Turk J Zool
31 (2007) 35-46

Gonad Development and Sex Ratio of Sharptooth Catfish (*Clarias gariepinus* Burchell, 1822) Cultured under Laboratory Conditions

Şehriban ÇEK*, Erdal YILMAZ

Mustafa Kemal University, Faculty of Fisheries and Aquaculture, Tayfur Sökmen Campus 31034 Serinyol Hatay - TURKEY

Received: 24.11.2005

Abstract: In the present work, gonad development and sex ratio of sharptooth catfish (*Clarias gariepinus*) cultured under laboratory conditions over 365 days from hatching were investigated histologically and morphologically. The maturation stage for both males and females was detected 295 days after hatching. Five and 6 developmental stages were indicated for testis and ovaries, respectively. The developmental pattern of ovaries was categorised as the group-synchronous type. The sex ratio of the 200 sampled fish was 90:110 (male:female) and this difference was not significant ($P > 0.05$). Additionally, final live mean weight of the males was higher than that of the females ($P < 0.05$). The beginning of vitellogenesis was in April and ovarian development peaked in July. These results suggest that 1-year-old male and female *C. gariepinus* can be used as brood stock for seed production under standard laboratory conditions.

Key Words: *Clarias gariepinus*, histology, gametogenesis, morphology, sex ratio

Standart Laboratuvar Koşullarında Yetiştirilen Karabalıkların (*Clarias gariepinus*, Burchell, 1822) Gonad Gelişimi ve Cinsiyet Oranı

Özet: Mevcut çalışmada laboratuvar koşullarında yetiştirilen karabalıkların gonad gelişimleri yumurtadan çıktıktan sonra 365 gün boyunca histolojik ve morfolojik olarak incelenmiştir. Hem erkek hem de dişiler için yumurtadan çıktıktan sonraki 295. günde eşeysel olgunluğa ulaştığı belirlenmiştir. Testislerin oluşumunda 5, ovaryumların oluşumunda ise 6 gelişim aşaması saptanmıştır. Ovaryum gelişim paterni grup senkronize olarak kategorize edilmiştir. Deneme süresince örneklenen 200 balık için cinsiyet oranları 90 erkek ve 110 dişi olarak belirlenmiş olup, bu farklılık önemli bulunmamıştır ($P > 0,05$). Ayrıca erkeklerin ortalama canlı ağırlıklarının dişilerinkinden daha yüksek olduğu tespit edilmiştir ($P < 0,05$). Vitellogenezin başlangıcı Nisan ayında kaydedilmiş ve ovaryum gelişimi Temmuzda pik yapmıştır. Bu sonuçlar, bir yaşındaki erkek ve dişi *C. gariepinus*'un standart laboratuvar koşullarında yavru elde etmek amacıyla kullanılabileceğini önermektedir.

Anahtar Sözcükler: *Clarias gariepinus*, histoloji, morfoloji, gametogenez, eşey oranı

Introduction

The sharptooth catfish is a freshwater fish that has a wide distribution from South and Central Africa to the Middle East and Turkey. Although catfish aquaculture is one of the fastest growing activities in Africa, Europe, and America, the aquaculture of *C. gariepinus* has not yet been performed in Turkey, although it has an especially high aquacultural potential for southern Turkey (Yalçın et al., 2001). In addition to rapid growth, resistance to stress and feasible reproduction in captivity have made it a good candidate for aquaculture in Africa and Europe. To date, many studies have been carried out to develop reproduction and culture techniques for *C. gariepinus* (Fagbenro and Jauncey, 1995; Richter et al., 1995; Barnhoorn et al., 2004; Ali and Jauncey, 2005). Cyclic changes in the gonads (ovaries and testes) have also been examined in a few closely related species, including the African catfish, *Clarias lazera* (Hogendoorn 1979), Natal mountain catfish, *Amphilius natalensis* (Marriott et al., 1997), Japanese catfish, *Silurus asotus* (Kumakura et al., 2003), and freshwater catfish, *Mystus montanus* (Arockiaraj et al., 2004).

*E-mail: scek@mku.edu.tr

It is extremely important to understand the mechanism linking gonad development and reproductive performance in order to improve culture techniques. It has been reported that the reproductive performance of *C. gariepinus* in the natural environment was extremely different from that in laboratory conditions (Richter et al., 1995). The reproductive biology of *C. gariepinus* under laboratory conditions in America, the Netherlands, Malaysia, Portugal, and South Africa has been well documented through a long history of research. In contrast, the reproductive biology of Turkish *C. gariepinus* has not been studied. It is only recently that we have begun to broaden our knowledge of *C. gariepinus* in terms of aquaculture potential and reproductive biology. In addition to the limited information available on Turkish *C. gariepinus*, none of the studies have described gametogenesis, reproductive cycle, and sex ratio. The only study of the reproductive biology and aquaculture potential of *C. gariepinus* is that by Yalçın et al. (2001). In their study, some reproductive characteristics of *C. gariepinus* were investigated under natural conditions (not including histological observation of gamete development). They concluded that *C. gariepinus* is an important component of the freshwater fish species of southern Turkey. This species could provide a much-needed additional source of fresh protein for local consumption, but would have to be grown in hatcheries, using minimum-cost production methodology. This would necessitate the establishment of self-sustaining populations of *C. gariepinus* to provide a source of larvae. At present, the culture of *C. gariepinus* is still not undertaken by the private sector, but this species is being cultured by us, on a research basis, in our university. In other words, *C. gariepinus* production and farming have not been established in Turkey.

Moreover, one of the most important factors necessary in the successful culturing of a fish species is obtaining a basic understanding of its key biological processes. The most important of these biological processes is the fish's reproductive cycle and formation of gametes. In the present study, the reproductive cycle of *C. gariepinus* was examined under standard laboratory conditions. The methods used included measurement of the number of oocytes at different stages of development, determination of the sex ratio, and a histological and morphological study of the female and male gonads.

