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Morphological evaluation of spermatogenesis in Lake Magadi tilapia (*Alcolapia grahami*): A fish living on the edge

M.B. Papah[a,*], S.M. Kisia[a], R.O. Ojoo[a], A.N. Makanya[a], C.M. Wood[b,c], G.D. Kavembe[d], J.N. Maina[e], O.E. Johannsson[f], H.L. Bergman[g], P. Laurent[b], C. Chevalier[b], A. Bianchini[h], L.F. Bianchini[h], D.W. Onyango[a]

[a] Dept. of Veterinary Anatomy and Physiology, University of Nairobi, 30197-00100 Nairobi, Kenya
[b] Dept. of Biology, McMaster University, Hamilton, ON, Canada L8S 4K1
[c] Marine Biology and Fisheries, Rosenstiel School, University of Miami, FL 33149, USA
[d] Dept. of Range and Wildlife Sciences, South Eastern University College, 170-90200 Kitui, Kenya
[e] Dept. of Zoology, University of Johannesburg, South Africa
[f] Great Lakes Laboratory for Fisheries and Aquatic Sciences, Dept. of Fisheries and Oceans, Canada, Burlington, ON, Canada L7R 4A6
[g] Dept. of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA
[h] Instituto de Ciências Biológicas, Universidade Federal do Rio Grande (FURG), 96201-900 Rio Grande, RS, Brazil

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ABSTRACT

Spermatogenesis in Lake Magadi tilapia (*Alcolapia grahami*), a cichlid fish endemic to the highly alkaline and saline Lake Magadi in Kenya, was evaluated using light and transmission electron microscopy. Spermatogenesis, typified by its three major phases (spermatocytogenesis, meiosis and spermiogenesis), was demonstrated by the presence of maturational spermatogenic cells namely spermatogonia, spermatocytes, spermatids and spermatozoa. Primary spermatogonia, the largest of all the germ cells, underwent a series of mitotic divisions producing primary spermatocytes, which then entered two consecutive meiotic divisions to produce secondary spermatocytes and spermatids. Spermatids, in turn, passed through three structurally distinct developmental stages typical of type-I spermiogenesis to yield typical primitive anacrosomal spermatozoa of the externally fertilizing type (aquasperm). The spermatozoon of this fish exhibited a spheroidal head with the nucleus containing highly electron-dense chromatin globules, a midpiece containing ten ovoid mitochondria arranged in two rows and a flagellum formed by the typical 9 + 2 microtubule axoneme. In addition, the midpiece, with no cytoplasmic sheath, appeared to end blindly distally in a lobe-like pattern around the flagellum; a feature that was unique and considered adaptive for the spermatozoon of this species to the harsh external environment. These observations show that the testis of *A. grahami* often undergoes active spermatogenesis despite the harsh environmental conditions to which it is exposed on a daily basis within the lake. Further, the spermiogenic features and spermatozoal ultrastructure appear to be characteristic of Cichlidae and, therefore, may be of phylogenetic significance.

1. Introduction

Lake Magadi tilapia, *Alcolapia grahami* is a small cichlid teleost endemic to Lake Magadi, a southern-most Rift Valley Lake in Kenya (Coe, 1966; Seegers and Tichy, 1999; Walsh et al., 2001). This fish is the only vertebrate species thriving in this lake which presents arguably one of the most hostile environmental conditions for survival of any teleost on earth; with osmolality ~ 600 mOs mL^{-1}, alkalinity ~ 380 mmol L^{-1}, pH ~ 10, temperatures as high as 42 °C, extreme diurnal fluctuation of dissolved O_2 and intense ultra-violet solar radiation (Reite et al., 1974; Johansen et al., 1975; Narahara et al., 1996; Wilson et al., 2004). In order to survive in this extremely hostile and stressful environment, this fish has developed amazing physiological, morphological and behavioural adaptations (Maina, 1990, 2000; Wood et al., 1994, 2012; Laurent et al., 1995; Narahara et al., 1996; Bergman et al., 2003; Wilson et al., 2004), the most important and unique one being urea excretion instead of ammonia (Randall et al., 1989; Wood et al., 1989), a phenomenon that is completely obligate (Wood et al., 2002). In addition, the internal environment of the fish is unusual, with exceptionally high extracellular and intracellular pHs (Johansen et al., 1975; Wood et al., 1994) and moderately elevated urea concentrations (Wood et al., 1989, 1994; Wilson et al., 2004) relative to other teleosts. Despite increased research interests on this fish in the recent past, focusing

* Corresponding author. Tel.: +254 724366910.
E-mail address: papah@uonbi.ac.ke (M.B. Papah).

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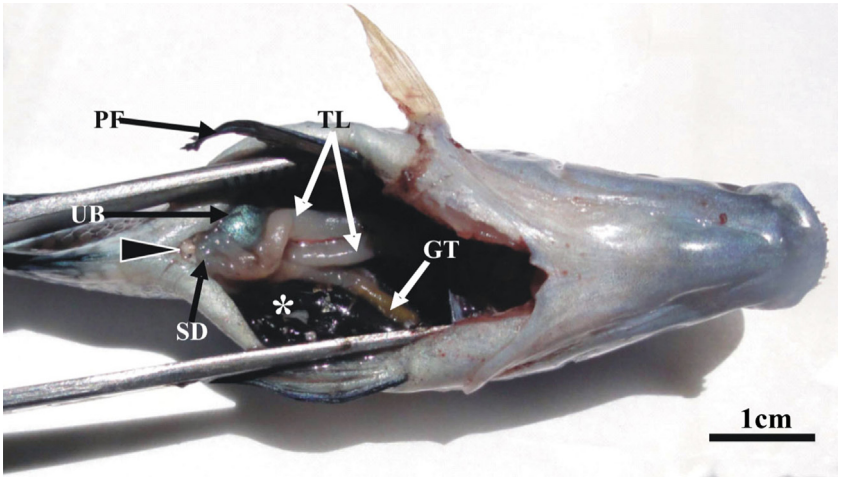

Fig. 1. A specimen of an adult *A. grahami* with the abdomen opened and part of the viscera removed to show testicular lobes (TL) *in situ*. The common spermatic ducts (SD), the pelvic fin (PF), the parietal peritoneum lined by a thin membrane highly laden with a dark pigment (asterisk) are also shown. GT denotes part of the caudal gut, UB the urinary bladder while the arrowhead points to the genital papilla.

mainly on its diverse adaptive strategies, very little is known about its reproductive biology. So far, only behavioural aspects of brooding and breeding have been documented. For example, it is known that this fish exhibit external fertilization (Coe, 1966; Seegers and Tichy, 1999), an indication that its spermatozoon is tolerant of the high salt, high pH and high alkalinity content which characterize the waters of the lake. In our previous work, we also observed that these fish exhibit highly skewed sex ratios, a phenomenon we largely attributed to the harsh conditions of the lake (unpublished).

However, information on the testicular structure and spermatogenesis in Lake Magadi tilapia still remains largely unknown (Onyango and Kisia, 2008), similar to other species within the genus *Alcolapia*.

In the present study, the testicular structure of mature *A. grahami* freshly collected from their native habitat was described both at histological and ultrastructural levels, with the main goal of determining the progression of spermatogenesis in this fish in the face of the extreme external environmental conditions of the lake and the unusual internal environmental conditions of the animal. Ultimately, this study aimed at generating information that will be useful in the understanding of the male *A. grahami* reproductive biology.

2. Materials and methods

2.1. Fish samples

Adult *A. grahami* were collected in July 2010 by use of a seine net from the Fish Springs Lagoon on the periphery of Lake Magadi (see Coe, 1966; Narahara et al., 1996; Wilson et al., 2004 for maps). They were quickly transferred to 20-l plastic buckets ¾-way filled with Lake Magadi water then taken immediately to an outdoor laboratory set up on the balcony of one of the buildings generously provided by Magadi Soda Company. A total of ten adult fish with a mean weight of 8.86 g (range 3–15.12 g) and length 8.2 cm (range 6–10 cm) were anaesthetized by transfer into ice-cold Magadi water at a temperature range of 0.5–1 °C for 3–5 min (see Pic, 1978; Wood et al., 2012), and then euthanized by cephalic concussion. A ventromedian incision was then made on each fish to expose the gonads for sex determination. Six of them were male and were therefore recruited in this study while the remaining four female fish were discarded. The remaining intact fish were returned to the lake.

2.2. Tissue processing

The testes were excised and fixed by immersion into 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2–7.4) and stored at 4 °C for at least 24 h. Thereafter, the tissues were rinsed in the same buffer, post-fixed in 2% aqueous osmium tetroxide (OsO_4) for 2 h, dehydrated in ascending concentrations of ethanol (50%, 70%, 90%, 95% and 100%), cleared in propylene oxide, infiltrated and then embedded in araldite resin mixture. Semi-thin (1 μm thick) sections were then obtained from a Reichert® ultra microtome, stained with Toluidine blue and examined and photographed using a Leica® DM 500 light microscope. Ultrathin (70–100 nm thick) sections were subsequently obtained from the same blocks, mounted on copper grids, stained with uranyl acetate and counterstained with lead citrate, examined and photographed using a Philips® CM12 Transmission Electron Microscope (TEM).

