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Maturation of African catfish, *Clarias gariepinus*, in condition of seasonal climate of Uzbekistan

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Abstract
The goal of present study was to examine African catfish (*Clarias gariepinus, Burchell*, 1822) maturation (gonadogenesis and oogenesis) under outdoor conditions of Uzbekistan in 2016. Gonad stages of maturity were characterized using common 5 stage scale. Oogenesis was divided into 5 stages, which followed one after the other. Artificial reproduction, eggs hatching and fry rising were carried out in February (2016) in indoor facilities. In the middle April fry (19.4 g) were stored in outdoor tanks. African catfish matured at the age of 8 months. Fish matured when the body weight was 185 – 200 g, total length was 28-30 cm for females and 180-185 g and 26-26.5 cm for males, relatively. The diameter of ripe oocytes was 1.0-1.2 mm.

Keywords: Aquaculture, African catfish, *Clarias gariepinus*, artificial reproduction, gonadogenesis, oogenesis

1. Introduction
Extensive efforts were carried out for finding fish species suitable for aquaculture production in different regions. The African catfish (*Clarias gariepinus, Burchell*, 1822) is omnivorous, bottom feeders and tropic water fish species [1]. It is selected as a promising candidate due to its high environmental tolerance and easily controllable breeding habit [2, 3]. The African catfish is a freshwater fish that has a wide distribution from South and Central Africa to the Middle East and Turkey [4, 5, 6]. The reproductive biology of African catfish in area and under controlled conditions has been well documented through a long history of research. Initial trials were done for artificial breeding of catfish [1, 2, 3, 4, 5, 6].
Uzbekistan is situated in temporary highly continental climate, far to the north from species area. Summer is hot, average monthly air temperature is higher than 27 ºC in July, often day temperature is about 40 ºC. Winter is rather cold, average monthly temperature in January is about -3 ºC; water bodies can be covered by ice for 1-1.5 month [7].
In Uzbekistan, experiments with African catfish culturing were begun at the hatchery of Scientific-Experimental Station for Aquaculture Development, Ministry of Agriculture and Water Management, Tashkent region, in 2014. Fish shows fast growth from May up to the early October; during that period they reach from 0.3-0.5 g up to 1200-2000 g as in earth ponds so in cages and flow through tanks [our unpublished data]. In local open conditions African catfish cannot survive from the end of October to the middle of April [our unpublished data]. As a result, catfish becomes popular aquaculture object among local fish farmers. 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 maturation and formation of gametes. African catfish mass artificial reproduction technology development is needed in Uzbekistan environments.
The goal of the present study was to examine African catfish maturation (gonadogenesis and oogenesis) under open water bodies environments in Uzbekistan.

2. Material and Methods
The present study was conducted at the hatchery of Scientific-Experimental Station for Aquaculture Development since the April up to the November 2016.
African catfish broodstock was formed from fish seeds introduced from the Netherlands in 2015 and kept in flow-through tanks during vegetation season.

Several matured fish were stored in aquaria since the October 2015 up to the April 2016. Water temperature in aquaria was regulated by thermostats and kept at level 22-26 ºC. In February, African catfish larvae were obtained through the artificial reproduction method with using of common carp pituitary injections [3]; dose of injection was 4 mg/kg of fish body weight. Males were sacrificed, opened vertically, and the milt squeezed from the testes onto the eggs. Fertilization was done artificially by the dry method. The eggs hatched in incubation jar. After hatching, larvae were stored in aquaria to the middle of April. After that fry having 19.4 ±0.05 g in weight were stored in outdoor tanks with stocking rate 20 fish/m^3.

Water temperature in outdoor tanks was favourable for African catfish up to the early October; average daily water temperature was 18.5 – 28.2 ºC. Dissolved oxygen level was higher than 3.3 mg/L. During the experiment the larvae were first fed powdered aqua feed, juveniles and older fish were fed pellets.

From the age of 1 month up to 8 months: 10-18 fish were sampled every month, and their body weights and total lengths were recorded. Finally, they were sacrificed and their sexes were recorded. The sex and maturity stage of each fish were determined. The maturity stages were determined by visual examination of the gonads and using a five-point maturity scale [8]. The gonad samples of the sacrificed fish were fixed in 4% formalin, dehydrated, embedded in paraffin, and then sectioned at 5-6 mm and stained with Heidenhain's iron hematoxylin [9]. Oocyte development was determined histologically under light microscopy. Oocytes were classified by developmental stage adapted from Makeeva [10].

