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Induction of Artificial Reproduction of Common Carp in Winter by Temperature Program in Conditions of Uzbekistan

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Annotation: In December 2022, 4-year-old matured females and males of common carp (Cyprinus carpio) were placed in the RAS and the control group in an earthen pond. In the RAS and in the pond, the water was 14-15 °C. Since the beginning of January, the water temperature in the RAS began to increase by 1°C every two days. In early February, the fish from the RAS and from the ponds were injected by pituitary injections according to routine method of artificial reproduction. The fish from the RAS gave mature eggs and sperm, and the eggs were successfully incubated. The fish from the ponds did not respond to the injections, their gonads remained at stage III – IV.

Keywords: Common carp, Cyprinus carpio, artificial reproduction, Recirculating aquaculture system, Uzbekistan.

Common carp (Cyprinus carpio) is one of the main objects of aquaculture in the world. It is cultured mainly in earthen ponds. In Uzbekistan, common carp is also one of the main objects of pond fish farming (Kamilov Yuldasov, 2021; FAO, 2022). In accordance with the geographical features (southern zone of temperate climate) and the seasonality of the climate, table common carp is grown for 2 years: in the first year to fingerlings (20-25 g), wintering is carried out from November to early April, table fish (1000 g and more) is grown in second year of fish life. The advent of RAS (recirculating aquaculture system) makes it possible to try to manage artificial reproduction in the

winter and growing fingerlings until early April, which was the goal of this work.

Material and methods

In mid-December 2022, 4-year-old common carp spawners were brought to the RAS of the Institute of Zoology from the fish hatchery of the Uzbekistan research Institute of Fish Farming. The fish were selected based on the expression of secondary sexual characteristics during the transfer of fish from fattening ponds for wintering; 6 females and 2 males of common carp were brought in. During the fishing, the water temperature in the ponds was 10-11°C. The fish were transferred to the RAS tanks and placed separately by sex. Initially, the water temperature in the tanks was kept at 14-15°C. Since the beginning of January 2024, the water temperature in the RAS has been artificially increased in the following mode. On one day, it was increased by 1°C, then kept at the same level for 24 hours. Then it was increased again by 1°C, and so on. By January 18, the water temperature in the RAS was raised to 20°C. The spawners were kept in this condition for 2 weeks. In addition, 6 females (control group) were brought to an earthen pond located next to the RAS, where the temperature was not regulated.

The water temperature was measured with an accuracy of 0.1 °C in the RAS and in the pond. In the RAS, when the water temperature was gradually increased, the common carp spawners were fed with ‘Aller Aqua’ feed for growing commercial fattening carp, the diet was kept at a level of 3% of the fish biomass in the tank per day. It was visually noted that common carp of both sexes actively ate all the feed. To understand the effect of temperature programs on the maturation process of common carp in the winter, we analyzed the state of the gonads under conditions of water in open facilities (ponds). For this purpose 6 females of common carp that were kept in ponds were dissected, and the maturity stage of the gonads was visually determined on a 6-point scale. (Pravdin 1966). The stages of embryonic development of common carp were determined according to Makeeva (1992).

Results

In February, an experiment on artificial reproduction of common carp began: on 3rd February, 2 females and 2 males of the control group were transplanted from the earthen pond into the RAS tanks. On the same day, 4 experimental females from the RAS and females from the pond were injected with preliminary injections of acetonated pituitary gland of mature common carp at a rate of 0.2 g/kg of body weight of the fish. After 12 hours, all females were given injections at a rate of 2 g/kg of body weight, and one injection was given to the males.

An attempt was made on February 4th to obtain mature sexual products from fish. The fish were taken out of the water one-by-one, the anal area was wiped dry and the abdominal area was pressed from the head to the anal opening. All females from the RAS easily gave up mature eggs. The eggs were collected in a plastic cup. The sperm was also milked from the males (after wiping the genital area dry) into test tubes. Then the sperm was poured into the eggs, water was added, that is, artificial insemination was carried out. De-adhesion was carried out with milk. It took 24 minutes to mix the eggs. Matured but unfertilized carp eggs were spherical in shape and yellow-greenish in color. Unfertilized eggs had a diameter of 1.05-1.36 mm.

The females that were caught from the ponds (not in the RAS) did not respond to the injections. An autopsy revealed that their gonads were at stage III-IV maturity.