3. Results

3.1. Testicular morphology

Grossly, the testis of a mature *A. grahami* appeared as bilobed, creamy-white, elongate organs located in the coelomic cavity whose parietal peritoneum was lined entirely by a thin membrane heavily laden with a dark pigment (Fig. 1). These lobes converged caudally to connect with the common spermatic duct and were suspended from the dorsal body wall by the mesorchium. Histologically, the testicular parenchyma which was enclosed by a connective tissue capsule (tunica albuginea), comprised the germinal and interstitial compartments, with the former organized as seminiferous lobules terminating blindly beneath the capsule (Fig. 2A). Each lobule was defined by the lamina propria on whose basement membrane rested the germinal epithelium (spermatocysts). These spermatocysts, occurring in various developmental stages and sizes, were distributed along the entire length of the lobular lumen. Essentially, these spermatocysts comprised a clone of germ cells at the same stage of development circumscribed by the cytoplasmic extensions of Sertoli cells, hence, forming sites of spermatogenesis in this fish (Fig. 2B). At the end of spermatogenesis,

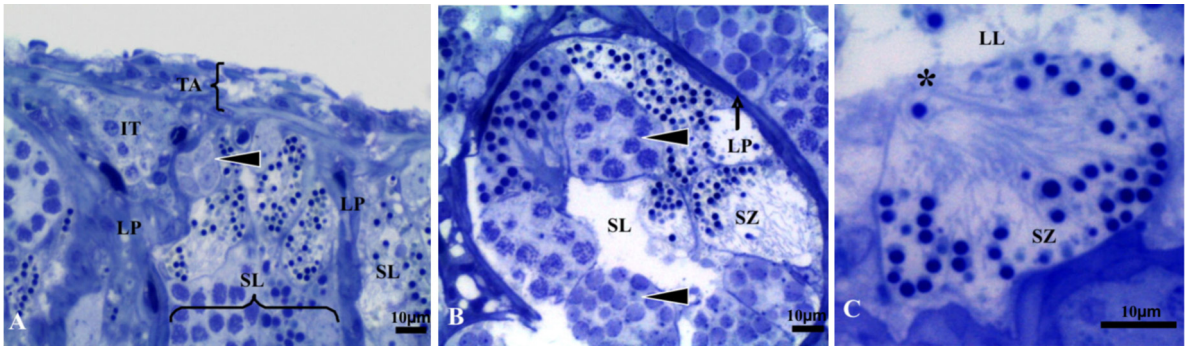

Fig. 2. Light micrographs of the testicular parenchyma in Lake Magadi tilapia. (A) Seminiferous lobules (SL) bounded by lamina propria (LP), and ending blindly at the tunica albuginea (TA). Interstitial tissue (IT) is evident. Toluidine blue. (B) A cross-section of the seminiferous lobule (SL) containing spermatocysts at varying stages of development (arrowheads) formed by the encystment of germ cells by Sertoli cell cytoplasmic processes. Lamina propria (LP) and spermatozoa (SZ) are shown. Toluidine blue. (C) An opening spermatocyst (asterisk) containing spermatozoa (SZ) that are yet to be released into the lobule lumen (LL).

spermatocysts opened to release spermatozoa into the lumen (Fig. 2C).

3.2. Spermatogenic cells

In this species, all the developing germ cells were confined within their respective spermatocysts in the seminiferous lobules (Fig. 2B). On the basis of the nuclear size and their respective morphological characteristics, four successive germ cell-types in this species were identified, namely spermatogonia, spermatocytes, spermatids and spermatozoa.

3.3. Spermatogonia

Spermatogonial germ cells observed in the testis of Lake Magadi tilapia comprised two types: primary (type A) and secondary (type B) spermatogonia distributed randomly along the lobular wall.

***Primary (type A) spermatogonia*:** These were the largest of all the spermatogenic cells present in the testis of *A. grahami*. Two types were identified; the undifferentiated and differentiated type A spermatogonia, distinguished mainly by their nuclear sizes as well as the number of cells in a cyst. The undifferentiated spermatogonia were spheroidal solitary cells distributed along the entire seminiferous lobules (Fig. 3A). On average, the nuclear diameter of these cells measured 8.47 ± 0.24 μm ($n = 20$), and hence, they were the largest of all the spermatogenic cells in Lake Magadi tilapia. They underwent mitotic division giving rise to differentiated spermatogonia distributed throughout the lobules. These differentiated spermatogonia, whose nuclear diameter measured on average 7.32 ± 0.25 μm ($n = 20$), occurred in clones of 2–8 spheroidal cells within a cyst (Fig. 3B). Ultrastructurally, the undifferentiated type A spermatogonia presented scanty, electron-lucent euchromatin material distributed uniformly in their large nuclei. In addition, their conspicuous cytoplasm contained characteristic electron-dense substances positioned contiguous to a cluster of mitochondria. These inter-mitochondrial dense substances were frequently found adjacent to the nuclear envelope. Mitochondria often occurred in clusters within the cytoplasm and were round in shape with ill-defined cristae (Fig. 3C).

***Secondary (type B) spermatogonia*:** Each differentiated type A spermatogonium further underwent mitotic division giving rise to secondary (type B) spermatogonia. Histologically, type B spermatogonia were spheroidal in shape with centrally located nuclei containing sparse, lightly condensed and irregularly distributed clumps of chromatin material (Fig. 4A). Ultrastructurally, the chromatin material in these cells appeared to have greater density compared to the primary spermatogonia. The cytoplasm also contained aggregates of ovoid mitochondria with ill-defined cristae, found in close association with inter-mitochondrial dense substances as in the primary spermatogonia (Fig. 4B). These cells were comparatively smaller in size with a nuclear diameter measuring, on average, 6.2 ± 0.17 μm ($n = 21$).

3.4. Spermatocytes

Two types of spermatocytes were observed in the testis of Lake Magadi tilapia: primary and secondary spermatocytes. Primary spermatocytes were formed subsequent to the last mitotic division of type B spermatogonia. The most frequently recognized primary spermatocytes were those of prophase I of the first meiotic division, distinguished on the basis of their nuclear size, density and characteristics of chromatin condensation and distribution. Zygotene spermatocytes, whose nuclei diameter measured 6.05 ± 0.20 μm ($n = 18$), presented relatively electron-dense patches of coarse chromatin material with numerous short synaptonemal complexes (Fig. 5A). Pachytene spermatocytes, with their nuclei diameter measuring 6.33 ± 0.14 μm ($n = 25$) exhibited relatively increased density and a greater amount of the coarse chromatin material but few synaptonemal complexes compared to their immediate predecessors. Their nuclei were clearly outlined by a typical nuclear envelope comprising the inner and outer layers (Fig. 5B). Diplotene spermatocytes, whose nuclear diameter measured, on average, 6.32 ± 0.19 μm ($n = 20$), revealed more-or-less spheroidal nuclei enclosed by a double layered nuclear envelope. Chromatin material was organized into thick clumps of more electron-dense patches compared to pachytene spermatocytes. In addition, there were a few synaptonemal complexes within the nucleus (Fig. 5C). The cytoplasm contained numerous ovoid mitochondria and vesicles. Completion of the first meiotic division in primary spermatocytes resulted in secondary spermatocytes. The latter cells were rarely encountered due to their short lifespan since they quickly entered the second meiotic division to form spermatids. Nonetheless, some cysts containing a few intact secondary spermatocytes were encountered in some parts of the seminiferous lobule together with a number of metaphase figures of the second meiotic division (Fig. 6). Ultrastructurally, secondary spermatocytes exhibited electron-dense nuclei measuring 3.25 ± 0.18 μm ($n = 14$).

3.5. Spermatids

Three maturational stages of spermatids were identified in *A. grahami* during the course of spermatogenesis, namely early, intermediate and late spermatids. These maturational stages were identified on the basis of their characteristic condensation and distribution of chromatin material within their nuclei, development of the midpiece and flagella, and exocytosis of residual cytoplasm to

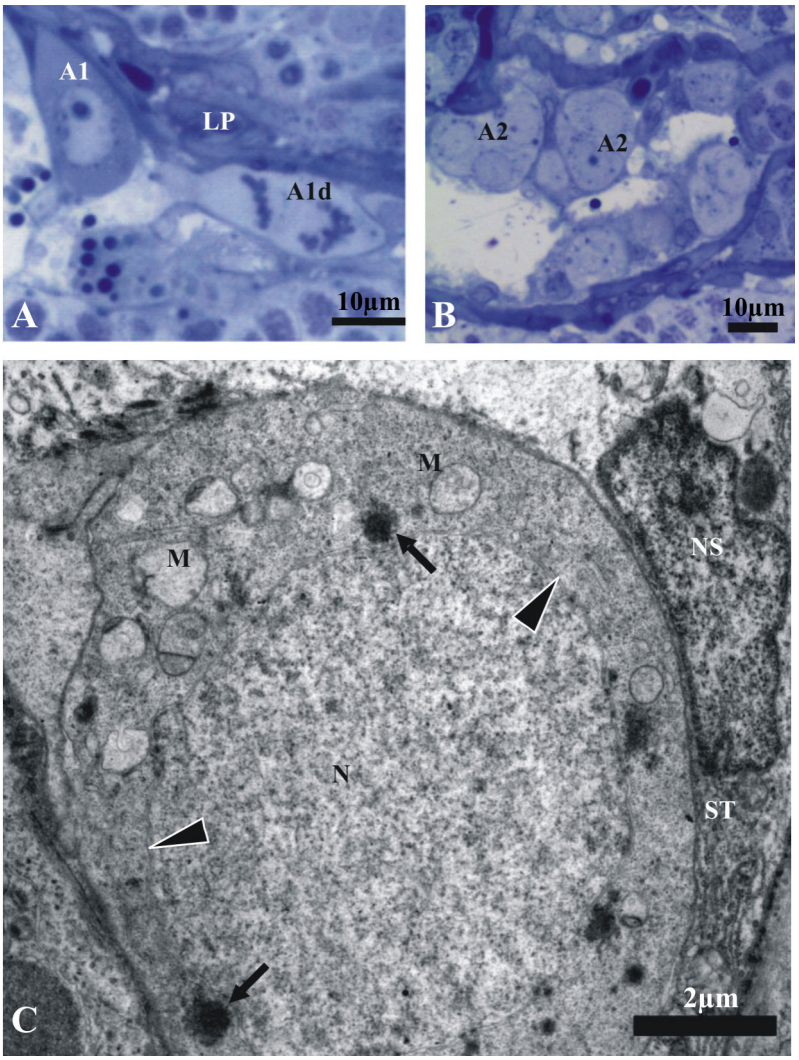

Fig. 3. Primary spermatogonia in Lake Magadi tilapia testis. (A) A semi-thin section showing intact (A1) and dividing (A1d) undifferentiated primary spermatogonia. Lamina propria (LP) is evident. Toluidine blue. (B) A semi-thin section showing cysts containing differentiated primary spermatogonia (A2). Toluidine blue. (C) An electron micrograph showing undifferentiated primary spermatogonia with the nucleus (N) containing sparse electron-lucent chromatin material. In the cytoplasm are mitochondria (M) and electron-dense substances (arrows) found adjacent to the nuclear envelope (arrow heads). A Sertoli cell (ST) resting on the basement membrane (asterisks) with its nucleus (NS) containing coarse electron-lucent chromatin material is evident.

