 μm. **a.** General view of the spermatozoon. **b.** View of head and midpiece regions showing one mitochondrion. h: head; m: mitochondrion; mp: midpiece; t: tail. **c-f.** Transmission electron micrographs (TEM). **c.** Sagittal section through the centriolar complex showing the nuclear fossa containing the proximal and distal centrioles linked to each other by a disk of electron-dense material (arrowhead). The proximal centriole is associated with a cross-striated cylindrical body lying inside a satellite nuclear notch. Scale bar: 0.5 μm. **d.** Oblique section through the centriolar complex showing the distal centriole linked to the nuclear envelope by electron-dense structures. Scale bar: 0.5 μm. **e.** Sagittal section at head and midpiece level showing the mitochondrion. Scale bar: 0.5 μm. **f.** Cross section through the flagellum showing the 9+2 axonemal pattern (arrow). Scale bar: 0.25 μm. ax: axoneme; cc: cytoplasmic canal; dc: distal centriole; m: mitochondrion; n: nucleus; nn: satellite nuclear notch; pc: proximal centriole; rf: radial fibres, lp: lateral plate.

2004). Although sharing with them the main structural aspects, the spermatozoon of *Sparus aurata* differs from those of the other sparids in a number of features, the most striking of which is the satellite nuclear notch adjacent to the proximal centriole, lodging a cross-striated cylindrical body. A satellite nuclear notch, containing some electron-dense material, has been reported by Gwo et al. (1993) and Gwo (1995) in the spermatozoa of three other sparids, namely *A. schlegeli*, *A. latus* and *Sparus sarba*. It is therefore likely that this character is shared with other sparids belonging to the genera Acanthopagrus and Sparus and may be of systematic importance. However, its function remains uknown. The peculiarity of the satellite nuclear notch in *S. aurata* is the cross-striated cylindrical body contained inside it, which is a kind of satellite apparatus of the centriolar complex, resembling the striated rootlet of Elopomorphs (Jamieson, 1991) and the cross-striated fibrous body of the garpike Lepisosteus osseus (Lepisosteiformes; Afzelius, 1978). A striated rootlet, located at the level of the distal centriole, has also been reported in *A. latus* (Sparidae; Gwo, 1995), *Leuciscus souffia* (Cypriniformes; Baccetti et al., 1984) and *Poecilia latipinna* (Cyprinodontiformes; Grier, 1973). The origin and function of this cross-striated body remain unknown, although it has been suggested that it might be a short flagellar root, perhaps a kind of an abortive flagellum (Mattei et al.,1981; Mattei, 1988).

The presence of cytoplasmic structures associated with the basal body and axoneme are of widespread occurrence in teleost sperm, of both type I and type II (Eiras-Stofella et al., 1993; Gwo et al., 1993, 1994; Gwo 1995; Lahnsteiner and Patzner, 1997; Hara and Okiyama, 1998; Abascal et al., 2002). These structures are likely to be involved in the anchoring of the flagellar apparatus to the sperm head, thereby enabling the centriolar complex to withstand the torque generated by the movement of the flagellum (Gwo, 1995). The more or less strong anchorage of the flagellum may also determine sperm resistance to procedures involved in artificial fertilization and gamete preservation, as has been demonstrated in carp sperm, which is very sensitive to strong agitation (Billard et al., 1995). The morphological features of such anchoring structures may vary considerably in different species. Afzelius (1979) suggested that the anchoring fiber apparatus, mitochondria and centriolar complex can be used as indicators of relationships among metazoan phyla. The osmiophilic lateral plate anchoring the distal centriole to the nuclear envelope in *S. aurata* closely resembles the basal foot described in the spermatozoon of *A. latus* (Gwo, 1995).

A common source of interspecific variation in the sperm structure in fishes is the number of mitochondria contained in the midpiece (Baccetti et al., 1984; Mattei, 1991) and this holds true for the family Sparidae. One mitochondrion is present in *S. aurata* (present study) as in *D. sargus*, *D. puntazzo*, *P. erythrinus*, *B. boops* and *P. major*, two in *A. australis*, *L. rhomboids* and *R. sarba*, three in *A. latus* and *A. berda*, four in *A. schlegeli* and *A. probatocephus*. The duration of sperm motility has been related to the size of the midpiece (Billard et al.,1995), which, in turn, depends to some extent on the number and/or size of mithocondria stored in it (Baccetti et al., 1984). As it is known, ATP produced by mitochondrial respiration is the main energy source for sperm motility. Thus, the number of mitochondria might be related to sperm motility (Lahnsteiner and Patzner, 1995), contributing also to a more or less successful gamete preservation (Labbé et al., 1998).