Materials and Methods

Experimental Fish and System

The present study was conducted at the Aquaria Research Unit of Mustafa Kemal University, Hatay, Turkey, between 30 August 2003 and 30 July 2004. Sharptooth catfish larvae were obtained through the artificial reproduction method described by Hogendoorn (1980). Female broodstock fish were selected from the local fish supplier on the basis of their swollen abdomens and they were taken alive to the aquaria unit. Male broods were sacrificed, opened vertically, and the milt squeezed from the testes onto the eggs. Fertilisation was done artificially by the dry method. The eggs hatched within 1 or 2 days at 26 ± 1 ºC. After hatching, 300 larvae were selected randomly and assigned to 5 groups (5 aquariums), each having 60 fish averaging 4.35 ± 0.05 mm in length. The larvae were stored in a fibreglass tank (1000-l capacity). The aquarium system was housed inside an experimental room with a natural photoperiod (12 h light and 12 h dark). Each aquarium had a 96-l water volume capacity and was continuously aerated using a pump. During the experiment the larvae were first fed Artemia, which were propagated daily from Artemia cysts. After 20 days they were fed powdered trout feed (Epac ALFA 1 300-500 µm; Inve, Aquamaks, Turkey). Juveniles were fed trout feed pellets (IDL ALFA 2.2 mm; Inve, Aquamaks, Turkey). A static water system was used, and 80% of the water in each aquarium was changed daily, before the morning feed. From the age 1 month up to 6 months, 4-5 fish from each aquarium were sampled and, after this, from the age of 6 months up to 12 months (a 6-month period), 10 fish from each of the tanks were sampled every month, and their body weights and total lengths were recorded. Finally, they were sacrificed and their sexes were recorded.

Histological Procedures and Statistical Analysis

At the end of the experiment, all fish from each tank were sampled and their body weights and lengths were recorded. Subsequently, they were anaesthetised in 0.04%, 2-phenoxethanol (Sigma Chem. Dorset, UK). The gonads of the sacrificed fish were taken and then fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, and then sectioned at 5-mm and stained with haematoxylin and eosin for histological evaluation (Çek et al., 2001, Çek, 2006). Gonad development was determined histologically by light microscopy. The

developmental stages of testes and ovaries were determined for each fish. Spermatozoa classification was based on the histological criteria adapted from Grier (1981). Oocytes were classified by developmental stage adapted from Bromage and Cumaranatunga (1987).

Differences between sex ratios based on secondary characteristics (males grow larger and develop external genital papillae) and gonad histology were analysed by chi-squared (χ^2) test (Zar, 1984). Differences in growth were determined with the Kruskal-Wallis one-way analysis of variance by ranks (SPSS 10.0 for Windows) followed by Duncan's multiple range test (Zar, 1984). The significance level for differences was set at $P = 0.05$.

In order to determine the monthly changes in the number of oocytes at different developmental phases (1-6), oocytes at different developmental stages were counted using the method of Kopiejewska (2003) adapted from Marable (Abercrombie's formula, 1962),

where

$$N = \frac{T}{T + D} n$$

N = number of oocytes in a selected phase,

T = section thickness,

D = arithmetic mean of diameters of 20 oocytes in a selected phase,

n = number of sections of oocytes in a selected phase in 3 serial sections.

Results

General Morphology of the Gonads during the Study

In December (3 months into the experiment), gonads were inactive and undifferentiated, and it was not possible to distinguish males from females. At this time, ovaries and testes were a thin ribbon-like structure, creamy white, and translucent. The ovaries and testes were attached to the dorsal lateral lining of the peritoneal cavity. In January, it was still not possible to distinguish males from females, although the gonads were larger than in previous months. In February, females were distinguished from males. In March and April, ovaries were reddish-brown and granular in appearance, and testes were expanded and beginning to coalesce, but still appeared white. In April, genital papillae were recognisable. In May, the ovaria membranes were very thin and matt green eggs were visible. When the abdomens were squeezed, no ova were released (Table 4). Testes were larger and genital papillae were also larger than the previous month. At the end of July, very transparent and light green eggs were observed and when the abdomens were squeezed gently, free eggs were visible (Table 4). In August, the testes were large and grey-white; however, when the abdomens were squeezed no free spermatozoa were recorded. Moreover, the genital papillae were very large and reddish.

Phases of Oocyte Development

The process of oogenesis was classified with respect to the appearance of nuclei and nucleoli, and distribution of cytoplasmic inclusions. The phases of oocyte development in teleosts is traditionally divided into 3 stages: primary growth phase (PGP), secondary growth phase (SGP), and finally the maturation including hydration phase (MHP). In this study, a similar pattern of classification was followed (Table 1).

Ovaries were in the resting stage from October until March. During this period the ovaries of the catfish contained mainly oocytes at the chromatin and perinucleolar stage (Figure 1a and b). SGP began in March and ended in May (Figure 1c and d). Mainly 2 oocyte stages, vesicle formation and exogenous yolk formation, were distinguished in the ovaries of *C. gariepinus*. The latter differed from the former by the presence of a yolk precursor protein, vitellogenin (Figure 2a). Exogenous yolk formation was characterised by the presence of yolk granules. The yolk granules stained pink with haematoxylin and eosin and were first detected close to the microvilli of the oocyte membranes and later in the ooplasm (Figure 2a). As development progressed these yolk granules coalesced to form larger yolk globules (Figure 2b). At the same time they were pushed towards the centre of the oocytes (Figure 2b). The largest oocytes were stage 6 oocytes in mature ovaries. They were completely yolk-filled structures (Figure 2c). During the breeding season, from May to July, ovaries were in the post-vitellogenic or post-ovulation stage (Figure 2d). A full-grown ovary sometimes was found to enter a phase of regression when environmental conditions were not suitable. This was occasionally observed in May (Table 4).

Table 1. Phases of oocyte development in *Clarias gariepinus*, based on histological criteria from September 2003 to July 2004.