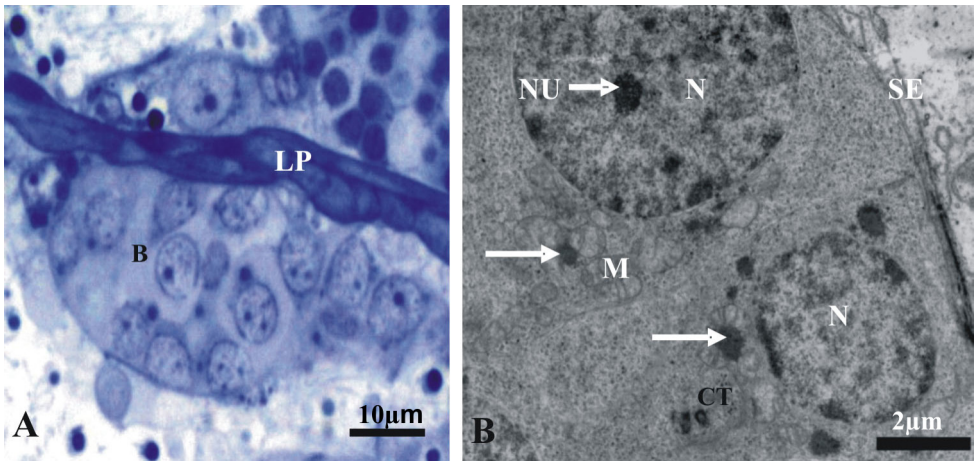

Fig. 4. Secondary spermatogonia in *A. grahami* testis. (A) A photomicrograph showing a cyst containing type B spermatogonia (B). Lamina propria (LP) is evident. Toluidine blue. (B) An electron micrograph of type B spermatogonia with nuclei (N) containing clumps of relatively high electron-dense heterochromatin material compared to primary spermatogonia. In the cytoplasm are inter-mitochondrial dense substance (arrows), mitochondria (M), centriole (CT) and a portion of a thin Sertoli cell cytoplasmic extension (SE). The nucleolus (NU) within the nucleus is evident.

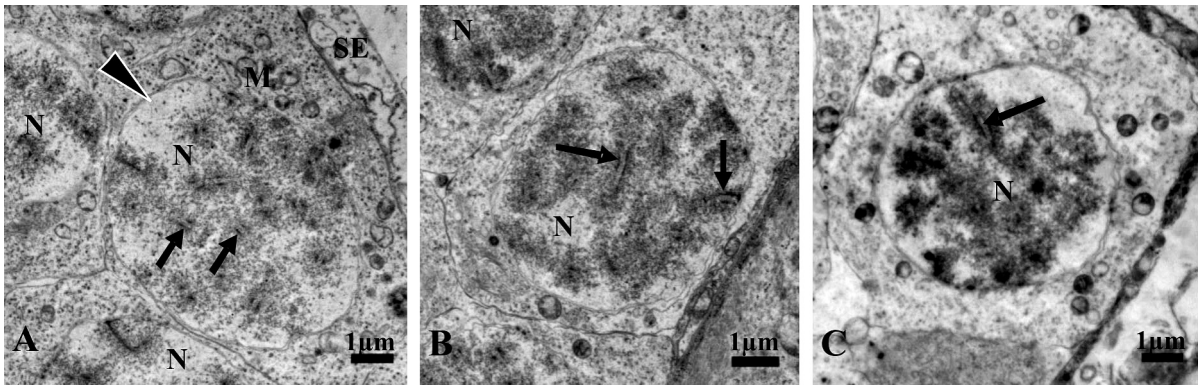

Fig. 5. TEM micrographs of primary spermatocytes in *A. grahami* testis. (A) Zygotene spermatocytes with nuclei (N) containing electron-lucent chromatin material with numerous synaptonemal complexes (arrows). Nuclear envelope (arrowhead), mitochondria (M) and a relatively thin Sertoli cell cytoplasmic extension (SE) are observed. (B) Pachytene spermatocytes with nuclei (N) exhibiting clusters of chromatin material with relatively increased electron-density. Notice the relatively decreased amount of synaptonemal complexes (arrows). (C) A diplotene spermatocyte whose nucleus (N) contains patches of thick electron-dense irregular clumps of chromatin material and a few synaptonemal complexes (arrows).

form spermatozoa. Early spermatids, which in most cases appeared as closely packed cells, were generally ovoid to spheroidal in shape with spheroidal nuclei measuring 3.40 ± 0.18 µm ($n = 20$) in diameter. Their nuclei contained heterogeneous chromatin material and the nuclear envelope appeared either irregular or indistinct, indicating its re-formation immediately after the second meiotic division (Fig. 7A). Furthermore, these cells possessed a more-or-less symmetrical cytoplasm around their nuclei with generalized distribution of ovoid mitochondria. Few developing flagella were observed within their interstices. Intermediate spermatids, whose nuclei measured approximately 3.44 ± 0.12 µm ($n = 20$) in diameter, contained uniformly distributed fine granular homogeneous chromatin material enclosed by a double layered nuclear envelope. Their cytoplasm, containing vesicular structures, demonstrated asymmetry where it seemed to surround the distal pole of the nucleus around the developing flagellum (Fig. 7B). At this stage, the point of attachment of the plasma membrane to the distal centriole appeared to have moved together with the centriolar complex (diplosome) towards the nucleus, hence, creating the future cytoplasmic canal. An indentation into the nucleus then formed at the point where the proximal centriole made contact with the nuclear envelope. Concomitantly, a single flagellum was developing from the basal body of the distal centriole distally, resulting in a tangential orientation of the diplosome-flagellar axis with respect to the nucleus (Fig. 7B).

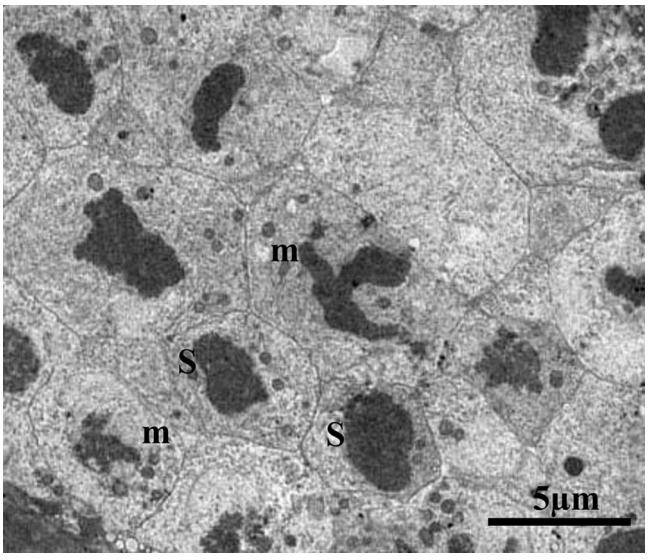

Fig. 6. An electron micrograph of a cyst containing secondary spermatocytes (S) with some of these cells undergoing 2nd meiotic division (m). Notice the highly electron-dense chromatin material in the nuclei.

Late spermatids, with nuclear diameter measuring 2.75 ± 0.13 µm ($n = 20$), formed the final phase of spermatogenesis in Lake Magadi tilapia. At this stage, the initially fine, granular and generally homogeneous chromatin material associated with the intermediate spermatids began to condense into foci of highly electron-dense, homogeneous chromatin, appearing as dark globules (Fig. 8A). Eventually, most of the nucleus was filled by the compacted chromatin material, distributed irregularly against an electron-lucent matrix (Fig. 8B). Organelles within the cytoplasm were displaced to the distal end of the nucleus, leaving a thin layer of cytoplasm proximally appearing as lamellations. Most of the cytoplasm in the late spermatids was later shed off and phagocytosed by the cytoplasmic processes of the Sertoli cell walling the cyst at this stage, resulting in their thick appearance (Fig. 8C). As the condensation continued, the nuclear indentation initially formed in the intermediate spermatids continued to extend deep into the nucleus, forming the nuclear/implantation fossa. The entire diplosome, which initially was tangential to the nucleus in the intermediate spermatids, now became perpendicular to the nucleus and inside the nuclear fossa, demonstrating the complete rotation of the nucleus along the diplosome-flagellar axis (Fig. 8D).