The spermatozoon of *S. aurata* resembles those of other sparid species previously investigated, sharing with them some main ultrastructural features, such as location of the centriolar complex and insertion of the flagellum with respect to the nucleus. However, they differ in a number of characteristics, such as the number of mitochondria, presence of satellite nuclear notch and organization of cell organelles, with particular reference to the anchoring apparatus of the flagellum. Further research on other sparid species will help determine whether such differences bear taxonomic implications at the family level.

References

Abascal F.J., Medina A., Megina C. and Calzada A. (2002). Ultrastructure of *Thunnus thynnus* and *Euthynnus alletteratus* spermatozoa. J. Fish Biol. 60, 147-153.

Afzelius B.A. (1978). Fine structure of the garfish spermatozoon. J. Ultrastr. Res. 64, 309-314.

Afzelius B.A. (1979). Sperm structure in relation to phylogeny in lower Metazoa. In: The spermatozoon: Maturation, motility and surface properties. Fawcett D.W. and Bedford J.M. (eds). Urban and Schwarzenberg, Baltimore & Munich. pp. 243-251.

Baccetti B. (1986). Evolutionary trends in sperm structure. Comp. Biochem. Physiol. 85, 29-36.

Baccetti B., Burrini A.G., Callaini G., Gilbertini G., Mazzini M. and Zerunian S. (1984). Fish germinal cells. I. Comparative spermatology of seven cyprinid species. Gamete Res. 10, 373-396.

Billard R., Cosson J., Crim L.W. and Suquet M. (1995). Sperm physiology and quality. In: Broodstock management and egg and Larval Quality. Bromage N.R. and Roberts R.J. (eds). Blackwell Science, Oxford. pp. 25-52.

Eiras-Stofella D.R., Gremski W. and Kuligowski S.M. (1993). The ultrastructure of the mullet *Mugil curema Valenciennes* (Teleostei, Mugilidae) spermatozoa. Rev. Brasil. Zool. 10, 619-618.

Glauert A.M. (1975). Fixation methods. In: Fixation, dehydration and embedding of biological specimens. Pratical methods in electron microscopy. Glauert A.M. (ed). Elsevier, North-Holland Biomedical Press. pp 73-110.

Grier H.J. (1973). Ultrastructure of the testis in the teleost *Poecilia latipinna* spermiogenesis with reference to the intercentriolar lamellated body. J. Ultrastruct. Res. 45, 82-92.

Gwo J.C. (1995). Spermatozoan ultrastructure of the teleost fish *Acanthopagrus latus* (Perciformes: Sparidae) with special reference to the basal body. J. Submicrosc. Cytol. Pathol. 27, 391-396.

Gwo J.C., Gwo H.H. and Chang S.L. (1993). Ultrastructure of the spermatozoon of the teleost fish *Acanthopagrus schlegeli*

(Perciformes: Sparidae). J. Morphol. 216: 29-33.

Gwo J.C., Gwo H.H., Kao Y.S., Lin B.H. and Shih H. (1994). Spermatozoan ultrastructure of two species of grouper *Epinephelus malabaricus* and *Plectropomus leopardus* (Teleostei, Perciformes, Serranidae) from Taiwan. J. Submicrosc. Cytol. Pathol. 26, 131-136.

Gwo J.C., Kuo M.C., Chiu J.Y. and Cheng H.Y. (2004). Ultrastructural of Pagrus major and Rhabdosargus sarba (Perciformes: Sparidae: Sparinae). Tissue Cell 36, 141-147.

Gwo J.C., Chiu J.Y. Lin C. Y., Su Y. and Yu S.L. (2005). Spermatozoal ultrastructure of four Sparidae fishes: *Acanthopagrus berda*, *Acanthopagrus australis*, *Lagodon rhomboids* and *Archosargus probatocephus*. Tissue Cell 37, 109-115.