The fertilized eggs were placed in two Zugger apparatuses, which in turn hung on plastic barrels of the incubation apparatus for silver carp "Amur". All apparatuses were connected with hoses to the general recirculation system (Fig. 1). The treatment with methylene blue from saprolegniosis was carried out according to the regulations. For this, the Zugger apparatus were disconnected from the general RAS water supply system, half of the water was drained from the apparatus, a dye solution was added and then water was fed into the apparatus for 8 minutes, which was drained not into the general water flow system in the installation. The fact is that the dye could have a negative effect on the biofilter. This aspect still requires solutions so that the treatment of eggs from saprolegniosis

does not destroy the biofilter biofilm.

Fig. 1. Obtaining eggs and incubation of fertilized carp eggs in Zugger apparatus.

Fertilization of the eggs occurred as soon as the sperm entered the egg and water was added to plastic dish. The fertilized eggs were sticky and round. As they developed, they became translucent. The diameter of the fertilized eggs became slightly larger and fluctuated within 1.27-1.69 (on average 1.48 + 0.06 mm). 30 minutes after fertilization, the eggs were yellowish-white, their diameter reached 1.41-1.42 (1.44 + 0.05) mm. We analyzed the embryonic development of eggs in Zugger apparatus at a constant water temperature in the RAS. To do this, we took 10-15 eggs from the device and quickly placed them under the "Microphoto - 5 PO - 1" device, sketched the contours, and poured the eggs back into the Weiss devices. They also took another sample of 5-10 eggs and examined them under a binocular to determine the stages of embryonic development.

Stage I (activation of the egg). All fertilized eggs formed a blastodisc after 10 minutes. This is in good agreement with the known literature data on common carp in the Tashkent region during the usual periods of artificial reproduction.

Stage II – multiple divisions, when a series of mitotic divisions of the cell nucleus and cytoplasm occur, but there is no noticeable growth in the size of the eggs. As a result of such divisions, a multicellular growth was formed on the yolk – the future embryo. In fact, we only noted from the description that the first cleavage, which divided the blastodisc into two blastomeres or 2 separate cells of the same size, was observed 28-30 minutes after fertilization. In the samples collected after 1 hour and 35 minutes, most of the eggs already had a 4-cell stage.В пробах через 2 часа все икринки имели уже по 8 бластомеров. У карпа икринки несколько мутные, поэтому количество бластомеров надо разглядывать тщательно.

In the samples after 2 hours and 30 minutes, more than 60% of the eggs can already be considered to have the 16-cell stage (blastomeres).

In the samples after 3 hours after fertilization, we already determined the presence of eggs with 32 blastomeres (more precisely, the blastomeres were already smaller than in the previous review.

In the samples after 4 hours after fertilization, we found that more than 80% of the eggs had reached the morula stage in development. According to literary data, this is the stage when division led to the formation of 64–256 cells. It was clearly visible that in all the eggs, the blastomeres were dividing and were collected around one pole of the eggs, this was their former animal pole.

Stage III of development is the formation of the blastula. We detected the blastula in half of the eggs in the sample 5 hours after fertilization. It was clearly visible that the eggs had a blastoderm, it was near the yolk sac and formed a dome-like structure around the yolk sac. In studies of the embryonic development of fish, specialists noted the importance of achieving this stage in development, since the first visually noticeable differentiation of the cells of the future embryo's body occurs, the embryonic shield is transformed into a thickened edge of the blastoderm. Indeed, in the samples collected 6 hours after fertilization, two cellular layers of the blastoderm are already noticeable. Epiboly was clearly visible - the blastodisc grows onto the yolk sac. The picture called the yolk plug is clearly visible.

We detected the next stage (stage V of embryonic development in carp) after 10 hours. The embryo already had a clearly visible body located along the axis of the egg around the yolk sac. It was already possible to distinguish (differentiate) the tail section of the embryo, the future head.

Specialists with more powerful microscopic equipment note that at this stage, differentiation of a number of organs from the head section to the tail section occurs. There is also already a mesoderm in which individual somites appear.

In our samples, we found signs of the appearance of the Kupffer's follicle 18 hours after fertilization.

The next important thing in the embryonic development of carp is the appearance of the tail section from the yolk sac, which characterizes the development of the embryo before the beginning of stage VI. We found the beginning of this in samples after 19 hours, and in more than half of the eggs in the sample - after 29 hours.