Primary Growth Phase (PGP)	a) Chromatin nucleolar stage (stage 1): This stage was characterised by a large nucleus in the central position, surrounded by little cytoplasm (Figure 1a). At this stage the diameter of oocytes was 3.17 ± 0.19 µm. b) Perinucleolar stage (stage 2): Nucleus increased in size and nucleolus increased in number. Balbiani bodies appeared in the cytoplasm. At the end of this stage balbiani bodies were distributed all over the cytoplasm (Figure 1b). These stages were detected from November 2003 to 2004 July. Oocyte diameter ranged from 5.85 ± 0.87 to 6.75 ± 0.35 µm.
Maturation and Hydration Phase (MHP)	Stage 3 oocytes: Cortical vesicles were detected for the first time. These were usually spherical structures that appeared at random at various depths in the ooplasm. They provided the first evidence for initiation of the secondary growth phase and appeared usually as empty unstained vacuoles (Figure 1c). The diameter of the stage 3 oocytes was 14.71 ± 2.12 µm. Stage 4 oocytes: The nucleus consisted of many nucleoli and continued to enlarge, becoming very irregular in shape. The zona radiata was more conspicuous. The process of vacuolisation was completed by the formation of 2 rows of vacuoles (Figure 1d). Stage 4 oocytes were 29.25 ± 0.88 µm in diameter. Stage 5 oocytes: Yolk granules were first detected only between vacuoles and later in the cytoplasm free from them. The nucleus showed a significant number of projections into the cytoplasm. The development of the egg shell was completed with the zona radiata and vitellin membrane. These 3 stages were observed from April to July. The diameter of stage 5 oocytes was 64.80 ± 3.41 µm.
Secondary Growth Phase (SGP)	This stage was distinguished by migration of the nucleus to the animal pole, where it remained, but the nuclear membrane disintegrated. The nucleus was smaller in size. The nucleoli were smaller than the previous stage, and hardly distinguishable in the nucleus. The layers of oocytes were thinner than those of stage 5 oocytes. However, during oocyte maturation and ovulation, the zona radiata increased rapidly in size (Figure 2c). After the germinal vesicle breakdown, the oocytes ovulated into the ovarian lumen and the post ovulatory follicle remained in the ovary (Figure 2d). These 2 stages were detected from May to the end of July. Oocyte diameter at these stages ranged from 105 ± 1.97 to 125 ± 5.95 µm.

Phases of Spermatogenesis during Catfish Development

The paired testes of adult *C. gariepinus* were composed of numerous finger-like projections, which extended from their respective sagittal axes. The bilateral testes fused together in their posterior region to form the seminal vesicle. The tubules of the seminal vesicle contained non-germinal epithelia and were shorter and thinner than those of the anterior region. Formation of spermatozoa in the *C. gariepinus* was divided into 5 stages in samples taken from the anterior part of the gonads (Tables 2 and 4).

Phases of spermatogenesis were distinguishable on the basis of their characteristic nuclear and cytoplasmic morphologies. All stages of spermatogenesis, including ruptured spermatozoa, were detected in the sperm ducts (Figure 3a). Lobules containing numerous spermatocytes, from early stages (spermatogonia) to complete spermatogenesis (spermatocytes, spermatids and spermatozoa), were observed (Figure 3a,b; Table 4). However, in the posterior part of *C. gariepinus*'s gonads, free spermatozoa were occasionally recorded. This part of the testes contained mostly spermatogonia and spermatocytes. The anterior part of the testes contained mostly free spermatozoa. At the age of 6 months they were clustered with their heads attached to the lobules, but subsequently (at the age of 8 months) the lobular walls broke down, and sperm became unattached and lay free in the lumen (Figure 3c). In the resting period (from August to May), the testes contained only spermatogonia. In May, June, and July, the anterior part of the testes contained spermatids and ruptured spermatozoa.

Distribution of Oocytes Numbers

The developmental pattern of sharptooth catfish ovaries was categorised as the group-synchronous type because the ovaries of sexually matured fish in July 2004 consisted mainly of stage 6 oocytes and primary growth phase (stages 1 and 2). Changes in the numbers of oocytes in different developmental stages in the ovaries are shown in Figure 4. From February 2004 to Mach

