Features of puberty in female African Clary catfish in high-tech industrial aquaculture

Elena Romanova¹, Minzifa Mukhitova¹, Vasiliy Romanov¹, Vaselina Lyubomirova¹, Ludmila Shadieva¹ and Tatyana Shlenkina¹
¹Ulyanovsk State Agrarian University named after P.A., Stolypin, Novy Venets Blvd., 1, Ulyanovsk, 432017, Russia
E-mail: vvr-emr@yandex.ru

Abstract. There is a growing interest in African Clary catfish as a perspective object of industrial fishery in the Russian aquaculture in the last decade. This interest is due to the high growth rate of Clary catfish, its delicious meat and valuable caviar. This species has multiport spawning, so it is possible to get caviar from catfish several times a year. However, in high-tech industrial system of fish breeding, biology of fishes is modified, so that the African Clary catfish loses the ability to reproduce and produce full roe.

For reproduction and production of full roe in industrial aquaculture, artificial spawning technologies are used with the use of hormonal drugs that induce the maturation of reproductive cells. The study is devoted to the dynamics of puberty in female African Clary catfish to identify the starting age of its possible use in the reproductive process and in the production of caviar. The results showed that at the age of 8 months, the reproductive system of females reached maturity, females were able to reproduce posterity. However, its reproductive performance in this period, in particular caviar productivity, was very low. The age of 12 months can be considered the starting age for caviar production. Caviar productivity continued to grow, reaching a maximum at the age of 18-24 months, and depended on the weight of females. Working fertility of females and gonadosomatic index at the age of 12-24 months were characterized by increasing dynamics. The size and weight of oocytes during this period reached definitive sizes.

1. Introduction
African Clary catfish is a widespread aquaculture object in Europe, Asia, Africa and America [1, 2]. This species has the highest growth rate. For six months of cultivation, it is able to gain a weight of 1.5 kg or more [3, 4]. The growth potential of African Clary catfish in conditions of artificial breeding is currently not fully disclosed [4, 5]. The interest in this promising species has been growing steadily in Russia in the last decade [3, 4]. African Clary catfish is gradually being introduced in industrial aquaculture of many regions of Russia.

This fish species is characterized by a delicacy meat, popular in the dietary and baby foods, and precious caviar. The African Clary catfish has multiport spawning, to get caviar from catfish is possible several times a year [5 - 7]. However, a natural way to spawn of Clary catfish under artificial cultivation is impossible. This is due to the fact that in high-tech industrial fish breeding systems, fish biology undergoes significant changes and African Clary catfish loses the ability to reproduce naturally [6, 7]. In industrial aquaculture reproductive cells of this species never naturally ripen, and the gonads stop in its development at the fourth, the penultimate stage of development (on a scale

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To obtain full roe and then the posterity of African Clary catfish, artificial spawning technologies are used in industrial aquaculture [8, 9]. For the maturation of reproductive cells, hormonal preparations are used that stimulate maturation of the reproductive system. Under the influence of hormones, reproductive cells - oocytes and sperm cells, mature for artificial spawning [9, 10] and for obtaining raw roe. Therefore, the cultivation of marketable fish is really to get more product - caviar of African Clary catfish [10]. Currently in Russia and abroad caviar production of African Clary catfish is not exist, although the biological characteristics of this species testify to the reality of this idea. To get the caviar of Clary catfish all-season, it is necessary to overcome the seasonality of reproduction. In representatives of this species in its natural habitat, the content of gonadotropin in the pituitary gland has a seasonal dynamics, in winter the hormonal level is 4-6 times lower than in summer. Whether the same pattern persists in breeding in an artificial environment that levels the effect of natural factors is not yet known. Perhaps the use of hormones will solve this problem.

Further success in the development of industrial aquaculture of African Clary catfish depends on the level of our fundamental knowledge about the features of fish biology in artificially created breeding conditions, so further research in this direction is relevant.