Noticeable pigmentation of the eyes, even greater development of the tail section were found in samples collected 32 hours after fertilization. It is noted that the pectoral fin is laid down, the Kupffer's follicle disappears and the tail section separates from the yolk sac. Soon, carp embryos sometimes began to sharply turn around their axis, which was clearly visible in a binocular magnifying glass.

45 hours after fertilization, in samples collected in the Weiss apparatus, we found the beginning of stage VII of development. This was clearly revealed by the noticeable development of the embryonic respiratory system, the appearance of blood flow through the heart (small thickening) and the yolk sac, visible under a microscope. It was noticeable that the head of the embryo straightened out. The embryos began to spin (move) almost constantly in the shells of the eggs.

62 hours after fertilization, we clearly noticed the beginning of the hatching of the embryos.

At the next - VIII stage of embryonic development - the carp develops gill arches and jaws. Free embryos have a clearly visible large yolk sac. Their head is bent downwards. The front part of the body is expanded. At this stage, the development of the gill arches occurs.

Free embryos lie quietly soon after hatching. In the drawings of their body contour in the "Mikrofot - 5 PO - 1" device, it was quite easy to determine the length of the prelarvae, which was 5.0 - 5.29 mm. In the binocular microscope, we clearly saw that the mouth of the free embryos was still closed. The pectoral fins were still rudimentary. Melanophores are clearly visible from the head to the tail end.

Important indicators are the body size of the organisms. 24 hours after hatching, the larvae in the samples we collected from the Amur device had a body length of 5.69 - 6.09 mm. After 4 days, they were all actively swimming, had a well-developed jaw apparatus, and reached a body length of 7.02 - 7.69 mm. Note that we determined the appearance of a gas bubble (in fact, that is why the larvae were already actively swimming). We noticed that the tail fold was rounded.

We also looked at the samples collected 7 days after fertilization. The larvae had already reached 8.84 - 9.1 mm in length.

We can assume that the first attempt to achieve carp maturation by heating the water to spawning

values in winter in Uzbekistan was successful.

Discussion

Uzbekistan is located in the south of the temperate climate in the Central Asia between 37° and 45° N, 56° and 73° E. That is, Uzbekistan is a deeply continental country in the very center of the largest continent. In such environments summer is hot (average daily air temperature in July is around 27 - 29 ºC) but winter is rather cold (average daily air temperature in January is 0 - -2ºC) (Salikhov et al, 2001). The average daily water temperature warms up above 14°C (the beginning of growth of warm-water fish, including common carp) in the first half of April, and drops below 14°C (the end of vegetation) at the end of October. At the same time, the period of fast growth of warm-water fish (when the average daily water temperature warms up above 20-22°C) is only about 4.5-5 months.

Let us point out the features of carp maturation in ponds in this region. In spring, yearlings of carp usually reach a standard body length of 10.0–25.0 cm and a total body weight of 29.2–100 g. In all yearlings, the gonads are still at maturity stage I. The gonads are visible as thin threads from colorless to translucent brown. The fish cannot be visually determined by sex. In the second year, the gonads move to maturity stage II; in spring, the 2-year-old females reach a body length of 25–33 cm. Upon dissection, the fish can be visually distinguished by sex. In the third year, the gonads of females further develop; in spring, 3-year-old females reach a standard body length of 26–47 cm. Thus, in pond environments, common carp reach sexual maturity at the age of 3 years, having reached a standard body length of 30–32 cm. In Uzbekistan, females of the broodstock of common carp aged 3-5 years with a standard body length of 32-87 cm have an individual absolute fecundity within the range of 130-1830 thousand eggs. From them, 3-year-old females has absolute fecundity is 130-580 thousand eggs, in 4-year-old fish - 200-1000 thousand eggs, in 5-year-olds - 700-1800 thousand eggs. (Kamilov et al., 2020).

Thus, the 4-year-old female common carp that we used in the experiment were all re-matured.

As our experience has shown, under the conditions of temperature programs (slow increase of water temperature in tanks with breeder fish) it is possible to carry out artificial reproduction of common carp, for this the water temperature in tanks should be raised by 1 °C every 2 days to the level of 20-22 °C. In our case it took 1 month. The obtained eggs of common carp can be further grown to fingrelings until the middle of spring, and they can be grown to marketable fish in 1 year instead of 2 years as is currently the case in Uzbekistan.

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