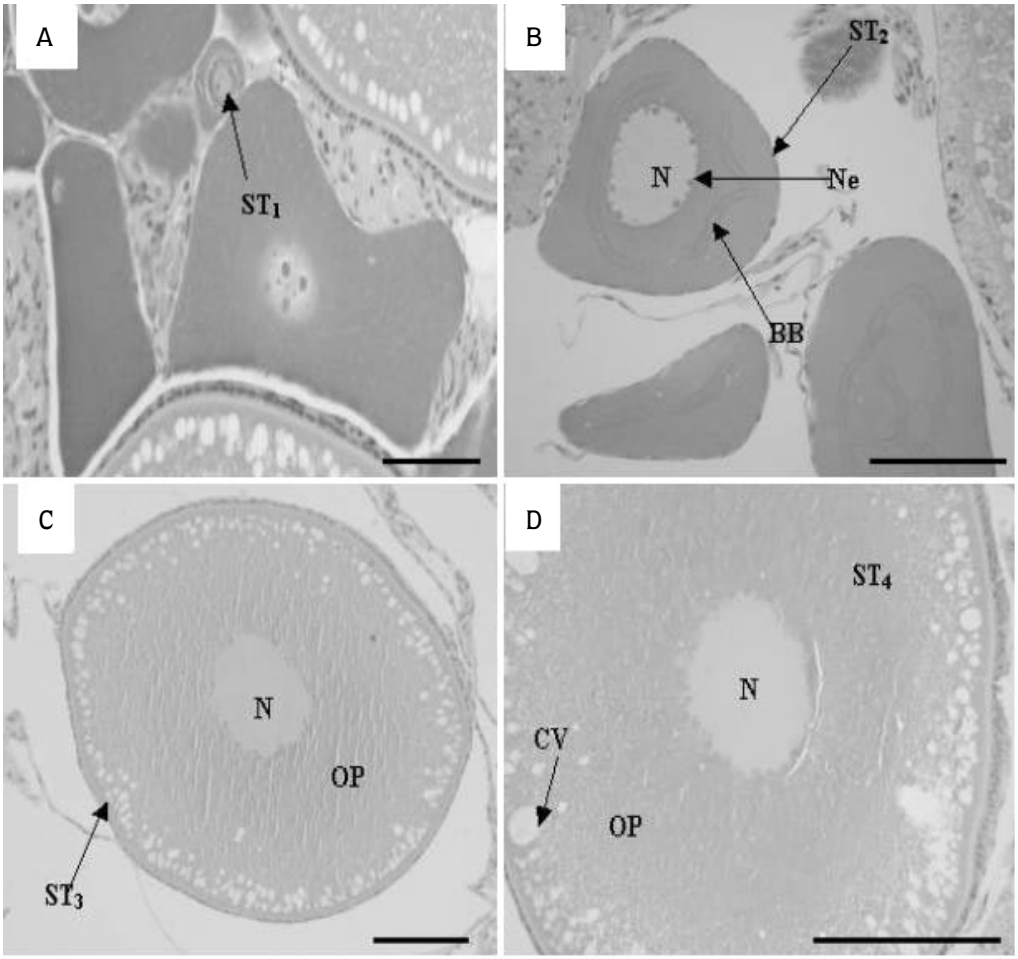

Figure 1. Sections of African catfish ovaries in different stages of oogenesis. A. Oocytes at primary growth phase (St1: stage 1 oocytes). B. Oocytes at primary growth phase (BB: Balbiani bodies; St2: stage 2 oocytes). C. Oocytes at secondary growth phase (CV: cortical vesicles; St3: stage 3 oocytes). D. Oocytes at secondary growth phase (St4: stage 4 oocytes; N: nucleus; Ne: nucleoli; OP: ooplasm; CV: cytoplasmic vesicle). All scale bars = 200 µm.

2004, ovaries of immature females mainly consisted of PGP oocytes (stages 1 and 2) and a few oocytes at the cortical vesicle stages were observed. In April 2004, primary and secondary yolk globule stages (stages 3 and 4) were detected. Oocytes at stage 5 also first appeared in April and the number of these oocytes increased in May. Stage 6 oocytes were first detected in June and the number of these oocytes reached their maximum in July (Figure 4). The greatest numbers of oocytes present in the ovaries were chromatin nucleolar and stage 2 oocytes in February. With the appearance of vitellogenic oocytes, the number of oocytes gradually decreased. The number of stage 2 oocytes also gradually decreased from February to July (Figure 4). From June to July, some atretic oocytes were also observed in the ovaries, with a decrease in stage 5 oocytes.

Growth and Sex Ratio

At the beginning of the experiment the mean weight and length of larvae were 0.0015 ± 0.0001 g and 0.515 ± 0.005 cm, respectively. At the end of the experiment the males were 140.45 ± 5.05 g, whereas the females weighed 123.45 ± 4.44g ($P \leq 0.001$). The sex ratio observed was close to the expected ratio of 1:1 (male:female) from all the aquariums. The sex ratio of the 200 sampled fish at the end of the experiment was 90:110 (male: female) and the difference was not significant ($P > 0.05$) (Table 3).

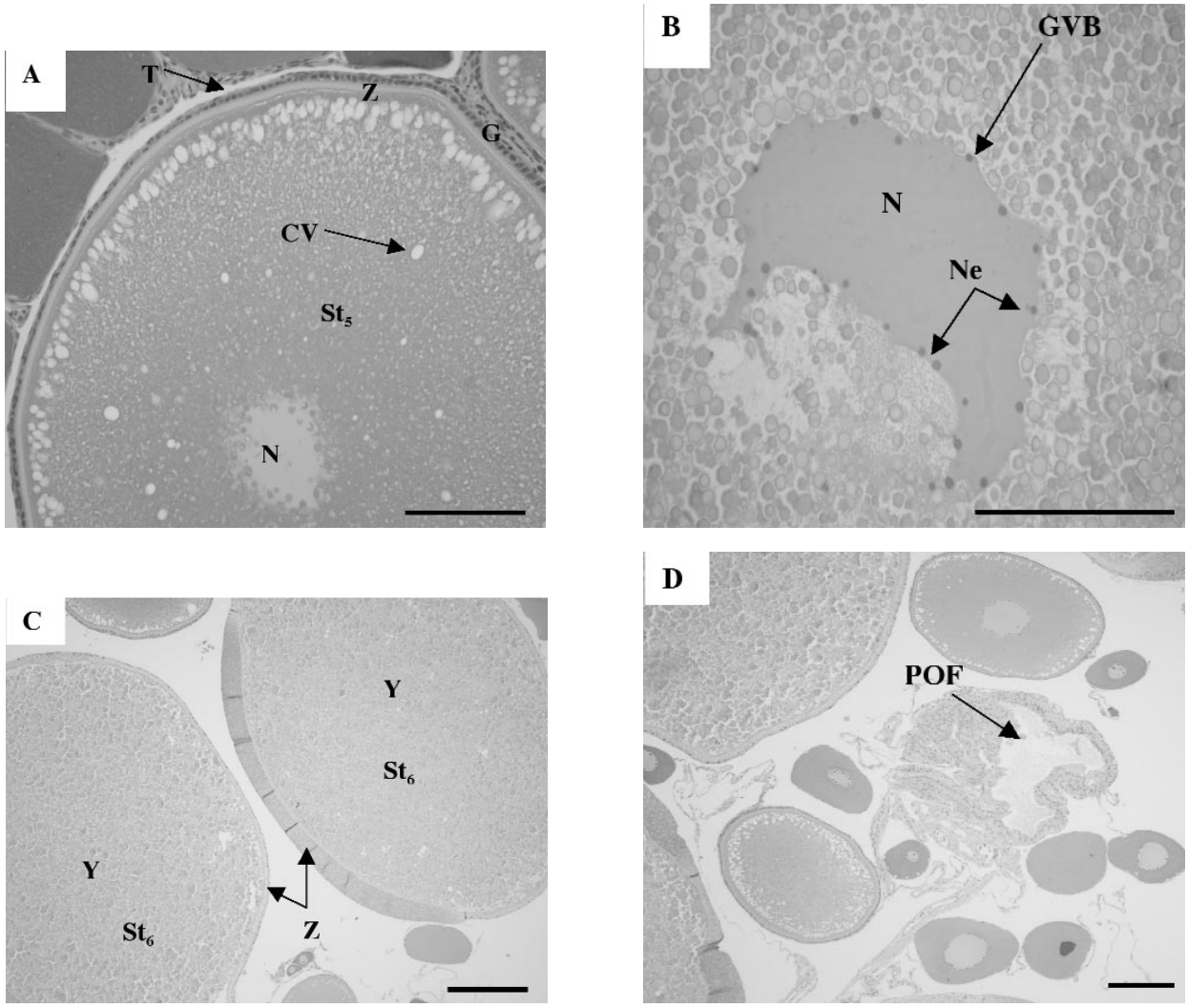

Figure 2. Sections of African catfish ovaries in different stages of oogenesis. A. Oocytes at stage 5 (St_5: stage 5 oocytes). B. Oocytes at maturation phase (arrow shows germinal vesicle breakdown; GVB), (St_6: stage 6 oocytes). C. Zona radiata underwent changes during oocyte maturation and ovulation. D. Arrow shows post-ovulatory follicle (POF). N: nucleus; Ne: nucleoli; Y: yolk; CV: cytoplasmic vesicle; T: theca; Z: zona radiata; G: granulosa. All scale bars = 225 µm.