**Aim of work:** to study the dynamics of puberty of female of African Clary catfish and determine the starting age of its use in the reproductive process and in the process of obtaining caviar for food purposes.

2. **Materials and methods**

The research was carried out in the laboratory of experimental biology and aquaculture of Ulyanovsk state agrarian University. The laboratory is equipped with 2 installations of closed water supply (ICW), 4 autonomous fish modules with a filtration on quartz sand, water treatment system, the apparatus Weiss for the cultivation of embryos, spawning school of African Clary catfish, and different age fish of its own reproduction.

During the experiments, we used biotechnological methods: hormonal stimulation of gonad maturation, in vitro fertilization, cultivation of embryos, cultivation of live launch feed – nauplii of Artemia.

Morphophysiological studies were conducted, monitoring the growth and development of dimensional-weight parameters, evaluation of fertility, control of quantity, quality and maturity of the reproductive products, the study of the stages of embryonic development of the caviar and the environmental factors determining this development.

Cytological and histological studies of gonads in different age groups of males and females, starting from the early stages of ontogenesis, were carried out to identify the timing of puberty of Clary catfish and readiness for technological impact of the caviar.

The gonad maturity was assessed on the Kiselevich scale, which distinguished five stages of maturity - from I to V and VI stage-of spawned individuals.

Stage (I juvenile) - sub-adult – juvenales. Gonads undeveloped, firmly attached to the inner side walls of the body (on the sides and below the swim bladder) and are represented by long, narrow cords or tapes, which is impossible to visually determine the sex; visible area protoplasmic growth, dispersed between numerous oogonia.

Stage II - maturing individuals or developing reproductive products after spawning. Gonads began to develop; darkened thickenings are formed on the cords, in which the ovaries and testes are already recognized. The caviar is so small that it’s not visible to the naked eye. Ovaries from testicles (soft roe) in this period differ in that along the first on the side facing the middle of the body, there is a fairly thick blood vessel.

Stage III - individuals whose reproductive glands are still far from maturity, but sufficiently developed. Ovaries significantly increased in size, fill from 1/3 to 1/2 of the entire abdominal cavity and filled with small opaque, whitish caviar, clearly visible to the naked eye. When cutting the ovary, caviar is difficult to break away from the internal partitions of the body, always form lumps of several...
pieces together.

Stage IV - individuals whose genitals have reached almost maximum development. The ovaries are very large and fill up to 2/3 of the entire abdominal cavity. Caviars are large, transparent and flow when pressed. When the incision of the ovary and the scraping of cut with scissors, the caviars are scraped alone.

Stage V - fluid individuals. Caviar and soft roe are so mature that freely flow not drops and squirt with the slightest pressure. If you hold the fish upright by the head and shake it, the caviar and soft roe flow freely.

Stage VI - spawned individuals. At this stage, the reproductive products are swept out. Ovaries and testicles are very small, flabby, inflamed, dark red. Often in the ovary there remains a small number of small caviars that undergo fatty degeneration and dissolve. After a few days, the inflammation disappears, and the gonads pass to stage II, and then to stage III.

To investigate the dynamics and timing of puberty of females, we conducted cytological and histological studies of gonads, starting from the early stages of ontogenesis. Histological and cytological studies of the gonads included: measuring the size of oocytes using a micrometer ocular, microscopical research of morphology and structural features of the oocytes, evaluation of its degree of maturity and saturation of the yolk, the distribution of yolk, features of localization of the nucleus, the condition of the shells.

In our work, we determined several fertility indicators: individual fertility, multiplying the number of oocytes in 1 g by the weight of gonads; relative fertility (RF) - as the number of ovules per unit body weight of the female; working fertility, which was calculated as the number of caviars obtained from one female when decanting caviar during artificial spawning. Obtained artificially strained caviar is not all suitable for fertilization. According to the average fertility of females determine the number of males required for artificial insemination. Characteristics of the degree of development of female gonads is of theoretical and practical importance in assessing the readiness of the ovaries to spawn.