3.6. Spermatozoa

These cells, resulting from the final transformation of late spermatids, had nuclei measuring 1.82 ± 0.08 µm ($n = 40$) in diameter, and contained highly condensed chromatin material covering the entire nucleoplasm. They were therefore, arguably, the smallest germ cells in this fish. These cells exhibited faintly stained masses of flagella projecting behind the dark stained heads within the lumen (Fig. 9A). At maturity, a typical *A. grahami* spermatozoon was composed of the head and a single flagellum (Fig. 9B and C), the latter being surrounded by the midpiece proximally. The head generally round, comprised a large conspicuous nucleus situated at the centre (Fig. 9B). There was no evidence of an acrosome or an acrosomal vesicle on the head. The nucleus was packed with compacted, highly electron-dense chromatin material, organized as spheroidal masses closely attached to each other. As in late spermatids (Fig. 8B), the cytoplasm showed several lamellar configurations running transversely from the irregular nuclear envelope to the plasma membrane (Fig. 9B and C). Excess cytoplasm cast

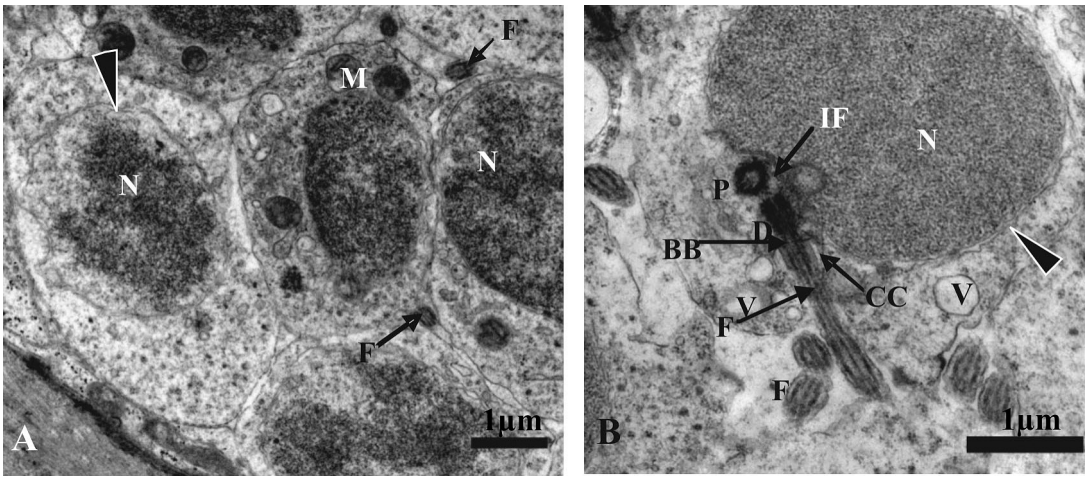

Fig. 7. TEM micrographs of spermatids in *A. grahami*. (A) Early spermatids immediately after differentiation from secondary spermatocytes. Their nuclei (N) contain irregularly distributed clumps of coarse heterochromatin. Notice the irregular outline of the nuclear envelope (arrowhead) surrounded by relatively symmetrical cytoplasm containing ovoid mitochondria (M). At the interstices of the cells are developing flagella (arrows). (B) An intermediate spermatid where the proximal centriole (P), located at the indentation fossa (IF) on the nuclear envelope (arrowhead), and the distal centriole (D) with its basal body (BB) where the flagellum (F) grows from are all oriented tangential to the nucleus (N). Vesicular structures (V), cytoplasmic canal (CC) and other flagella (F) axonemes are also evident.

off by the developing spermatozoa as residual bodies occupied the intercellular spaces (Fig. 9B). Distal to the head, the remaining cytoplasm initially displaced from the head region, surrounded the base and most of the proximal portion of the flagellum symmetrically. This cytoplasm and its contents then formed the midpiece of the mature spermatozoon, which measured about 1.5 µm in length. The cytoplasmic portion at the distal end of the midpiece was limited by a blind ending plasma membrane. Hence, a sagittal section of an *A. grahami* spermatozoon depicted a midpiece made up of a bilobed structure separated centrally by the flagellum (Fig. 9C). The most notable feature of the midpiece was the presence of round mitochondria within the cytoplasm, arranged in two transverse layers: the proximal and distal layers, both surrounding the centrally located axoneme (Fig. 10A). The proximal layer was in close proximity with the distal centriole typified by its nine microtubule triplets (9+3) pattern (Fig. 10B). The entire midpiece therefore appeared to comprise about 10 mitochondria, distinctly isolated from each other by a thin cytoplasmic matrix (Fig. 10A and B). At this

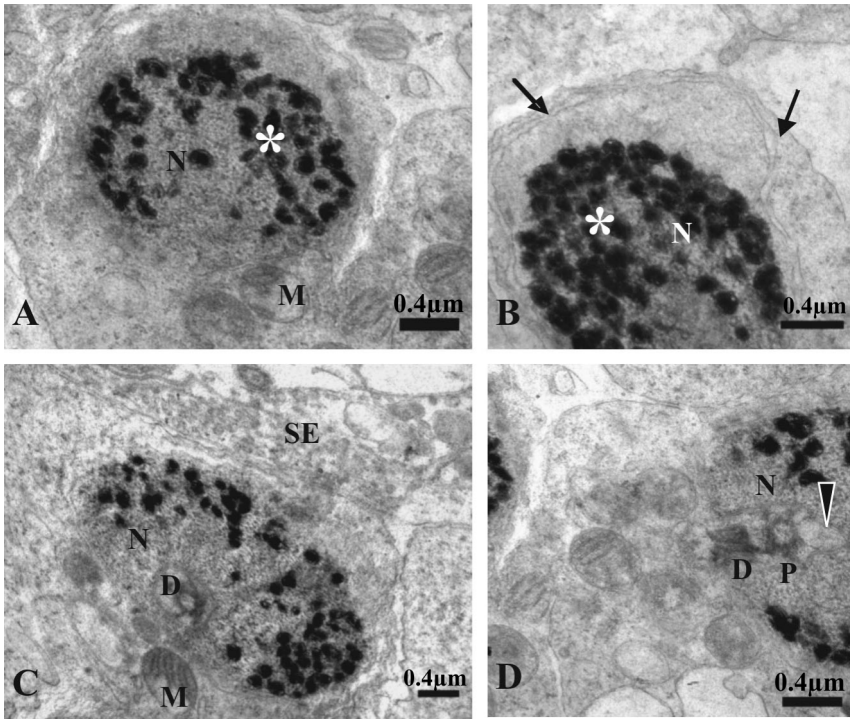

Fig. 8. TEM micrographs of late spermatids in Lake Magadi tilapia. (A) Head of the late spermatid exhibiting initial phase of chromatin condensation and compaction into highly electron-dense globules (asterisk) within the nucleus (N). Mitochondria (M) on the distal end of the cell are evident. (B) Head of the late spermatid showing the nucleus (N) under advanced phase of chromatin compaction evidenced by increased chromatin globules (asterisk). Lamellar configurations (arrows) in the cytoplasm proximal to the nucleus are present. (C) Transverse section of the late spermatid at the proximal portion of the midpiece. Notice the relatively thick Sertoli cell cytoplasmic extension (SE) indicative of its phagocytotic activity. The nucleus (N), distal centriole (D) and a mitochondrion (M) are evident. (D) Oblique section of the proximal portion of the midpiece showing the longitudinal orientation of the diplosome proximal (P) and distal (D) centrioles to the nucleus (N), and the deep seated nuclear fossa (arrowhead).

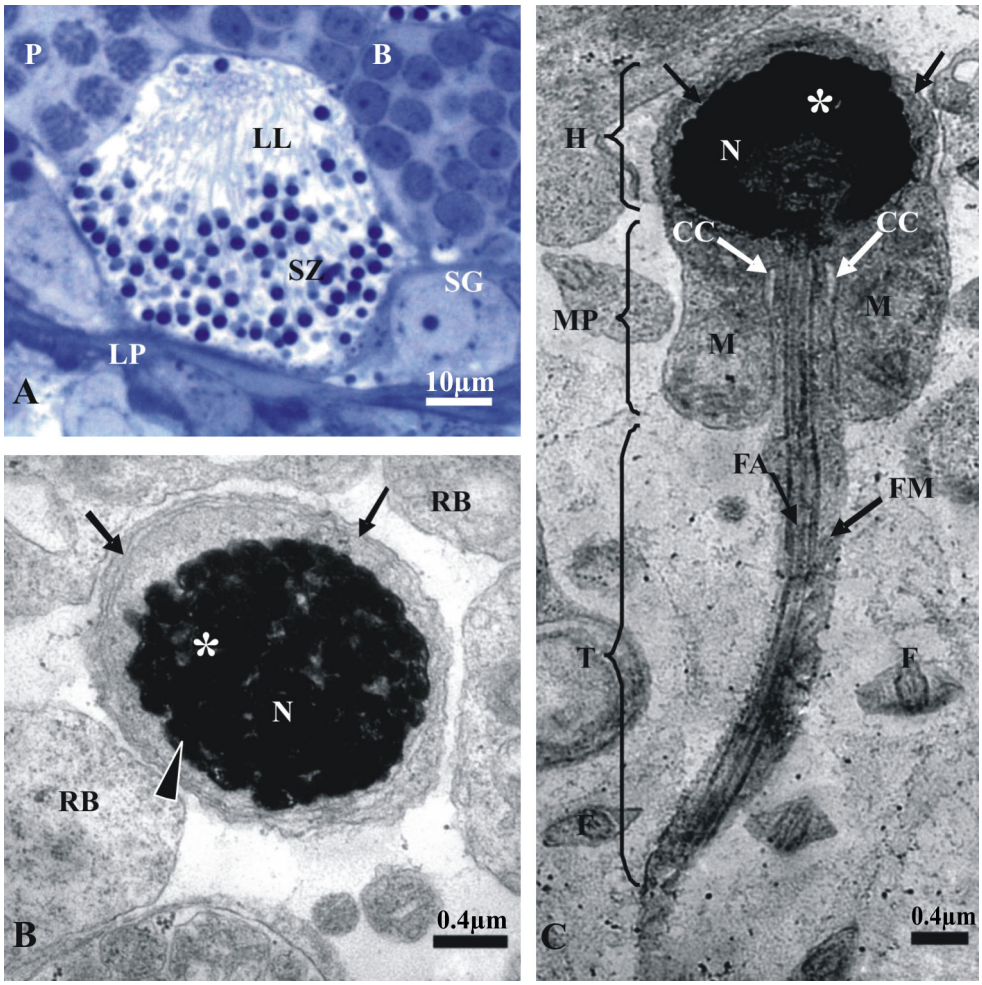

Fig. 9. Spermatozoa in Lake Magadi tilapia. (A) A photomicrograph of spermatozoa within the lobular lumen (LL). Notice the dark staining heads connected to faintly staining tails within the lumen. Primary spermatogonium (SG), secondary spermatogonia (B), pachytene spermatocytes (P) and lamina propria (LP) are evident. Toluidine blue. (B) A TEM micrograph showing a transverse section of a spermatozoon head. Notice the highly electron-dense globules of chromatin material (asterisk) packed within the nucleus (N). Residual bodies (RB) are observed. (C) A longitudinal section of a spermatozoon of *A. grahami* showing the major parts: head (H), midpiece (MP) and tail (T). The nucleus (N) is capped with a thin cytoplasm containing lamellar configurations (arrows). Notice that the midpiece containing mitochondria (M) ends blindly distally. The cytoplasmic channel (CC) and the flagellar axoneme (FA) covered by the flagellar membrane (FM) and flagella (F) of other neighbouring spermatozoa are evident.

stage, the cytoplasmic canal became more apparent and elongated compared to the same in spermatid stages. This cytoplasmic canal shared a common base with the flagellum proximally, and was open distally, facilitating a direct communication with the external environment (see Figs. 9C and 10A). Still at the level of the midpiece, the flagellum was centrally positioned within the narrow, symmetrical cytoplasmic canal. The flagellar axoneme comprised 9 distinct microtubule peripheral doublets and a central pair enclosed by a plasma membrane along its entire length (Fig. 10C and D).