Discussion

The study indicated that *C. gariepinus* matured 1 year after hatching under standard laboratory conditions with a constant temperature of 26 ± 1 ºC and a natural photoperiod (12 h light and 12 h dark). A previous study of wild *C. gariepinus* reported that the age of first maturity is 1 year, when the body weights of the fishes are approximately 108 g for females and 113 g for males (Yalçın et al., 2001). Although our results were similar to those observed in the natural habitat of *C. gariepinus*, the growth rate of the fish reared under laboratory conditions was faster. In the present study male and female fish reached 140 and 123 g of body weight, respectively. The rapid growth in *C. gariepinus* may have been due to the feeding conditions. The spawning season of *C. gariepinus* lasts from June to July under laboratory conditions. This finding was also similar to that of Yalçın et al. (2001). Although their study was conducted on *C. gariepinus* living in the Asi River (Hatay, Turkey), no major difference was found between the natural and laboratory spawning times of the fish. Nonetheless, our study somehow differs from those by Cavaco et al.

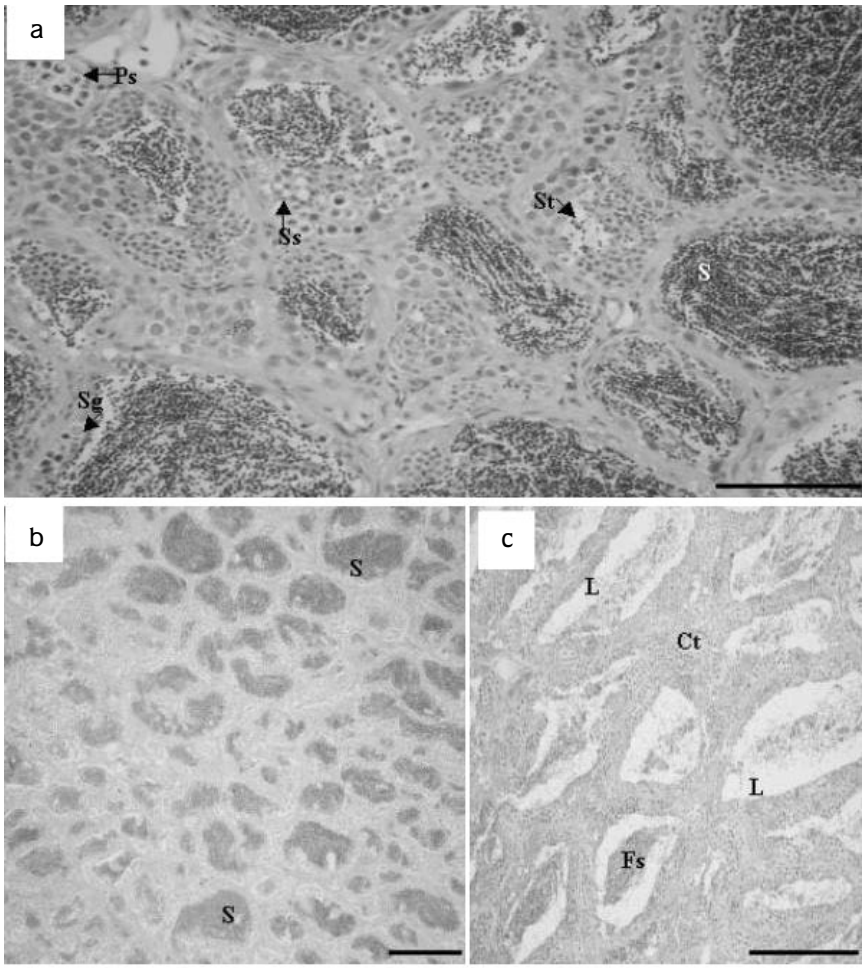

Figure 3. Testis development of African catfish: A-All stages of spermatogenesis B-Testis development at maturation stage C- Ruptured free spermatozoa are shown L; Lumen, Ct; connective tissue, Sg; Spermatogonia, Ps; Primary spermatocytes, Ss; Secondary spermatocytes, St; spermatids, S; spermatozoa, Fs; free spermatozoa. Scale bar= 200 µm (A), 160µm (B,C).

(1997) and Schulz et al. (1994). They observed early maturation in male *C. gariepinus* (spermatozoa first detected at 6 months of age). In the present study spermatozoa were first detected at 9 months of age. Early maturation in fish has been achieved either by genetic selection or better nutrition, revealing a correlation between maturation and growth (Le Bail, 1996). In their study, precocious maturation may have been achieved as a result of the feeding conditions and genetic selection. Full maturation of *C. gariepinus* was found to be 12 months of age in both studies. Schulz et al. (1994) stated that maturity is related to age in *C. gariepinus*; however, our observations showed that maturity was related to size rather than age. Age cannot be totally excluded in the determination of puberty, but the age of puberty appears to decrease with size. On the basis of our observations, we propose that puberty depends more on size than age, since in the same aquarium (all fish were the same age) the larger ones matured earlier than the smaller ones.

The seasonality of spawning imposes a considerable problem in African catfish aquaculture (Brzuska, 2003); however; Richter et al. (1995) and Viveiros et al. (2002) were able to prolong the spawning season of *Clarias gariepinus* with a constant temperature and photoperiod regime.

Table 2. Phases of spermatogenesis in *Clarias gariepinus*, based on histological criteria from September 2003 to July 2004.