3. Results
The process of development of the female reproductive system includes the formation of the reproductive gland (gonadogenesis) and the transformation of initially undifferentiated cells into mature female reproductive cells – gamete (gametogenesis).

The study of the development of the reproductive system of catfish began at 10 weeks age (2.5 months). The average weight of the catfish during this period amounted to 43.5±4.5 g. According to the results of our research, reproductive glands at this age are undeveloped, fit snugly to the inside walls of the body and presents as a long narrow cords or tapes, which are still impossible to visually determine the sex.

Opening of the abdominal cavity of catfish 12 weeks age (3 months) and 14 weeks age (3.5 months), who reached a mass of 64±5.2 g and 85±5.9 g, respectively, showed that the female reproductive glands are poorly developed and are represented by thin transparent-vitreous strands. Morphologically, at this age it is possible to distinguish females from males. At the age of 3-4 months, the ovaries corresponded to the I stage of maturity - the stage of immature individuals. The oocytes of protoplasmic growth, located between numerous oogonia, were viewed by microscopy. At I stage of maturity, diameter of oocytes was 0.16±0.01 mm and a weight of 0.52±0.02 mg. In the process of maturation of the gonads in females with the age increased the diameter of the oocytes and its weight (figures 1, 2).
Figure 1. Oocyte diameter at different stages of gonad maturation.

At the age of 17 weeks (4 months), weight of Clary catfishes ranged from 111±12.2 g to 206.3±16.7 g. The gonads in this weight and age category were even more advanced in its development. When headless carcass weight 99.4±6.7 g gonadosomatic index was 0.05%. Darkened thickenings were formed on “cords”, in which it was already possible to define ovaries and testicles. The caviars were very small and could only be seen under a microscope. Gonad development corresponded to stage II - maturing individuals. The diameter of the oocytes was averaged 0.38±0.02 mm and its mass was 0.89±0.02 mg (figure 1, 2). Oocytes were transparent, was not rich yolk, stage of vitellogenesis was not completed. Thus, at the age of 4-5 months, the stage II of gonad development was observed, which was largely determined by the size of the individual.

Figure 2. Oocyte mass at different stages of gonad maturation.

At the age of 5-6 months, females had a mass of 580±95 g. Development of ovarian cytologically and histologically corresponded to stage III by Kiselevich. Ovules at stage III of maturity were significantly increased in diameter to 0.82±0.06 mm, and its mass increased to 1.17±0.04 mg. Morphologically and histologically gonads are still far from maturity, but they are much more developed than at the previous stage. The ovules fill from 1/3 of the abdominal cavity and are filled with small opaque, whitish caviars, clearly visible to the naked eye. If you cut the ovary, caviars, when
you try to scrape it from the walls of the ovary, come off it with difficulty, form a lump, sticking together for several pieces. With weight of female headless carcass of 490±46g, the gonadosomatic index was 4.1%. Absolute individual fertility of females in this period did not exceed 22000 oocytes. The oocytes of different sizes were found in ovaries from 0.5 mm to 1.04 mm. The average size of the oocytes was 0.82±0.06 mm (figure 1, 2). The oocytes were at different stages of maturation. Caviars were pale green, yolk, forming a zone of fallow in the photo, a small amount; in larger oocytes there are inclusions of yolk in the center. At the age of 6-7 months, the females of Clary catfish reached marketable weight 726.6±33.4 g.

In the period of intensive development of the reproductive system, the females have significantly lagged behind males in growth, on average, 180-200 g. In this age, the stage of development of the ovaries was defined as the transition from III to IV. The ovaries were greatly enlarged and filled up to 2/3 of the entire abdominal cavity. Caviars are large, transparent and flowed out when pressed. When the ovary was cut and scraped, the eggs were separated individually and in groups. The gonadosomatic index increased to 6.3% (Fig. 3).