3. Results

African catfish maturation stages were sufficiently observed according to generally accepted ones. The stages of 118 individuals were:

- **Stage 1:** Immature virgin. Ovary were thin ribbon-like structure, creamy white and translucent; visually, it was difficult to select fish by sex.
- **Stage 2:** Not Ripe, developing virgin. Gonads were slightly expanded, become thicker. Gonads easily could be differed by sex. Ovaries were translucent, testes were milky white. Testes gonad edges after cutting remained sharp, not melted. As opposed to cyprinids and other local fishes, gonads of African catfish occupied about one fourth of the length of the peritoneal cavity (Fig. 1).

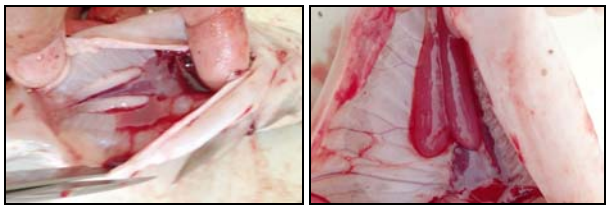

Fig 1: African catfish male (on the left) and female (on the right) gonads on 2nd stage of maturity, Uzbekistan.

- **Stage 3**: Almost Ripe. The ovary appreciably expanded and had some large ova visible. The ovary was brownish green in color. Blood capillaries were visible around the ovary. Male gonad edges after cutting rapidly melted. Gonads of both sexes reminded short and occupied from one third to half of the abdominal cavity. Oogoniums, previtellogenic and vitellogenic eggs could be found in ovaries.
- **Stage 4:** Ripe. The gonads were fully formed, opaque, browngreen in color. The eggs were visible with the naked eye. The ovaries occupied from one third to two third of the peritoneal cavity. A highly developed capillary network was visible. No ova were released when the abdomen was squeezed (Fig. 2). Oogoniums, previtellogenic, vitellogenic and ripe eggs could be found in ovaries.

Fig 2: African catfish female gonad on 4th stage of maturity (1st maturation), Uzbekistan.

- Stage 5 (running ripe) and stage 6 (spent) were not observed in present work.
- Oogenesis can be divided into 5 stages, which follow one after the other:
- **Stage 1 (Primary growth, oogonium stage):** The oogoniums were the smallest gametes and not yet containing yolk. Oogonium was characterized by a large nucleus in the central position, surrounded by little cytoplasm. The number of primary oocytes increased through mitotic division.
- **Stage 2 (Pre-vitellogenesis stage):** The oogonium turned into oocytes. Nucleus increased in size and in number. Within this period oocyte didn't contain yolk. At the end of the stage the oocyte increased its size to 150-200 micron.
- **Stage 3 (Vitellogenesis stage):** During this period gamete increased its size strongly. Cortical vesicles (1-2 rows) were detected for the first time. They were clearly visible a little outside of the centre of the oocyte. Within this stage the yolk of the oocyte was formed. The process of vacuolization was completed by the formation of 2 rows of vacuoles. During this phase yolk formation in the oocyte increased; the origin of the proteins needed for this process was outside the oocyte (the liver). The oocyte increased to its final size of 1–1.2 mm. They remain in this stage until environmental factors (rainfall and water level rise or a hormonal injection) let them ovulate.
- **Stage 4 (Ripe egg, ovulated egg):** The yolk accumulated into one droplet, consequently the egg became transparent and the nucleus migrated completely to the outer-side (animal pole) of the egg. Either through natural or artificial processes (the administration of hormones) the oocyte would be ovulated or liberated from the ovarian tissue.
- **Stage 5 (Atretic egg):** The egg which was not well ovulated will be re-absorbed (or recycled) again by the fish and they can distinguished by their "flushy" appearance. The present study failed to observe this stage.

In April-May (when fish were at the age of 1-2 months), the gonads were inactive and undifferentiated, and it was not possible to distinguish males from females. At that time, ovaries and testes were a thin ribbon-like structure, creamy white, and translucent. Oocytes on stage 1 were visible on histological sections.

In June (at the age of 3 months), the ovaries and testes were attached to the dorsal lateral lining of the peritoneal cavity. Females were easily distinguished from males. Fish increased their size to 40 – 58 g of body weight; the gonads were at second stage of maturity. The most developed oocytes were on 2nd stage (Fig. 3).