Spermatogonia (ST_1)	The spermatogonia were the largest cells in the germinal tissue of the C. gariepinus testes. The nucleolus was large and lay close to the centre of the nucleus. These cells divided mitotically and formed primary spermatocytes. Somatic cells around the spermatogonial cells were clearly visible (Figure 3a).
Primary Spermatocytes (ST_2)	The primary spermatocytes were smaller than the spermatogonial cells and their daughter cells. They were spherical and presented as small groups (nests). Primary spermatocytes divided meiotically and produced secondary spermatocytes. They had no visible nuclear membrane and the chromatin material occupied most of the cell (Figure 3a). This stage was first observed at 2 months of age.
Secondary Spermatocytes (ST_3)	Secondary spermatocytes were morphologically similar to primary spermatocytes, though somewhat smaller and more basophilic. Their nucleolus was not clearly detected. The differentiating germ cells continued to have a close morphological relationship with the cyst cells, which formed the cytoplasmic processes extending between the spermatogonial cells (Figure 3a). This stage was first observed at 4 months of age.
Spermatids (ST_4)	Secondary spermatocytes continued meiotic division and produced spermatids. They were smaller than the secondary spermatocytes, irregular in shape, and very strongly basophilic (Figure 3b). This stage was first observed at 7 months of age.
Spermatozoa (ST_5)	At 9 months of age, all stages of spermatogenesis were clearly detectable. Transformation of spermatids into mature spermatozoa consisted of a reorganisation of the nucleus and cytoplasm, together with the development of a flagellum. No cell division was visible (Figure 3c).

Table 3. Sex distribution and ratio (%) for the different aquaria stocked with *Clarias gariepinus*.

Number of Aquariums	Sex distribution	Sex ratio (%)	
	(Male:Female:Intersex) M:F:I	M:F	χ^2
(I)	19:21	47.5:52.5	-
(II)	18:22	45:55	-
(III)	20:20	50:50	-
(IV)	16:24	40:60	-
(V)	17:23	42.5:57.5	-

The sex ratio was not significantly different from the expected 1M:1F ($P > 0.05$) in each aquarium ($n = 40$).

Our study briefly described the histological characteristics of oocyte development in *C. gariepinus*, which was divided into 3 phases: PGP, SGP, and maturation. The maturation phase included hydration and stage 6 oocytes. As in other teleosts, oogonia of *C. gariepinus* proliferated and turned into primary oocytes, which subsequently grew within follicles, formed cortical alveoli, entered vitellogenesis, underwent maturation, and finally ovulated. The changes that occurred during these phases were similar to those reported for catfish and some other teleosts (Richter et al., 1995; Anibeze and Inyang, 2000; Arockiaraj et al., 2004; Olaleye, 2005; Jalabert, 2005). *C. gariepinus* is a seasonal spawning species (Clay, 1979; Richter et al., 1995; Yalçın et al., 2001).

Examined ovaries showed oocytes in various stages and different sizes. At the maturation stage, the major part of the ovary was occupied by stage 6 oocytes in July, which comprised a synchronous population of larger oocytes, defined as a clutch. Yet, a large number of

Table 4. Monthly changes in gonad morphology, histology, and maturity stages of *Clarias gariepinus*.

Month	Gonadal Condition (Morphology)		Gonadal Condition (Histology)		Maturity Stage	
	Male	Female	Male	Female	Male	Female
Feb	Testes very thin and fine like ovaries, colourless, thread-like, situated close to vertebral column.	Ovaries colourless, thread-like, oocytes distinct only after histological study. Situated close to vertebral column.	Spermatogonia were visible, more somatic cells than spermatogonia, primary spermatocytes also recorded.	Large nucleus in central position, surrounded by little cytoplasm, nucleoli increased in number.	Immature (ST1,2)	Immature (ST1 , 2)
Mar	Were larger than previous month. One side slightly longer than the other, creamy white and smooth.	Ovaries were also larger than previous month. Distinct under light microscope (without histological study), white or yellowish-white.	Few primary spermatocytes and spermatogonia visible, somatic cells were clearly greater in number.	Oocytes growing rapidly; germinal vesicle increased in size, more or less oval, nucleoli increased in number.	Maturing (ST1,2,3) (Virgin)	Maturing (ST1 , 2 , 3) (Virgin)
Apr	Testes expanded, beginning to coalesce, still white and smooth.	Ovaries reddish-brown and granular in appearance to the naked eye, differs from matured ones in colour (quite mat-green in case of matured ovary).	More spermatogonia, somatic cells, and spermatocytes were recorded.	Cortical vesicles were detected. In some oocytes yolk granules were visible, secondary growth phase of oocytes was completed. Some oocytes were completely matured.	Maturing (ST1,2,3,4)	Maturing (ST1 , 2 , 3, 4, 5)
May	Testes are elongate, bulged, and dark- reddish, some red spots visible with naked eye. Milt was not observed.	Both sides of ovaries were mat-green. Eggs clearly discernible, opaque. Eggs run under strong pressure.	Unlike ovaries, suddenly, just before maturation, all stages of spermatogenesis were visible. It seems that differentiation in male catfish completed just before maturation.	Germinal vesicle irregular in shape, nucleoli reduced in number.	Matured (ST1,2,3,4,5)	Matured (S T 1 , 2 ,3,4,5,6)
Jun	Very white, red spots are still visible on both sides of testes. Drops of milt were not even observed under pressure.	Both sides of ovaries were light-green and eggs were transparent, filled ventral cavity. Eggs run under slight pressure.	Spermatids and spermatozoa, darkly-stained.	Micropyles were observed. In ovaries, the nuclear membrane disintegrated. The nucleus was smaller in size.	Fully Matured (ST1 , 2 , 3, 4, 5)	Fully Matured (ST1,2,3,4,5, 6)
Jul	As if testes were dorsoventrally flattened. Milt under pressure did not run.	Very light-green eggs were visible from outside of the genital opening, even without any pressure.	The spermatogonia were greater in number than the previous stage.	The nucleoli were smaller than the previous stage, and hardly distinguishable in the nucleus. In some ovaries post ovulatory follicles were recorded.	Spawning (ST1 , 2 , 3, 4, 5)	S p a w n i n g (ST1,2,3,4,5, 6)

previtellogenic and vitellogenic oocytes were also detected among the mature oocytes, which comprised a more heterogeneous population than the population from which the clutch were recruited. On the basis of this description, *C. gariepinus* might be classified as having a group-synchronous type ovary development.