Figure 3. The gonadosomatic index depending on the age of females.

Absolute individual fertility of females did not exceed 40000 oocytes. The oocytes in the ovaries were found with definitive size of 0.94±0.03 mm and weight 1.37±0.04 mg (figure 1, 2). Microscopic studies of oocytes showed that it was saturated with fallow yolk and close to mature. The yolk in 70% of the oocytes was dispersed throughout the cell, and in 30% was concentrated at the vegetative pole. Polarization of the nucleus in the oocytes at this stage has not yet been detected.

When females reach 8 months of age, its reproductive system can be considered formed. According to morphological and histological criteria, females at this age are ready to spawn. The size of caviar amounted to 1.72±0.03 mm with a mass of 1.67±0.05 mg. The caviar had a dark-green color. At the age of 7-8 months, with a headless carcass weight of 975±73 g, the gonadosomatic index of females was still low and did not exceed 6.8% (figure 3). Absolute individual fertility of females did not exceed 50000 oocytes. The oocytes were of definitive size, saturated with yolk except for a narrow layer of wall vacuoles and a thin layer of cytoplasm around the nucleus. The nucleus in most oocytes was located at the animal pole.

At the age of 8 months, more than 80% of female of African Clary catfish reached a market weight of 900 to 1100 g, ovaries were sufficiently developed and corresponded to the IV stage of maturity. However, it was possible to get caviars from females at the age of 8 months after hormonal stimulation only in a small amount, as evidenced by the value of the gonadosomatic index.

At the age of 10-12 months, with a headless carcass weight of 1280±195 g, the ovarian maturity coefficient varied widely from 7.4 to 14%. Minimum individual fertility of females in this age exceeds 58000 oocytes. Females were subjected to hormonal stimulation with surfagon. After stimulation of artificial spawning females weighing 1000-1200 grams by 12-month of age, an average of 150 g of full roe were received in first portion, with a maximum of 250 g (figure 4).
The state of maturity of ovaries subjected to hormonal induction corresponded to V (the highest stage of maturity - fluid individuals). The color of the caviar after ovulation was dark green. The number of oocytes in 1 g of caviar was 956±93 cells, an indicator of working fertility exceeded 80000 oocytes.

Microscopy of oocytes showed that oocytes are mature and full of yolk of fallow, which is localized in most of the oocytes at the vegetative pole; in 93% of the oocyte nucleus was displaced to the animal pole in the area of the micropyle, around oocytes visible shiny shell. The oocytes reached a definitive sizes, its average diameter was 1.78±0.15 mm. As females of Clary catfish belong to the batch-spawning species in which ovules ripen also in portions, the size of oocytes in the ovaries ranged from 1.6 to 2.3 mm.

Thus, at the age of 10-12 months, weighing more than 1000 g, the females of African Clary catfishes can be used in reproductive technologies and begin to operate in the mode of caviar donor, selecting the most prolific in the spawning school. Studies have shown that the number and quality of female caviar depended not only on its age, but also on its weight. As puberty was rising the growth of gonadosomatic index and absolute individual fertility of fish was noted.

Summarizing the obtained results, it should be noted that gonadosomatic index in 3-4 months was only 0.05% in 4-5 months - 2.9%, in 6-7 months and 6.5%, 7-8 months 10% in 12 months and 13.3% , i.e. in the process of maturation has increased by more than 260 times. Such a high level of generative exchange affects the weight-growth indicators of females. Females during puberty lag behind in growth and weight gain from males. The mass of oocytes in 3-4 months amounted to 0.52±0.02 mg, in 7-8 months. – 1.67±0.05, and in 12 months reached 1.72±0.05 mg, i.e. increased more than 3.3 times during the maturation of gonads.