3.7. Sertoli cells

Sertoli cells in *A. grahami* appeared as thin or flattened somatic cells within the germinal compartments exhibiting cytoplasmic processes that formed a wall around spermatogenic cells in various developmental stages (spermatocysts) (Fig. 2B). Ultrastructurally, these Sertoli cells generally rested on the basement membrane separating the lobular system and the lamina propria (Fig. 3C). They presented highly irregular pleiomorphic nuclei containing coarse electron-lucent chromatin material diffusely distributed. The cytoplasmic processes encysting various germ cells seemed to vary in thickness. While those processes encysting type B spermatogonia (Fig. 4B) and spermatocytes (Fig. 5A) were thinner, those surrounding spermatids were generally thicker (Fig. 8C).

4. Discussion

The testicular parenchyma of *A. grahami*, comprising the germinal and interstitial compartments separated by a basement membrane and enclosed by the tunica albuginea, is a typical feature of both teleostean (Grier, 1981; Loir et al., 1995) García-López et al., 2005 and mammalian (Hermo et al., 2010) testicular tissue. In particular, the disposition of the germinal compartment with its distribution of primary spermatogonia among different groups of teleosts has necessitated the classification of the testicular architecture in this group of vertebrates into two major categories: lobular and anastomosing tubular types with each having either restricted or unrestricted spermatogonial distribution (Grier, 1981, 1993; Nagahama, 1983; Parenti and Grier, 2004). In this study, the disposition of the germinal epithelium with its distribution of primary spermatogonia in the testis of *A. grahami* conforms to the unrestricted lobular testis type described by Parenti and Grier (2004). Each testicular lobule in a mature *A. grahami* demonstrated

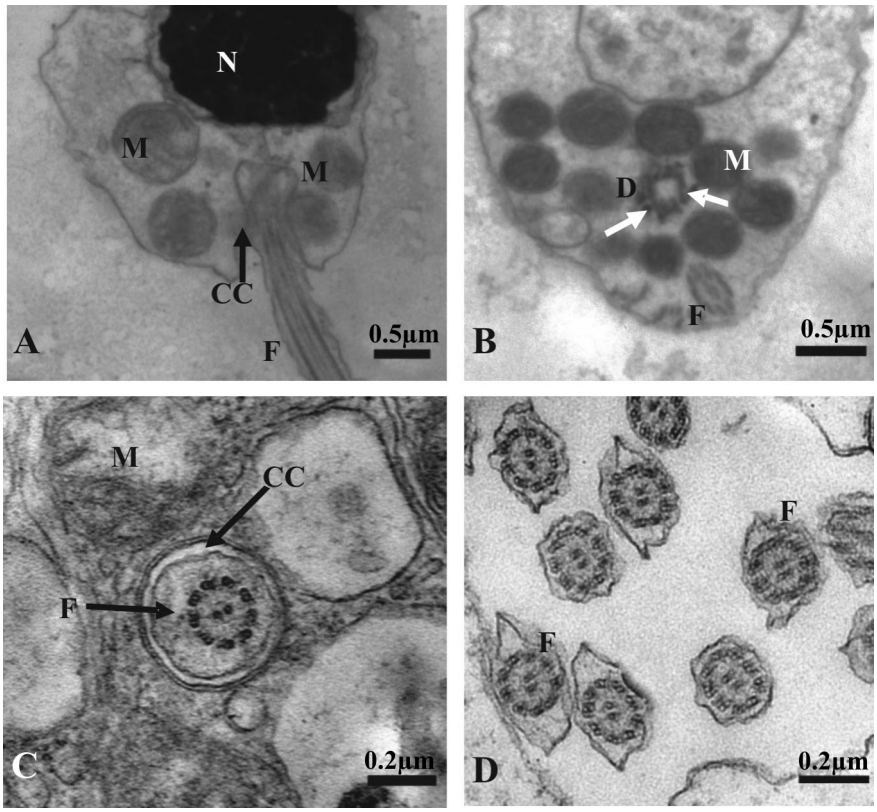

Fig. 10. TEM micrographs of spermatozoa in Lake Magadi tilapia. (A) A longitudinal section of the spermatozoon at the midpiece region showing the two transverse layers of mitochondria (M). The nucleus (N), cytoplasmic canal (CC), and flagellum (F) are shown. (B) An oblique-section of the proximal portion of the midpiece of a mature spermatozoon showing mitochondria (M) surrounding the distal (D) centriole. Notice the nine microtubule triplets (9 + 3) of the distal centriole (arrows) and the flagellum (F) distally. (C) A cross-section of the distal portion of the midpiece revealing the central location of the flagellum (F) within the cytoplasmic channel/canal (CC). M denotes mitochondria. (D) Cross-sections of the tails/flagella (F) revealing the 9 peripheral doublets and a central pair of the microtubule pattern of the axoneme enclosed by flagellar plasma membrane.

germ cell associations exclusively found in cysts with the exception of spermatozoa. This observation suggests that each cyst functions as an independent spermatogenic unit where germ cells undergo the entire spermatogenic process culminating in the liberation of spermatozoa into the lobule lumen. Taken together, these observations signify that *A. grahami* undergo complete cystic spermatogenesis as previously reported in the majority of teleosts (Mattei et al., 1993; Weltzien et al., 2002; Schulz et al., 2005, 2010; Chung, 2008; Leal et al., 2009; Chung et al., 2010; Shabana, 2012).

4.1. Spermatogenic cells

The morphological appearance of primary spermatogonia (undifferentiated and differentiated) in Lake Magadi tilapia exhibited features frequently shared by most other studied fish species (Quagio-Grassiotto and Carvalho, 1999; Koulish et al., 2002). Particularly conspicuous, was the presence of inter-mitochondrial dense substance in the cytoplasm located adjacent to the nuclear envelope. These inter-mitochondrial dense substances have previously been observed in the primary spermatogonia of a number of teleosts where they have been variously referred to as 'nuages', 'inter-mitochondrial cement', 'dense nuclear bodies' or 'dense germinal bodies' (Stoumboudi and Abraham, 1996; Muñoz et al., 2002; Fishelson, 2003; Fishelson et al., 2006; Jun et al., 2006; Chung, 2008; Chung et al., 2010). It has been demonstrated that these substances are units of RNA which detach from the nucleus to cross the nuclear membrane into the cytoplasm (Eddy, 1975). They have further been regarded as the early indicators of spermatogonia formation, but they disappear in later stages of germ cell development (Koulish et al., 2002; Fishelson et al., 2006). Ultimately, type A differentiated spermatogonia divide mitotically giving rise to secondary (type B) spermatogonia (Schulz et al., 2005, 2010). The presence of inter-mitochondrial dense substances in type B spermatogonia in this fish is a feature that has similarly been reported in other fish species (Quagio-Grassiotto and Carvalho, 1999; Koulish et al., 2002; Fishelson et al., 2006; Chung, 2008; Chung et al., 2010). The most advanced type B spermatogonia in Lake Magadi tilapia differentiated into primary spermatocytes after undergoing the final mitotic division in a similar fashion as previously reported by Nóbrega et al. (2009). The abundant spermatocytes in the testis of *A. grahami* were those under prophase I of the first meiotic stage of spermatogenesis, a common phenomenon frequently observed in germ cells at this stage of spermatogenesis both in mammals (França and Russell, 1998; de Rooij and Russell, 2000; Hermo et al., 2010) and teleosts (Fishelson et al., 2006; Nóbrega et al., 2009). Among the primary spermatocytes, pachytene spermatocytes were the largest. This finding is in general agreement with reports in the Nile tilapia (Schulz et al., 2005, 2010) and zebrafish (Leal et al., 2009). In guppies, however, zygotene spermatocytes have the largest volume (Schulz et al., 2010) while diplotene spermatocytes are the largest in mammals (França and Russell, 1998). In *A. grahami*, the most notable feature in primary spermatocytes was the formation of synaptonemal complexes frequently observed in zygotene and pachytene spermatocytes. The occurrence of synaptonemal complexes in this fish was consistent with its localization in the black porgy, *Acanthopragus schegeli* (Gwo and Gwo, 1993), *Sorubim lima* (Quagio-Grassiotto and Carvalho, 1999), *Thalassoma bifasciatum* (Koulish et al., 2002) and *Pampus argenteus* (Chung et al., 2010). The

first meiotic division in primary spermatocytes ends with the formation of secondary spermatocytes, which then proceeds through the short-lived second meiotic division to form spermatids (Silva and Godinho, 1983; Selman and Wallace, 1986; Quagio-Grassiotto and Carvalho, 1999). Therefore, the occurrence of relatively few cysts observed in the testis of Lake Magadi tilapia containing secondary spermatocytes may probably be as a result of the short life-span associated with these cells.