In the male sharptooth catfish, formation of spermatozoa was subdivided into 5 histological stages. Stage I testes-contained spermatogonia only, while stage II testes showed spermatogonia, primary spermatocytes, and meiotic germ cells. Stage III testes contained spermatogonia, primary and secondary spermatocytes,

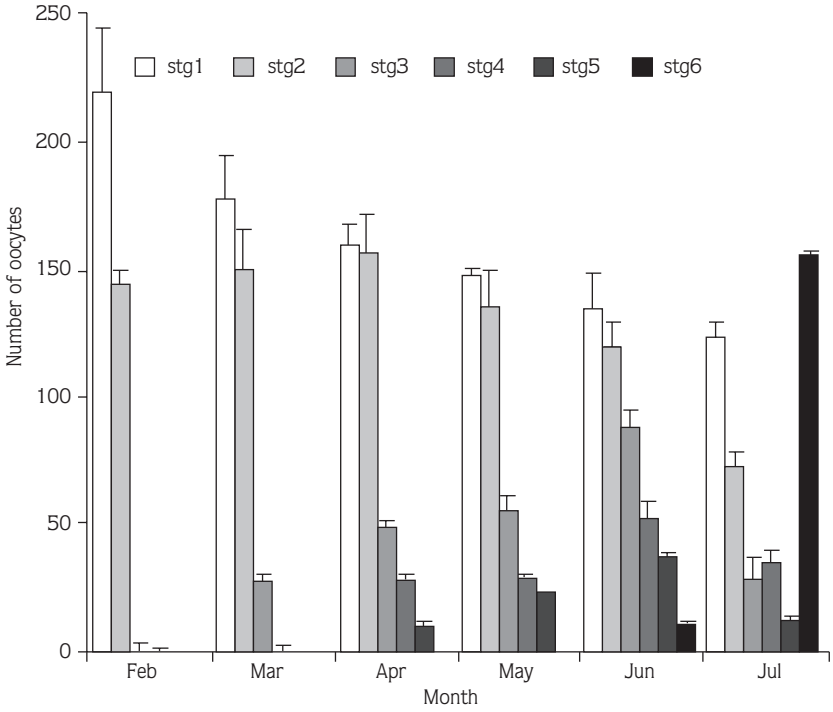

Figure 4. Monthly changes in the mean number of oocytes in the different developmental stages (1-6).

and meiotic germ cells. Stage IV testes contained spermatids, but no spermatozoa. In stage V testes, all germ cell stages, including spermatozoa, were present. These findings were similar to those reported by Grier (1981), Cavaco et al. (2001), and Cavaco (2005). In the present study, testes were of the unrestricted type (anastomosing tubular type) in which spermatogonia were distributed throughout the testes, and the primary and secondary spermatocytes were motionless during puberty. These findings were also similar to those published by Cavaco et al. (1997), Cavaco (2005), and Schulz et al. (1994). The only notable difference in the present results was that *C. gariepinus*'s milt was not obtained from the posterior part of the testes. It might be hypothesised that this posterior part of the testes of *C. gariepinus* is non-germinal. Since the anterior part of the testes contained germinal (spermatogenic) cells, milt was easily taken from this part of the testes after they were dissected. During the study milt was never taken; drops of milt could not even be taken when pressure was applied to the abdomen. We always had to sacrifice the fish in order to take milt. Milt was always taken from the anterior part of the testes. These findings are supported by those reported by Wu et al. (2001) and Viveiros et al. (2002).

Our results also showed that the sex ratio of *C. gariepinus* was 45% male and 55% female. These results were similar to those given by Anibeze and Inyang (2000) and Yalçın et al. (2001); however, our results contradict those published by Willoughby and Tweddle (1978). They reported that males were more plentiful than females in the natural environment. These differences may be attributed to endocrinal contamination in their study area and the effects of environmental contaminants on the endocrine system of *C. gariepinus*, as their study was conducted in the natural environment. Tyler et al. (1998) concluded that a large amount of androgen and oestrogen released into the environment has the potential to disrupt the endocrine system of fish.

In conclusion, *C. gariepinus* matured 1 year after hatching under controlled laboratory conditions. The beginning of vitellogenesis was in April and ovarian development peaked in July. Ovarian development was

the group-synchronous type. *C. gariepinus* in the Hatay region, similar to other species, such as walking catfish, *C. batrachus*, in Japan (Zairin et al., 1992); catfish, *C. macrocephalus*, in Asia (Tan-Fermin et al., 1997); and American catfish, *Rhamdia sapo*, in America (Espinash Ros et al., 1984); undergo normal gonadal development and growth under standard laboratory conditions. These results suggest that 1-year-old *C. gariepinus* can be used as brood stock for seed production in captivity.

Acknowledgement

We wholeheartedly thank Mrs. Esin ATİK-DOĞAN for helping us with the histological work.

References

Ali, M.Z and Jauncey, K. 2005. Approaches to optimising dietary protein to energy ratio for African catfish, *Clarias gariepinus* (Burchell, 1822). Aquacult. Nutr. 11: 95-101.

Anibeze, C.I.P. and Inyang, N.M. 2000. Oocytes structure, fecundity and sex ratio of *Heterobranchus longifilis* (Valenciennes 1840) in Idodo River basin (Nigeria) with comments on the breeding biology. J. Aquat. Sci. 15: 59-62.

Arockıaraj, A.J., Haniffa, M.A., Seetharaman, S. and Singh, S. 2004. Cyclic changes in gonadal maturation and histological observations of threatened freshwater catfish "Narikeliru" *Mystus montanus* (Jerdon, 1849). Acta Ichthyologıca et Piscatoria. 34: 253-266.

Barnhoorn, I.E.J., Bornman, M.S., Pieterse, G.M. and van Vuren, J.H.J. 2004. Histological evidence of intersex in Feral Sharptooth catfish (*Clarias gariepinus*) from an estrogens-polluted water source in Gauteng, South Africa. Environ. Toxicol. 19: 603-608.

Bromage, N.R. and Cumaranatunga, P.R.T. 1987. Oocytes development in the rainbow trout with special reference to vitellogenesis and atresia. Proceedings of the 3rd International Symposium on the Reproductive Physiology of Fish, (Eds. D.R. Idler, L.W. Crim, and J.M. Walsh). St John's, Newfoundland, Canada, p. 194.

Brzuska, E. 2003. Artificial propagation of African catfish (*Clarias gariepinus*): differences between reproduction effects after stimulation of ovulation with carp pituitary homogenate or GnRH-a and dopaminergic inhibitor. J. Anim. Sci. 48: 181-190.