Jamieson B.G.M. (1991). Fish evolution and systematics: Evidence from spermatozoa. Cambridge University Press. Cambridge. pp 1-319.

Jones P.R. and Butler R.D. (1988). Spermatozoon ultrastructure of *Platichthys flesus*. J. Ultrastruct. Mol. Struct. Res. 98, 71-82.

Labbé C., Maisse G. and Billard R. (1998). Fish sperm cryopreservation: what has to be improved. In: New species for mediterranean aquaculture. Enne G. and Greppi G.F. (eds). Elsevier. Paris. pp 97-110.

Lahnsteiner F. and Patzner R.A. (1995). Fine structure of spermatozoa of two marine teleost fishes, the red mullet, *Mullus barbatus* (Mullidae) and the white sea bream, *Diplodus sargus* (Sparidae). J. Submicrosc. Cytol. Pathol. 27, 259-266.

Lahnsteiner F. and Patzner R.A. (1997). Fine structure of spermatozoa of four littoral teleosts, *Symphodus ocellatus*, *Coris Julius*, *Thalassoma pavo* and *Chromis chromis*. J. Submicrosc. Cytol. Pathol. 29, 477-485.

Lahnsteiner F., Weismann T. and Patzner R.A. (1994). Fine structure of spermatozoa of the burbot, *Lota lota* (Gadidae, Pisces). J. Submicrosc. Cytol. Pathol. 26, 445-447.

Lahnsteiner F., Berger B., Weissman T. and Patzner R.A. (1996). Motility of spermatozoa of *Alburnus alburnus* (Cyprinidae) and its relationship to seminal plasma composition and sperm metabolism. Fish Physiol. Bioch. 15,167-179.

Leveroni Calvi S., Zoccarato I., Gasco L. and Andrione A. (1993). Effetto del diluitore e delle modalità di congelamento e scongelamento sulla motilità del seme crioconservato di carpa (*Cyprinus carpio* L.). Riv. Ital. Acquacol. 28, 187-195.

Leveroni Calvi S., Zoccarato I., Gasco L. and Andrione A. (1994). Effetto dell'aggiunta di trealosio e/o albumina e della concentrazione di metanolo sulla motilità del seme di carpa (*Cyprinus carpi*o L.). Riv. Ital. Acquacol. 29, 45-51.

Maricchiolo G., Genovese L., Laurà R., Micale V. and Muglia U. (2004). Fine structure of spermatozoa in pandora (*Pagellus erythrinus* Linnaeus, 1758) (*Perciformes Sparidae)*. Histol. Histopathol. 19, 1237-1240.

Mattei X. (1970). Spermiogenèse comparée des poissons. In: Comparative Spermatology. Baccetti B. (ed). Academic Press. New York. pp. 55-70.

Mattei X. (1988). The flagellar apparatus of spermatozoa in fish. Ultrastructure and evolution. Biol. Cell 63, 151-158.

Mattei X. (1991). Spermatozoon ultrastructure and its systematic implication in fish. Can. J. Zool. 69, 3038-3055.

Mattei C., Mattei X., Marchand B. and Billard R. (1981). Reinvestigation de la structure des flagelles spermatiques: cas particulier des spermatozoids à mitochondria annulaire. J. Ultrastr. Res. 74, 307-312 (in French).

Scala C. and Pasquinelli G. (1987). Gli elettroni secondari. In: Microscopia elettronica a scansione in biologia. CLUEB. Bologna. pp. 59-181.

Taddei A.R., Abelli L., Baldacci A., Barbato F., Fausto A.M. and Mazzini M. (1998). Ultrastructure of spermatozoa of marine teleosts *Umbrina cirrosa L.* and *Diplodus puntazzo* (Cetti), and preliminary observation on effects of cryopreservation. XXXIII International Symposium on New Species for Mediterranean Aquaculture. Alghero, April 22-24, pp. 407-413.

Van Look K.J.W. and Kime D.E. (2003). Automated sperm morphology analysis in fishes: the effect of mercury on goldfish sperm. J. Fish Biol. 63, 1020-1033.

Accepted August 1, 2006