4.2. Spermiogenesis

Ultrastructural studies on spermiogenesis in teleosts have revealed the presence of three distinct types: types I, II, and III spermiogenesis. These types have been identified on the basis of either the presence or absence of nuclear rotation and orientation of the flagellum to the nucleus (Jamieson, 1991; Lahnsteiner and Patzner, 1997; Shahin, 2006, 2007; Quagio-Grassiotto and Oliveira, 2008; Schulz et al., 2010). Apart from these three spermiogenic types, some fish species, for example, the cichlid *Oreochromis niloticus* (Lou and Takahashi, 1989), cyprinid *Cyprinus carpio* (Billard, 1986) and characid *Paracheirodon innesi* (Jamieson, 1991) demonstrate incomplete or partial nuclear rotation, hence, the flagellar axis is eccentrically oriented with respect to the nucleus. In this study, the entire process of spermiogenesis in *A. grahami* depicted a typical case of type I spermiogenesis where diplosome migration and growth of the flagellum, both disposed tangential to the nucleus, as well as establishment of a cytoplasmic canal, were a common occurrence in the early phase. This was followed by complete nuclear rotation, resulting in a flagellum that was oriented centrally within the cytoplasmic canal and perpendicular to the nucleus in the final spermatozoon. This type of spermiogenesis has been extensively described in the past (Jamieson, 1991; Mattei, 1991), and frequently occurs in many teleosts (Gwo and Gwo, 1993; Spadella et al., 2007; Chung, 2008; Chung et al., 2010; Shabana, 2012; Rey Vázquez et al., 2012) resulting in type I spermatozoa.

4.3. Spermatozoa

Ultimately, the final products of spermiogenesis in teleosts, the spermatozoa, are presented as two types; aquasperm and introsperm, based on the mode of reproduction of the fish. Most teleosts exhibit external fertilization, hence, have retained the simple primitive spermatozoa known as the aquasperm while the internally fertilizing ones have an advanced type of spermatozoa referred to as the introsperm (Jamieson, 1991; Mattei, 1991). In this study, the characteristic morphology of the spermatozoon of Lake Magadi tilapia typified by a round head, short midpiece (about 1.5 μm in length) and a few mitochondria (approx. 10) are features reminiscent of a typical primitive type of externally fertilizing sperm (aquasperm), similar to the descriptions of spermatozoa in other teleosts (Poirier and Nicholson, 1982; Mattei, 1988; Lo Nostro et al., 2003). This finding compares favourably with previous reports on the breeding and brooding behaviour of *A. grahami* where, among other things, it was concluded that Lake Magadi tilapia exhibits external fertilization (Coe, 1966; Seegers and Tichy, 1999).

4.4. Sertoli cells

In the testes of many teleosts, Sertoli cells rest on the basement membrane and separate the lamina propria from the germ cells in the lobular system (Grier, 1981; Loir et al., 1995; Fishelson et al., 2006). These cells are responsible for the formation of spermatocysts, which are the basic functional units of spermatogenesis in fish (Schulz et al., 2010). These cysts are formed by the Sertoli cell cytoplasmic processes frequently enclosing germ-cell clones derived from a single spermatogonium (Nóbrega et al., 2009). In Lake Magadi tilapia, these Sertoli cells enclosed all developing germ cells until spermiation, similar to what is reported in most teleosts (Fishelson et al., 2006; Rey Vázquez et al., 2012). In *A. grahami*, it was also observed that cysts of type B spermatogonia and primary spermatocytes possessed relatively thin Sertoli cell cytoplasmic processes compared to the thick ones enclosing advanced spermatids. It is speculated that the increase in these cytoplasmic structures is due to the increased need for lysosomic resources required for catabolism of phagocytized degenerated spermatids, spermatozoa, or residual bodies shed off during the period of spermiogenesis. Indeed, the importance of Sertoli cells in phagocytosis of residual bodies and undischarged germ cells after spermiation is well documented (Koulish et al., 2002; Lo Nostro et al., 2003; Chung et al., 2010; Rey Vázquez et al., 2012). Therefore, the nature of encystment in advanced spermatids in *A. grahami* by the Sertoli cell cytoplasmic processes is not a new phenomenon and may be serving the same purpose as previously suggested.

4.5. Spermiogenic and spermatozoal features of phylogenetic significance

It is generally believed that the various types of spermatozoa formation results in a diverse variety of spermatozoa which are specific and highly conserved among various taxonomic units in teleosts (Mattei, 1988; Schulz et al., 2010). This phenomenon has, therefore, been exploited as a potent tool for phylogenetic analysis in fish (Jamieson, 1991; Quagio-Grassiotto and Oliveira, 2008; Burns et al., 2009; Schulz et al., 2010). In this regard, the rounded head of the spermatozoa of *A. grahami*, with no evidence of an acrosome or acrosomal vesicle, conforms to what is known in related fish species belonging to the family, Cichlidae (Lou and Takahashi, 1989; Matos et al., 1995; Quagio-Grassiotto et al., 2003). However, this phenomenon contrasts with those of other cichlids namely *Satanoperca jurupari* (Matos et al., 2002) and *Cichlasoma dimerus* (Rey Vázquez et al., 2012), which have cylindrical heads. It also appears to differ significantly with those of other families within the suborder Labroidei to which *A. grahami* belongs. The Embiotocid *Cymatogaster aggregata*, for example, possesses spermatozoa with elongated heads (Gardiner, 1978), while those in the family Pomacentridae (Mattei, 1991; Lahnsteiner and Patzner, 1997) have kidney shaped heads.

In teleosts, especially during the advanced stages of spermiogenesis, chromatin material undergoes gradual condensation processes, presumably as histones are replaced by the more basic protamines (Alfert, 1956; Louie and Dixon, 1972). In *A. grahami*, the gradual compaction and condensation of chromatin material into foci of small electron-dense globules characterized the terminal stages of spermiogenesis. Consequently, the spermatozoa arising from the spermiogenic process bore highly compacted electron-dense chromatin globules, reflecting the clustered form of chromatin condensation previously described by Jamieson (1991) and Mattei (1991), and found in a number of related cichlids (Lou and Takahashi, 1989; Matos et al., 1995; Quagio-Grassiotto et al., 2003; Fishelson, 2003). This form of chromatin condensation contrasts with the homogeneous (Jamieson, 1991; Mattei, 1991; Rey Vázquez et al., 2012) and floccus (Gusmão-Pompiani et al., 2009) types.

Nuclei of spermatozoa of most teleosts are reportedly penetrated by a nuclear fossa whose position, shape and prominence vary among different taxonomic units (Jamieson, 1991; Mattei, 1991). This phenomenon is well illustrated in families under the suborder Labroidei. In this regard, the Embiotocid *C. aggregata*, for example, shows no apparent nuclear fossa (Gardiner, 1978) while

the family Pomacentridae presents a simple depression on the nuclear outline instead of an actual nuclear fossa (Mattei, 1991; Lahnsteiner and Patzner, 1997). In *C. dimerus* a cichlid, the nuclear fossa is reportedly shallow (Rey Vázquez et al., 2012). In this study, the spermatozoa of *A. grahami* presented an actual nuclear fossa, extending deep into almost half the depth of the nucleus. This fossa appeared to lie longitudinal to the nucleus following the complete rotation of the head. Similar findings have been reported in other related cichlids such as *O. niloticus* (Lou and Takahashi, 1989) and *Cichla intermedia* (Quagio-Grassiotto et al., 2003). However, unlike *A. grahami*, the nuclear fossae in these two cichlids are eccentrically oriented within the nucleus owing to the partial nuclear rotation which they undergo.

In the course of spermiogenesis, the spermatids in *A. grahami* exhibited gradual displacement of the cytoplasm towards the distal aspect of the cell, hence, forming the midpiece of the spermatozoa. Ultimately, the distal aspect of the midpiece was limited by the plasma membrane ending blindly in a lobe-like pattern, a feature that appears unique for this fish among other related cichlids. In addition, the midpiece in *A. grahami* lacked a cytoplasmic sheath, which is a thin extension of the midpiece distally around the flagellum frequently observed in other cichlids (Lou and Takahashi, 1989; Quagio-Grassiotto et al., 2003; Fishelson, 2003). In particular, the cytoplasmic sheath in *C. intermedia* bears dilated edges at its distal-most end (Quagio-Grassiotto et al., 2003). The lack of the cytoplasmic sheath on the spermatozoa of *A. grahami* may be considered to contribute to increased flexibility of the flagella, thereby providing more propulsive force to move the spawned spermatozoa in the harsh aquatic environment towards the eggs for fertilization to occur. This feature, therefore, may be one of the possible adaptive strategies employed by this fish in maintaining its reproductive capacity against the harsh external conditions.

Within the midpiece of *A. grahami*, about 10 unfused spheroidal mitochondria arranged in two layers around the cytoplasmic canal were conspicuously present. This has close resemblance to the midpiece of most cichlids where the number of mitochondria ranges from 8 to 10 (Lou and Takahashi, 1989; Matos et al., 1995; Quagio-Grassiotto et al., 2003), with some recording a remarkable 20–24 mitochondria (Fishelson, 2003). In this case, *C. dimerus* having 36 mitochondria (Rey Vázquez et al., 2012) appears to be on the extreme end among the cichlids. Quagio-Grassiotto et al. (2003) reviewed the differences in the number of mitochondria among the families under the sub-order Labroidei, and it was established that they differ significantly from that in other members of the Cichlidae family. The flagellar axoneme in Lake Magadi tilapia was covered by the flagellar membrane, a continuation of the plasma membrane to the flagellum at the level of the midpiece. Related cichlids such as *O. niloticus* (Lou and Takahashi, 1989) and *C. intermedia* (Quagio-Grassiotto et al., 2003), possess similar flagellar membranes but, unlike *A. grahami*, they exhibit conspicuous lateral extensions referred to as side fins/ridges, thought to correspond to the undulating membranes occurring in a number of animal species (Lou and Takahashi, 1989). Other cichlids, *S. jurupari* (Matos et al., 2002) and *C. dimerus* (Rey Vázquez et al., 2012), possessing biflagellate spermatozoa, also exhibit prominent lateral fins. Among teleosts with externally fertilizing sperm, Ostariophysi (Characiform, Cypriniform, and Siluriform clades) have been reported to show no lateral fins (Burns et al., 2009).