Cavaco, J.E.B., Vischer, H.F., Lambert, J.G.D., Goos, H.J.Th. and Schulz, R.W. 1997. Mismatch between patterns of circulating and testicular androgens in African catfish, *Clarias gariepinus*. Fish Physiol. Biochem. 17: 155-162.

Cavaco, J.E.B. van Baal, J. and Bogerd, J. 2001. Steroid hormones activate gonadotrophs in juvenile male African catfish (*Clarias gariepinus*). Biol. Reprod. 65: 1807-1812.

Cavaco, J.E.B. 2005. Sex steroids and spermatogenesis in the African catfish (*Clarias gariepinus*). Archives of Androlog. 51:99-107.

Clay, D. 1979. Sexual maturity and fecundity of the African catfish (*Clarias gariepinus*) with an observation on the spawning behaviour of the Nile catfish (*Clarias lazera*). Zool. J. Linn. Soc. 65: 351-365.

Çek, Ş., Bromage, N.R., Randall, C. and Rana, K. 2001. Oogenesis, hepatosomatic indexes, and sex ratio in Rosy barb (*Puntius conchonius*). Turk. J. Fish. Aquat. Sci. 1: 133-141.

Çek, Ş. 2006. Early gonadal development and sex differentiation in rosy barb (*Puntius conchonius*). Anim. Biology. 56: 335-350.

Espinash Ros, A., Amutio, V.G., Mestre, J.P., Orti, G. and Nani, A. 1984. Induced breeding of the South American catfish, *Rhamdia sapo*. Aquaculture. 37: 141-146.

Fagbenro, O. and Jauncey, K. 1995. Growth and protein utilisation by juvenile catfish (*Clarias gariepinus*) fed dry diets containing co-dried lactic-acid-fermented fish-silage and protein feedstuffs. Biores. Tech. 51: 29-35.

Grier, H.J. 1981. Cellular organisation of the testis and spermatogenesis in fishes. Americ. Zool. 21: 345-357.

Hogendoorn, H. 1979. Controlled propagation of the African catfish, *Clarias lazera* (C&V) I. Reproductive Biology and Field Experiments. Aquaculture. 17: 223-333.

Hogendoorn, H. 1980. Controlled propagation of the African catfish, *Clarias lazera* (C&V) III. Feeding and Growth of fry. Aquaculture. 21: 233-241

Jalabert, B. 2005. Particularities of reproduction and oogenesis in teleost fish compared to mammals. Reprod. Nutr. Dev. 45: 261-279.

Kumakura, N., Sakai, K. and Takashima, F. 2003. Reproductive cycle and human chorionic gonadotropin-induced ovulation in hatchery reared Japanese catfish *Silurus asotus*. Fish. Sci. 69: 495-505.

Kopiejewska, W. 2003. Determination of frequency distribution of oocytes at different maturity phases in the ovaries of Roach, *Rutilus rutilus* (L.). Acta Ichthyologıca et Piscatoria. 33: 47-54.

Le Bail, P.Y. 1996. Growth-reproduction interaction in salmonids. In: Reproduction in Fish, Basic and Applied Aspects in Endocrinology and Genetics (Eds. Y. Zohar and B. Breton). Les Colloques de l'INRA, Paris, pp. 91-107.

Olaleye, V.F. 2005. A review of reproduction and gamete management in the African catfish *Clarias gariepinus*. Ife J. Sci. 7: 63-70.

Marable, A.W. 1962. The counting of cells and nuclei in microtome sections. Quarterly Journal of Microscopical Science. 103: 331-347.

Marriott, M.S., Booth, A.J. and Skelton, P.H. 1997. Reproductive and feeding biology of the Natal mountain catfish, *Amphilius natalensis* (Siluriformes: Amphiliidae). Env. Biol. Fish. 49: 461-470.

Richter, C.J.J., Eding, E.H., Verreth, J.A.J. and Fleuren, W.L.G. 1995. African catfish (*Clarias gariepinus*). In: Broodstock Management and Egg and Larval Quality (eds. N.R. Bromage and R.J. Roberts), Blackwell Science, Cambridge, pp. 242-277.

Schulz, R.W., Van der Corput, L., Janssen-Dommerholt, J. and Goos, H.J.Th. 1994. Sexual steroids during puberty in male African catfish (*Clarias gariepinus*): serum levels and gonadotropin-stimulated testicular secretion in vitro. J. Com. Physiol B. 164: 195-205.

Tan-Fermin, J.D., Ijiri, S., Ueda, H., Adachi, S. and Yamauchi, K. 1997. Ovarian development and serum steroid hormone profiles in hatchery-bred female catfish *Clarias macrocephalus* (Gunther) during an annual reproductive cycle. Fish. Sci. 63: 867-872.

Tyler, C.R., Jobling, S. and Sumpter, J.P. 1998. Endocrine disruption in wild life: a critical review of the evidence. Critical Reviews in Toxicology. 28: 319-361.

Viveiros, A.T.M., Fessehaye, Y., ter Veld, M., Schulz, R.W. and Komen, J. 2002. Hand-stripping of semen and semen quality after maturational hormone treatments, in African catfish *Clarias gariepinus*. Aquaculture 213: 373-386.

Wu, C., Patino, R., Davis, K.B. and Chang, X. 2001. Localization of estrogens receptor α and β RNA in germinal and nongerminal epithelia of the Channel catfish testis. Gen. Comp. Endoc. 124: 12-20.

Willoughby, N.G. and Tweddle, D.J. 1978. The ecology of the catfish *Clarias gariepinus* and Clarias *ngamensis* in the Shire valley. Malawi. Journal of Zoology (London) 186: 507-534.

Yalçın, S., Solak, K. and Akyurt, I. 2001. Certain reproductive characteristics of the African Catfish (*Clarias gariepinus* Burchell, 1822) living in the River Asi, Turkey. Turk. J. Zool. 25: 453-460.

Zairin, J.M., Furukava, K. and Aida, K. 1992. Changes in ovarian maturity in the tropical walking catfish, *Clarias batrachus* reared under 23-25 ºC. Nippon Suisan Gakkaishi. 58: 2033-2037.

Zar, H. 1984. Biostatistical Analyses. Prentice-Hall, New Jersey.