Selection of females in the spawning school was made from the age of 6 months. The main selection criterion at the 1st stage was the growth rate. Selected female weighing more than 1 kg. Females of first-year by mass significantly lagged behind males on average 20%. The spawning school was kept at a water temperature of 25-26 C. During spawning preparation, the water temperature was raised up to 28 C.

The caviar research productivity in the first year of life females showed that the lowest percentage of fertilization and the release of prenticing was observed in first-time spawning females. The first spawning we carried out at the age of 8 months. For hormonal stimulation surfagon was used at the rate of 5-7 \( \mu g/kg \) of weight.

With the increase in the mass of females during the second spawning, which was carried out at the age of one year, the percentage of fertilization of caviar significantly increased by 1.8 times compared to the first spawning females (P<0.05). The weight of females of the second year of life varied within 1100-1900 g and 85% of female gave a pronounced response to hormonal stimulation of oogenesis.
4. **Discussion**

In the course of the research, it was found that females of Clary catfish adapt well to the conditions of artificial breeding. After the first successful spawning with properly organized pre-spawning preparation, it is possible to achieve 80-85% efficiency in the return of caviars against the background of hormonal stimulation with surrogate. It is believed that the total amount of heat in the pre-spawning period, necessary for the complete maturation of fish oocytes, is 450 degree days.

The results show that the first time spawning females are characterized by low reproductive performance. With increasing weight and age of females significantly increased the average weight of the caviar. The increase in the number of caviar reflected the growth of the gonad maturity coefficient, i.e. the gonadosomatic index. It reached its maximum value at the age of 18 months.

The growth trend in the impregnation capacity of caviar peaked at the age of 18 months. The output of prenticing in relation to the number of caviar laid for incubation, significantly increased with increasing body mass of females.

Females of 8-10 months of age produced caviar in the amount of 7-11% of body weight, indicating their lack of readiness for spawning. Higher reproductive potential was observed in females over the age of one year. At this age, females produced up to 14-18% of caviar by body weight. It is known that caviar productivity is closely correlated with fish body weight. The correlation coefficient between these features usually exceeds 0.6-0.8.

Histological studies of ovaries showed that one-year-old females were fully mature individuals. In the evaluation of oocyte quality of Clary catfish of spawning school under 2 years, no abnormalities were found. The oocytes were of definitive size, fully mature and ready for fertilization. The oocyte shells were formed without any disturbances, the size of the cells corresponded to the biological characteristics of this species, no abnormal inclusions were observed in the cytoplasm. The caviar had a dark-green color.

The increase in fertility in the age aspect is associated with the ability to produce more ovules per unit of body weight. Therefore, the total volume of caviar increases, and, consequently, the absolute working fertility increases. The increase in fertility of females did not adversely affect the quality of caviar.

5. **Conclusion**

African Clary catfish is perspective object of industrial fish farming, with delicious meat and precious caviar. Clary catfish caviar can be obtained several times a year, since this species has multiportion spawning. However, in high-tech industrial fish farming systems, the biology of fish is changing so much that the African Clary catfish loses the ability to reproduce naturally and produce mature caviar. For reproduction and production full roe in industrial aquaculture, artificial spawning technologies are used with the use of hormonal drugs that induce the maturation of reproduction cells.

It was important for us to establish the age of puberty of females, on the basis of which it was possible to determine the starting age of obtaining caviar for food purposes and for reproduction. The results showed that by 8 months, the reproduction system of females reached maturity, females were able to reproduce. However, its reproductive performance during this period, in particular caviar productivity, was very low. After reaching reproduction maturity of females (in 8 months age), the reproductive performance continued to grow with age in the future. Caviar productivity reached a maximum at 18-24 months age and depended on the weight of females. The size and mass of oocytes during this period reached the limit values. Working fertility and gonadosomatic index of females aged from 12 to 24 months continued to grow, reaching maximum values. The age of 12 months can be considered as starting point in the technology of obtaining caviar for food purposes.

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