From the present findings, it can therefore be concluded that *A. grahami* exhibits regular spermatogenesis irrespective of the extremely severe environmental conditions in which they live. Further, the apparent absence of the cytoplasmic sheath and indistinct side fins/ridges in the spermatozoa of this fish compared to other cichlids may be considered to be a structural adaptation to the harsh external environment. The role of other somatic adaptive mechanisms, for example, nitrogen metabolism and urea excretion employed by this fish in ensuring normal body functions including spermatogenesis in the face of the harsh conditions in the lake cannot be overruled. In particular, the presence of the thin membrane heavily laden with a dark pigment on the parietal peritoneum may be presumed to provide protection to the testis, as well as other visceral organs against UV-radiation frequently reported in the area.

Studies on testicular structure and spermatogenesis in teleosts create possibilities, among other applications, for management, conservation and preservation of both endangered and/or valuable fish species, as exemplified in recent studies (Lacerda et al., 2006; Majhi et al., 2009; Nóbrega et al., 2009, 2010). Consequently, the findings of this study will further enhance conservation efforts of this reportedly "endangered" or "vulnerable" fish species (Bayona and Akinyi, 2006).

Ultrastructural investigations of spermiogenesis and sperm structure in Lake Magadi tilapia reveal some characteristics that are consistent with other members of the family Cichlidae. These features include condensation of spermatid chromatin material into multiple electron-dense globules and the presence of isolated mitochondria (about ten) around the proximal aspect of the sperm flagellum. These characteristics are now becoming more noticeable as more studies on cichlid reproduction continue to be undertaken, hence, they may be phylogenetically significant for this taxonomic clade. However, additional data from other members of this family are recommended to better test this hypothesis.

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References

Alfert, M., 1956. Chemical differentiation of nuclear proteins during spermatogenesis in the salmon. J. Biophys. Biochem. Cytol. 2, 109–114.

Bayona, J., Akinyi, E., 2006. *Alcolapia grahami*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. www.iucnredlist.org

Bergman, A.N., Laurent, P., Otiang'a-Owiti, G., Bergman, H.L., Walsh, P.J., Wilson, P., Wood, C.M., 2003. Physiological adaptation of the gut in the lake Magadi tilapia, *Alcolapia grahami*; an alkaline and saline adapted teleost fish. Comp. Biochem. Physiol. A 136, 701–715.

Billard, R., 1986. Spermiogenesis and spermatology of some teleost fish species. Reprod. Nutr. Dev. 26, 877–920.

Burns, J.R., Quagio-Grassiotto, I., Jamieson, B.G.M., 2009. Ultrastructure of spermatozoa: Ostariophysi. In: Jamieson, B.G.M. (Ed.), Reproductive Biology and Phylogeny of Fishes (Agnathans and Bony Fishes): Phylogeny Reproductive System Viviparity Spermatozoa, vol. 8A. Science Publishers, Enfield, NH, USA, pp. 287–387.

Chung, E.Y., 2008. Ultrastructure of germ cells, the Leydig cells, and Sertoli cells during spermatogenesis in *Boleophthalmus pectinirostris* (Teleostei, Perciformes, Gobiidae). Tissue Cell 40, 195–205.

Chung, E.Y., Yang, Y.C., Kang, H.W., Choi, K.H., Jun, J.C., Lee, K.Y., 2010. Ultrastructure of germ cells and the functions of Leydig cells and Sertoli cells associated with spermatogenesis in *Pampus argenteus* (Teleostei: Perciformes: Stromateidae). Zool. Stud. 49, 39–50.

Coe, J.M., 1966. The biology of *Tilapia grahami* (Boulenger) in Lake Magadi, Kenya. Acta Trop. 23, 146–177.

de Rooij, D.G., Russell, L.D., 2000. All you wanted to know about spermatogonia but were afraid to ask. J. Androl. 21, 776–798.