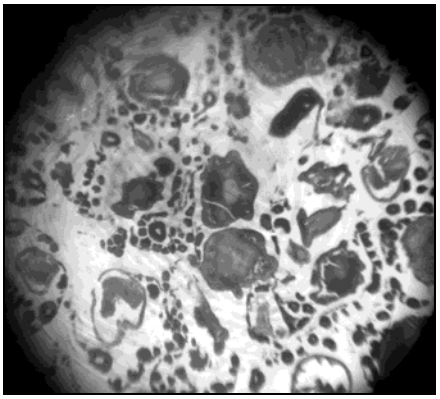

Fig 3: Paraffin section of African catfish female gonad in 3rd stage of maturity, Uzbekistan.

In September (at the age of 6 months), the gonads of both sexes passed into third stage of maturity. That was observed visually and obvious from histological sections. The most developed oocytes were on the stage of vitellogenesis, one row of vacuoles was appeared (Fig. 4). Also the oocytes on the pre-vitello genesis and vitellogenesis stages were present. Fish were 21.5-25 cm in length and 60 – 115 g in body weight.

In October (at the age of 8 months), the most of African catfish matured. The diameter of ripe oocytes were 1.05-1.18 mm the smallest matured females were 28 – 30 cm in body length and 185 – 200 g in body weight. The smallest males were 26 – 26.5 cm in length and 180-185 g in body weight. Matured females could be recognized by visual examination because the belly of was soft, relatively big and rounded. The genital opening was swollen, reddish and round-ended. The ripe oocytes in ovaries had reached definitive size. The testes were large and grey-white; however, when the abdomens were squeezed no free spermatozoa were recorded. Moreover, the genital papillae were large and reddish.

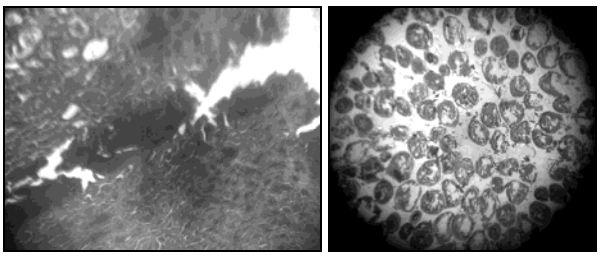

Fig 4: Paraffin sections of African catfish males (on the left) and females (on the right) gonads in 4th stages of maturity at age 8 months (1st maturation), Uzbekistan.

4. Discussion

African catfish mature in the wild within 1 or 2 years of age when they are 20-40 cm long and 150-500 g weight [1, 11]. Even in the north Turkey, African catfish mature at the age one year when the body weights were 108 g for females and 113 g for males [6]. In Malaysia (where African catfish was introduced), one-year-old African catfish females at weight 0.7-0.8 kg and males at weight 0.4-0.7 kg were matured [12]. Catfish reared in closed systems in a permanently high temperature are able to attain maturity at the weight of 400-600 g, at the age of 6-9 months [13]. The diameter of ripe oocytes in the wild is around 1.0-1.2 mm.

The present study indicated that African catfish matured at the age of 8 months under outdoor conditions of Uzbekistan. It should be noted that the experiment lasted from the April and covered the whole local vegetation season. Fish matured when the body weights were 185 – 200 g, total lengths were 28-30 cm for females and 180-185 g and 26-26.5 cm, relatively, for males. Although the present study results were similar to those observed in the natural habitat of the African catfish and in closed systems [3, 4, 5].

The size of first time matured African catfish are very small for culture species in compare with species that are cultured in Uzbekistan (traditional cyprinids namely common carp, *Cyprinus carpio,* silver carp, *Hypophthalmichthys molitrix*, bighead carp, *H. nobilis*, grass carp, *Ctenopharyngodon idella*, mature at the ages 3-4 years when the weight is 3-4 kg and total length 38 – 45 cm) [14, 15]. Early age of first maturation, small size of matured fish and fast growth are very favorable characteristics of African catfish for local aquaculture taking into consideration necessity of wintering in closed conditions. 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. At present, the culture of African catfish becomes popular as in small earth ponds so in cages and tanks in Uzbekistan, production of table catfish estimates at the level 700 – 900 tons per year and will rapidly increased due to propagation technology development.

5. Conclusion

The present study concluded that African catfish mature at the age of 8 months when the body weight was 185 – 200 g, total length was 28-30 cm for females and 180-185 g and 26-26.5 cm for males, relatively, in seasonal climate conditions of Uzbekistan, taking into consideration necessity of wintering in closed condition at the early ages.

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