

GRASS CARPS EMBRYONIC DEVELOPMENT UNDER CONDITIONS OF ARTIFICIAL REPRODUCTION IN TEMPERATE CLIMATE, UZBEKISTAN

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ABSTRACT:

Embryonic development of grass carp (*Ctenopharyngodon idella*) was studied; the species was introduced from China to Uzbekistan in the early 1960s. More than 10 generation changes were taken place in local conditions. Artificial reproduction by using gonadotropic stimulation of ripening and eggs incubation is main method to provide reproduction of the species in the country. Embryonic development passed normally. Embryonic development rate is some higher than in 1960-1980s in local conditions and higher than in the river Yangtze. Construction of water supply in hatchery provides more stable water temperature without noticeable changing in night.

KEYWORDS: grass carp, unfertilized egg, development, development, morula.

INTRODUCTION:

Grass carp (*Ctenopharyngodon idella* Val.) was introduced to Uzbekistan in the early 1960s as promising object for pisciculture and melioration in irrigation channels network. The species has the ability to adapt well in adverse environmental conditions and are known to have faster growth rate and high reproductive potency. Recently grass carp is one of the most important species in local fisheries (production is more than 3 thousand tons per year). Still 1960s, reproduction is conducted in a process called hypophyztion where inducing agent such (pituitary extract) is

administrated in the fish (Камилов, 1973; Камиловидр., 2003).

Knowledge about fish development process is essential for aquaculture. Therefore, studies on embryonic and early larval development are essential for successful artificial reproduction and fish seeds production (Makeeva, 1992; Balon, 1995; Nica et al., 2012). In Uzbekistan, studies of those processes for introduced common carp and silver carp were occurred in 1960s (Verigin et al., 1985). The aim of present work was to study grass carp embryonic and larval development under condition of artificial reproduction in Uzbekistan.

MATERIALS AND METHODS:

The research was conducted in Fish hatchery of Uzbekistan Research Institute of Pisciculture, Tashkent region, Uzbekistan, from May to June 2019.

In March, breeders of both species were fished in wintering ponds and stocked to ponds for before spawning keeping; females and males were stocked separately by sex to different ponds and kept there up to reproductive company. Routine technological management was done (Sbornik..., 1986). On May the 13th, 5 females and 5 males (at age 4-5-years old, 4-5.2 kg of total weight) were selected by sexual dimorphism and spawning readiness and transported to reproductive unit of hatchery. At 17:30, first dose of pituitary gland extract (0.3 mg/kg of fish body weight) had been injected for hypophyztion of grass carp females. At 10:45

(May the 14th) second dose for female (2 mg/kg) and single dose (3 mg/kg) for males were injected. After 12 hours fish were stripped out for the collection of matured egg and sperm in a plastic bowl. Females have given 580 – 1280 g of ovulated eggs. At 22:50, fertilization was done through mixing by shaking the bowl several times, the ovulated eggs were transferred into “Amur” (200 liters) incubation jars (0.8 - 1 mln of eggs / jar).

After fertilization, during 20 hours every 30 minutes of interval, the incubated eggs were observed to identify the developing stage (Makeeva, 1992). Later the eggs were observed at every hour of interval up to larvae exogenous feeding. All the sampled eggs and larvae were preserved in 0.1% formalin in plastic container. In the laboratory, egg and larvae samples were taken in separate slides with the help of brush. Optical microscope was used to observe embryonic and larval developmental stages. At the same time, measurement (diameter and length of egg and larva) were taken using apparatus for microfilm reading “Microfot-5 PO-1” with magnification 10*. Time of following stage approach was marked when less than 50 % of eggs in sample reached that stage.

RESULTS:

There are 4 basic phases (periods) of fish life cycle which are Embryonic Phase, Larval Phase, Fry Phase, Adult Phase and Senescence. According to A. Makeeva (1998), the Embryonic Phase includes (I) egg

activation and blastodisc formation; (II) cleavage, (III) blastula; (IV) gastrula; (V) organogenesis; (VI) tail bud; (VII) embryonic respiratory system, (VIII) gill-arch and jaw development (last stage often is separated to subperiod of out of egg cell development).

Water quality. During the period of reproductive company, water temperature was 21.1 – 23.1°C in ponds and 21.8-24.6°C in incubation jars. Level of dissolved oxygen 5,16 – 6,4 mg/l.

The grass carp early development had two primary periods, embryonic and posthatch. The characteristics of various stages of embryonic development were described as follows (Table 1).

Stage I: egg activation occurred, cytoplasm formed blastodisc at animal side.

Stage II (cleavage): included series of mitotic divisions of cell nucleus and cytoplasm without cell growth; consecutively 2-, 4-, 8-, 16-cell formed. Those four divisions occurred like meridians; following fifth division was parallel to yolk sac equator and led to generation of 32-, 64-, 128-, 256-cell stages. At the morula stage (64-256-cells), cells became smaller, the blastodisc was appeared mound-like and raised above the yolk, and the cytoplasm disappeared.

Stage III (blastula) included continuing divisions, the cell differentiation appeared. At the early-blastula stage, the blastodisc formed a half-sphere. At the late-blastula stage, the blastodisc began flattening and gradually expanded over the yolk.

Table 1. Timetable of embryonic and larvae development of grass carp in different environments.