Eddy, E.M., 1975. Germplasm and the differentiation of the germ cell line. Int. Rev. Cytol. 43, 229–280.
Fishelson, L., 2003. Comparison of testes structure, spermatogenesis and spermatocytogenesis in young, aging, and hybrid cichlid fish (Cichlidae, Teleostei). J. Morphol. 256, 285–300.
Fishelson, L., Delarea, Y., Gon, O., 2006. Testis structure, spermatogenesis, spermatocytogenesis, and sperm structure in cardinal fish (*Apogonidae*, *Perciformes*). Anat. Embryol. 211, 31–46.
França, L.R., Russell, L.D., 1998. The testis of domestic animals. In: Martínez-García, J., Regadera, J. (Eds.), Male Reproduction. Churchill Livingstone, Madrid, Spain, pp. 197–219.
García-López, A., Martínez-Rodríguez, G., Sarasquete, C., 2005. Male reproductive system in Senegalese sole, *Solea senegalensis* (Kaup): anatomy, histology and histochemistry. Histol. Histopathol. 20, 1179–1189.
Gardiner, D.M., 1978. Fine structure of the spermatozoon of the viviparous teleost, *Cymatogaster aggregata*. J. Fish Biol. 13, 435–438.
Grier, H.J., 1981. Cellular organization of the testis and spermatogenesis in fishes. Am. Zool. 21, 345–357.
Grier, H.J., 1993. Comparative organization of Sertoli cells including the Sertoli cell barrier. In: Russell, L.D., Griswold, M.D. (Eds.), The Sertoli Cell. Cache River Press, Clearwater, FL, USA, pp. 703–739.
Gusmão-Pompiani, P., Malabarba, L.R., Oliveira, C., Quagio-Grassiotto, I., 2009. Spermiogenesis and spermatozoa ultrastructure in the Serrasalminae (Ostariophysi: Characiformes) with further evidence on the relationships of the piranhas and pacus. Neotrop. Ichthyol. 7, 385–394.
Gwo, J.C., Gwo, H.H., 1993. Spermatogenesis in the Black Porgy, *Acanthopagrus schlegeli* (Teleostei: Perciformes: Sparidae). Mol. Reprod. Dev. 36, 75–83.
Hermo, L., Pelletier, R.M., Cyr, D.G., Smith, C.E., 2010. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 1: Background to spermatogenesis, spermatogonia, and spermatocytes. Microsc. Res. Tech. 73, 243–278.
Jamieson, B.G.M., 1991. Fish Evolution and Systematics: Evidence from Spermatozoa. Cambridge University Press, Cambridge, UK.
Johansen, K., Maloiy, G.M., Lykkeboe, G., 1975. A fish in extreme alkalinity. Respir. Physiol. 24, 159–162.
Jun, J.C., Chung, E.Y., Yang, Y.C., 2006. Ultrastructure of germ cells, cyst epithelial cells and interstitial cells during spermatogenesis of the stone flounder, *Kareius bicoloratus*. Kor. J. Ichthyol. 18, 311–318.
Koulish, S.C., Kramer, R.C., Grier, H.J., 2002. Organization of the male gonad in a protogynous fish, *Thalassoma bifasciatum* (Teleostei: Labridae). J. Morphol. 254, 292–311.
Lacerda, S.M.S.N., Batlouni, S.R., Silva, S.B.G., Homem, C.S.P., França, L.R., 2006. Germ cells transplantation in fish: the Nile tilapia model. J. Anim. Reprod. 3, 146–159.
Lahnsteiner, F., Patzner, R.A., 1997. Fine structure of spermatozoa of four littoral teleosts, *Symphodus ocellatus*, *Coris julis*, *Thalassoma pavo* and *Chromis chromis*. J. Submicr. Cytol. Pathol. 29, 477–485.
Laurent, P., Maina, J.N., Bergman, H.L., Narahara, A., Walsh, P.J., Wood, C.M., 1995. Gill structure of a fish from an alkaline lake: effect of short-term exposure to neutral conditions. Can. J. Zool. 73, 1170–1181.
Leal, M.C., Cardoso, E.R., Nóbrega, R.H., Batlouni, S.R., Bogerd, J., França, L.R., Schulz, R.W., 2009. Histological and stereological evaluation of zebrafish (*Danio rerio*) spermatogenesis with an emphasis on spermatogonial generations. Biol. Reprod. 81, 177–187.
Lo Nostro, F.L., Grier, H.J., Meijide, F.J., Guerrero, G.A., 2003. Ultrastructure of the testis in *Synbranchus marmoratus* (Teleostei, Synbranchidae): the germinal compartment. Tissue Cell 35, 121–132.
Loir, M., Sourdaine, P., Mendis-Handagama, S.M., Jégou, B., 1995. Cell–cell interactions in the testis of teleosts and elasmobranchs. Microsc. Res. Tech. 32, 533–552.
Lou, Y.H., Takahashi, H., 1989. Spermiogenesis in the Nile tilapia *Oreochromis niloticus* with notes on a unique pattern of nuclear chromatin condensation. J. Morphol. 200, 321–330.
Louie, A.J., Dixon, G.H., 1972. Trout testis cells. 1. Characterization by deoxyribonucleic acid and protein analysis of cells separated by velocity sedimentation. J. Biol. Chem. 247, 5490–5497.
Maina, J.N., 1990. A study of the morphology of the gills of extreme alkalinity and hyperosmotic adapted teleost *Oreochromis alcalicus grahami* with particular emphasis on the ultrastructure of the chloride cells and their modification with water dilution. A SEM and TEM study. Anat. Embryol. 181, 83–98.
Maina, J.N., 2000. Functional morphology of the gas-gland cells of the air-bladder of *Oreochromis alcalicus grahami* fish. An ultrastructural study on a fish adapted to a severe, highly alkaline environment. Tissue Cell 32, 117–132.
Majhi, S.K., Hattori, R.S., Yokota, M., Watanabe, S., Strussmann, C.A., 2009. Germ cell transplantation using sexually competent fish: an approach for rapid propagation of endangered and valuable germlines. PLoS ONE 4, e6132, http://dx.doi.org/10.1371/journal.pone.0006132.
Matos, E., Matos, P., Corral, L., Azevedo, C., 1995. Ultrastructural study of the spermatozoon of *Crenicichla saxatilis* Linnaeus, 1758 (Pisces, Teleostei) from the Amazon region. Braz. J. Morphol. Sci. 12, 109–114.
Matos, E., Santos, M.N.S., Azevedo, C., 2002. Biflagellate spermatozoon structure of the hermaphrodite fish *Satanoperca jurupari* (Heckel, 1840) (Teleostei, Cichlidae) from the Amazon River. Braz. J. Biol. 62, 847–852.
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 systemic implications in fishes. Can. J. Zool. 69, 3038–3055.
Mattei, X., Siau, Y., Thiaw, O.T., Thiam, D., 1993. Peculiarities in the organization of testis of *Ophidion sp.* (Pisces, Teleostei). Evidence for two types of spermatogenesis in teleost fish. J. Fish Biol. 43, 931–937.
Muñoz, M., Sàbat, M., Mallol, S., Casadevall, M., 2002. Gonadal structure and gametogenesis of *Trigla lyra* (Pisces: Triglidae). Zool. Stud. 41, 412–420.
Nagahama, Y., 1983. The functional morphology of teleost gonads. In: Hoar, W.J., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology. Academic Press, New York, USA, pp. 399–437.
Narahara, A.B., Bergman, H.L., Laurent, P., Maina, J.N., Walsh, P.J., Wood, C.M., 1996. Respiration physiology of the lake Magadi tilapia (*Oreochromis alcalicus grahami*), a fish adapted to a hot alkaline, and frequently hypoxic condition. Physiol. Zool. 69, 1114–1136.
Nóbrega, R.H., Batlouni, S.R., França, L.R., 2009. An overview of functional and stereological evaluation of spermatogenesis and germ cell transplantation in fish. Fish Physiol. Biochem. 35, 197–206.
Nóbrega, R.H., Greebe, C.D., van de Kant, H., Bogerd, J., de França, L.R., Schulz, R.W., 2010. Spermatogonial stem cell niche and spermatogonial stem cell transplantation in zebrafish. PLoS ONE 5, e12808, http://dx.doi.org/10.1371/journal.pone.0012808.
Onyango, D.W., Kisia, S.M., 2008. *Alcolapia grahami*: a fish inhabiting a highly alkaline environment of Lake Magadi Kenya. In: Sébert, P., Onyango, D.W., Kapoor, B.G. (Eds.), Fish Life in Special Environments. Science Publishers, Enfield, NH, USA, pp. 41–51.
Parenti, L.R., Grier, H.J., 2004. Evolution and phylogeny of gonad morphology in bony fish. Integr. Comp. Biol. 44, 333–348.
Poirier, G.R., Nicholson, N., 1982. Fine structure of the testicular spermatozoa from the channel catfish, *Ictalurus punctatus*. J. Ultrastruct. Res. 80, 104–110.
Pic, P., 1978. A comparative study of the mechanisms of Na^+ and Cl^- excretion by the gill of *Mugil capito* and *Fundulus heteroclitus*: effects of stress. J. Comp. Physiol. 123, 155–162.
Quagio-Grassiotto, I., Carvalho, E.D., 1999. The ultrastructure of *Sorubim lima* (Teleostei, Siluriformes, Pimelodidae) spermatogenesis: premeiotic and meiotic periods. Tissue Cell 31, 561–567.
Quagio-Grassiotto, I., Oliveira, C., 2008. Sperm ultrastructure and a new type of spermiogenesis in two species of Pimelodidae, with a comparative review of sperm ultrastructure in siluriformes (Teleostei: Ostariophysi). J. Comp. Zool. 247, 55–66.
Quagio-Grassiotto, I., Antoneli, F.N., Oliveira, C., 2003. Spermiogenesis and sperm ultrastructure in *Cichla intermedia* with some considerations about Labroidei spermatozoa (Teleostei, Perciformes, Cichlidae). Tissue Cell 35, 441–446.
Randall, D.J., Wood, C.M., Perry, S.F., Bergman, H.L., Maloiy, G.M.O., Mommsen, T.P., Wright, P.A., 1989. Urea excretion as a strategy for survival in a fish living in a very alkaline environment. Nature 337, 165–166.
Reite, O.B., Maloiy, G.M.O., Aasehaug, B., 1974. pH, salinity and temperature tolerance of Lake Magadi tilapia. Nature 247, 315.
Rey Vázquez, G., Da Cuña, R.H., Meijide, F.J., Guerrero, G.A., 2012. Spermatogenesis and changes in testicular structure during the reproductive cycle in *Cichlasoma dimerus* (Teleostei, Perciformes). Acta Zool. (Stockholm) 93, 338–350.
Schulz, R.W., Menting, S., Bogerd, J., França, L.R., Vilela, D.A., Godinho, H.P., 2005. Sertoli cell proliferation in the adult testis—evidence from two fish species belonging to different orders. Biol. Reprod. 73, 891–898.
Schulz, R.W., França, L.R., Lareyre, J.J., LeGac, F., Chiarini-Garcia, H., Nóbrega, H.R., Miura, T., 2010. Spermatogenesis in fish. Gen. Comp. Endocrinol. 165, 390–411.
Seegers, L., Tichy, H., 1999. The *Oreochromis alcalicus* flock (Teleostei: Cichlidae) from lakes Natron and Magadi, Tanzania and Kenya, with descriptions of two new species. Ichth. Explor. Freshwaters 10, 97–146.
Selman, K., Wallace, R.A., 1986. Gametogenesis in *Fundulus heteroclitus*. Am. Zool. 26, 173–192.
Shabana, N.M.A., 2012. Ultrastructural study of spermatogenic stages in the protandrous sparid fish *Diplodus cervinus cervinus* (Lowe, 1838) from the South Eastern Mediterranean coast. Afr. J. Biotechnol. 11, 7270–7285.
Shahin, A.A.B., 2006. Semi-cystic spermatogenesis and biflagellate spermatozoon ultrastructure in the Nile electric fish *Malapterurus electricus* (Teleostei; Siluriformes: Malapteruridae). Acta Zool. (Stockholm) 87, 215–227.
Shahin, A.A.B., 2007. A novel of spermiogenesis in the Nile catfish *Chrysichthys auratus* (Siluriformes: Bagridae) in Egypt, with description of spermatozoon ultrastructure. Zool. Res. 28, 193–206.
Silva, M., Godinho, H.P., 1983. Timing of some events of the gametogenesis in the male Nile tilapia, *Sarotherodon niloticus*. Arch. Anat. Mic. 72, 231–237.
Stoumboudi, M.T.H., Abraham, M., 1996. The spermatogenic process in *Barbus longiceps*, *Capoeta damascina* and their natural sterile hybrid (Teleostei, Cyprinidae). J. Fish Biol. 49, 458–468.
Spadella, M.A., Oliveira, C., Quagio-Grassiotto, I., 2007. Comparative analyses of spermiogenesis and sperm ultrastructure in Callichthyidae (Teleostei, Ostariophysi: Siluriformes). Neotrop. Ichthyol. 5, 337–350.
Walsh, P.J., Grosell, M., Goss, G.G., Bergman, H.L., Bergman, A.N., Wilson, P., Laurent, P., Alper, S.L., Smith, C.P., Kamunde, C., Wood, C.M., 2001. Physiological and molecular characterization of urea transport by the gills of the Lake Magadi tilapia (*Alcolapia grahami*). J. Exp. Biol. 204, 509–520.
Weltzien, F.A., Taranger, G.L., Karlsen, O., Norberg, B., 2002. Spermatogenesis and related plasma androgen levels in Atlantic halibut (*Hippoglossus hippoglossus* L.). Comp. Biochem. Physiol. A 132, 567–575.
Wilson, P.J., Wood, C.M., Walsh, P.J., Bergman, A.N., Bergman, H.L., Laurent, P., White, B.N., 2004. Discordance between genetic structure and morphological,

ecological, and physiological adaptation in Lake Magadi tilapia. Physiol. Biochem. Zool. 77, 537–555.

Wood, C.M., Perry, S.F., Wright, P.A., Bergman, H.L., Randall, D.J., 1989. Ammonia and urea dynamics in the lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment. Respir. Physiol. 77, 1–20.

Wood, C.M., Bergman, H.L., Laurent, P., Maina, J.N., Narahara, A., Walsh, P.J., 1994. Urea production, acid–base regulation and their interactions in the Lake Magadi tilapia, a unique teleost adapted to a highly alkaline environment. J. Exp. Biol. 189, 13–36.

Wood, C.M., Wilson, P., Bergman, H.L., Bergman, A.N., Laurent, P.G., Otiang'a-Owiti, G., Walsh, P.J., 2002. Obligatory urea production and the cost of living in the Magadi tilapia revealed by acclimation to reduced salinity and alkalinity. Physiol. Biochem. Zool. 75, 111–122.

Wood, C.M., Bergman, H.L., Bianchini, A., Laurent, P., Maina, J., Johansson, O.E., Bianchini, L.F., Chevalier, C., Kavembe, G.D., Papah, M.B., Ojoo, R.O., 2012. Transepithelial potential in the Magadi tilapia, a fish living in extreme alkalinity. J. Comp. Physiol. B 182, 247–258.