Embryonic and larvae development stages	Time after fertilization (hours : minutes)		
	Uzbekistan, 1960s	the Yangtze River	Our data
Blastodisc formation	0 : 40	-	0 : 40
Stage II (cleavage):			
2- cells	1 : 00	-	1:20
4 – cells	1 : 20	1 : 05	2 : 00
8 – cells	1 : 40	1 : 20	1 : 30
16 - cells	2 : 00	1 : 40	1 : 50
Large cell morula	2 : 30	2 : 45	2 : 00
Small cell morula	4 : 50	3 : 50	2 : 50
Blastulla	6 : 00	4 : 50	3 : 30
Early gastrula	7 : 10	8 : 55	4 : 00
Yolk plug (Blastopore closure)	12 : 10	10 : 30	9 : 20
Opticprimordium, notochord		12 : 50	14 : 45
Opticvesicle		16 : 05	17 : 00
Olfactory placode, tailbud	29 - 32 h	19 : 15	27 : 00
Hatching	34 : 00	37: 00	34: 30
Embryonic vascular system development	51: 00	47 : 00	47 : 40
Branchial – maxillary apparatus development	76 – 96 h	83: 00	68: 00
Larvae period			
Mixed feeding	108 – 144 h	167 : 00	82 : 00
Exogenous feeding	168 : 00	190: 00	122 : 00

Stage IV (gastrula) included process of homogeneous blastoderm division to germ rings. At 5 hours and 30 min after fertilization the blastoderm covered about 1/3 of the yolk cell, and the whole egg appeared round.

Stage V (organogenesis). The blastoderm (embryo body) covered almost the whole yolk, the head part was clearly visible, and the organ differentiation began: the notochord was clearly visible, the optic primordium was a long oval shape and clearly visible, somites appeared and numbered, the somite number was 10-13 pairs, nerve cord laid.

Stage VI (tailbud stage): the tail bud appeared, the eyes enlarged, the brain rudiment differentiated slightly, somites formed in tail bud. During this stage the heart rudiment appeared, the melanin was appeared; the unpaired fin fold rounded back part of embryo body. The muscular-effect was visible,

the embryo elongated, the embryo lashed occasionally.

Stage VII (embryonic vascular system development): the heart had two pulsating sections, the branchial arch laid, and the melanophore appearance on the head and back of embryo was visible. The head rectification occurred. The preanal fold was visible. The embryo rotated continuously. The embryo was ready to hatch.

Stage VIII (branchial – maxillary apparatus development). Hatching was noticed. Total length was 6.09 mm. The yolk sac was light in color and slightly transparent, the anterior portion of yolk sac was large, the posterior - narrow. Development of branchial and maxillary apparatuses was occurred. The air bladder bud was visible. Firstly mouth was presented as small pit, later the branchial arch were visible. The head extended straight out from the body. Eyes were pigmented (black).

The larvae still usually rested on the bottom but occasionally swam.

Further larvae period began with several successive development stages.

Stage I (mixed feeding). Back part of the air bladder filled with air, the larvae body straightened and rounded by unpaired fin fold. The pectoral fins enlarged. The yolk sac remained only as a narrow strip. The mouth was open and moved forward. The lower jaw began movement. Melanophores extended larvae body. The differentiation of maxillary was noticed, feeding began. The pectoral fin enlarged. Dorsal and anal finfolds began to separate from the caudal finfold. Larvae were able to swim normally.

Stage II (exogenous feeding, unpaired fin fold differentiation). The yolk sac was absorbed, the larvae fed with external food. The jaws were formed. The gill cover was formed. The provisory respiratory system organs reduced. The anterior portion of the dorsal finfold continued to differentiate and melanophores increased.

Stage III (rays in unpaired fin development). The initial rays were visible in unpaired fins, firstly in the caudal fin then in dorsal and anal ones. The posterior tip of the notochord curved slightly upward. The posterior margin of the caudal fin fold was crenulated. The operculum completely covered the gills. The ventral fin laid.

Stage IV (rays development in paired fins). The dorsal and anal fins were differentiated, the caudal fin was deeply forked, the pelvic fin lengthened and extended beyond the preanal finfold. The preanal finfold shrank slightly. The vertebral column was fully formed. The squamation stage began. Further silver carp entered the fry period.

DISCUSSION:

Grass carp is one of the most important cultured species in global freshwater aquaculture which is based on high adaptive potential of those species. At the same time, knowledge of biology peculiarities in specific region is the base for artificial reproduction. In Uzbekistan (Central Asia), artificial reproduction technology of both species is conducted since 1960s (Kamilov et al., 2003). Broodstocks consist from 3-5-year-old males and females, after that elder and larger fish are sold as high quality marketable good. Due to this method of broodstock formation used in the country, more than 10 generations of those invasive objects were completely changed in local environments. As our research has shown, grass carp has found favourable environments for embryonic and further development in Uzbekistan; adaptive potential of those species was enough to habitat under specific local conditions of semi-intensive aquaculture.

Suitable season for the grass carp reproduction occurs since the May, when water temperature warm up to 21 – 22°C. We compared our data of recent grass carp embryonic development with those from 1960s in Uzbekistan (breeders form introduced generation) and those from native area (River Yangtze, China) (table 1). Recently in Uzbekistan grass carp embryonic development passed normally. A blastodisc division begin during the first hour after fertilization, stages of morula – after 2 hours, blastula – 3.5 hours, gastrula - 4 hours, organogenesis – 14 hours, larvae hatching occurs after 34 hours after fertilization at water temperature 21-23°C, transition to mixed feeding of larvae – after 4 hours, to exogenous feeding – after 5 days.

Recently, embryonic development slightly increased in compare with 1960s. In pisciculture conditions of Uzbekistan, grass carp eggs develop noticeably faster than in the

River Yangtze (Yi et al., 2006). We relate that with more stable (in daytime and night) water temperature in incubation jar in fish hatchery. Water warms in pond from which it pumps to tanks (with total volume 90 t) then leads to incubation jars and do not cool strongly in the night. Construction of incubation unit use long-term experience in eggs incubation in the country.

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