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Review article

Protection of Resources Based on The Pseudobagrus Hwanghoensis Artificial Propagation

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Abstract

Due to the change of ecological environment and the increase of human intervention, the amount of rare fish resources in some water bodies becomes less and less. Pseudobagrus Hwanghoensis is also in serious decline in the mainstream of the Yellow River, with small populations still existing in the upper reaches of its tributary, Luohe River. The breakthrough in artificial propagation technology is an effective means to protect germplasm resources. We have developed a special sperm preservation solution for the Yellow River bullhead to prepare "artificial semen" and tested its quality with Cyto FLEX flow cytometry [1], which is a breakthrough of core technology to support the success of artificial breeding of the Yellow River bullhead. The average propagation and fertilization rate reached 93%, the hatching rate reached 75% and the survival rate of pond seedling reached 70%. A large number of fish species are bred by artificial breeding, respectively, in the main tributaries of the middle reaches of the Yellow River in Henan Province, the Luohe Guxian Reservoir and the lower reaches of the main channel of Changyuan, to carry out artificial stocking to replenish the natural resources of the bullhead in the Yellow River.

Keywords: Pseudobagrus hwanghoensis; artificial reproduction; proliferation and release

Introduction

The Yellow River bullhead (Pseudobagrus Hwanghoensis) belongs to Osteichthyes, Siluriformes, Siluridae and Pseudobagrus. Special medium sized economic fish are benthic and live in slow flowing rivers. This fish mainly feeds on benthic animals in the wild environment, which can reach sexual maturity in 3 years, with a body length of about 20cm and a maximum individual length of 1m. The spawning period is from mid-June to late July, and the number of eggs is small. Its high fat content, tender meat, delicious taste, has a high edible value and economic value. However, due to the construction of largescale water conservancy projects, water pollution, water reduction, manmade disorderly fishing and other reasons, the main river of the Yellow River and its main tributary resources have declined sharply, and if not protected, it may reach the edge of endangerment. In order to effectively protect the germplasm resources of the Yellow River bullhead, in recent years, we have collected parents and backup parents in Luohe River section of Lushi County, the main tributary of the Yellow River, carried out research on artificial propagation, and made breakthroughs in key technologies. After the successful cultivation

of pseudobagrus hwanghoensis seedlings, proliferation and release were carried out in the Guxian Reservoir of the Luohe River and the main road of the Yellow River in Changyuan respectively, which obtained good results initially.

Artificial Propagation of The Pseudobagrus Hwanghoensis

Parental Fish Breeding

Pond Conditions

The parent fish breeding pond is located in the Yiluo River Aquatic Biology Field Science Observation and Research Station in the Yellow River Basin of Henan Province. It has cement slope protection, sand and mud bottom, river water source, easy to enter and drain water into the pond. The pond covers an area of 300 square meters, and the water depth is kept between 1.5 and 2.0 meters.

Parental Fish Stocking

A total of 180 kg and 600 Pseudobagrus Hwanghoensis were stocked, and the parameters of the stocking parents are shown in Table 1.

Table 1: The situation of free Yellow River Pseudobagrus Hwanghoensis parents.

Parent	Full length (cm)	Body length (cm)	Body height (cm)	Head length (cm)	Snout length (cm)	Body weight (g)	Ration of gonad to body weight
Female	18.7	16.7	5.27	3.8	1.1	51.8	12.23%
Male	33.3	30.4	10.9	5.8	1.7	152.4	1.5%

Domestication of Parental Fish

Parent fish domestication with yellow catfish special floating feed, set up 1 * 1 m PVC pipe box under the feed Table 1, feed sprinkled in the box, every morning and evening feed a meal. Tame fish grabbed food at the surface, mastering the feeding amount each time until the fish did not grab food strongly.

Selection of Parental Fish

Selection of parental female fish to pick out the abdomen bulging, visible ovarian outline (short reproductive process), reproductive pores dilated (wide and round), hand touch eggs abdomen soft and elastic, no injury, weight of more than 100g individuals; Male parental fish abdomen is not swollen, the tail stalk is long and thin, the reproductive process is pointed and long and thin, the reproductive hole is closed, the sperm nest is serrated, it is not easy to squeeze out semen, the physique is strong, the body surface is not injured, the weight of more than 250g.

Injection of Parental Fish Oxytocin

The oxytocin of parental fish is an important part of artificial reproduction. When the water temperature of oxytocin is generally above 20°C (preferably stable in the range of 22 ~ 24°C), the parent fish should be oxytocin [2-3]. The thorax was injected twice with a depth of about 1cm. The injection was carried out twice, the first from 17:00 to 18:00 p.m., at a dose of LRH-A2 15-20 micrograms + HCG 10 IU per kilogram of female parental fish, halved for males; The injection interval was 12 hours, the next morning 5:00 to 6:00 to play the second injection, the dose was LRH-A2 15 microgram +HCG 800IU, and the male fish was halved. When the water temperature was 20-24 °C, the effect time was about 24 hours. After injection, the parent fish should be stimulated with flowing water, and the pool should be kept running water with a flow rate of 10-30 cubic meters per hour, so as to better oxytocin success. During injection, attention should always be paid to the water not spilling out of the pool. The dissolved oxygen in the pool is kept greater than 5.5mg/L. Keep the environment quiet and avoid the light.

Artificial Insemination

Because the estrus behavior of the parent fish is not obvious, after 24 hours of oxytocin, artificial insemination begins. The mature female fish are caught with squeezed eggs (the eggs are orange, with an average egg diameter of 2.0 mm) for semidry artificial insemination. When squeezing eggs, we should pay attention not to bring the pool water into the basin, so as not to affect the fertilization rate. Sperm motility is also closely related to fertilization rate [4-6]. Male fish can take out their spermary in advance to make “artificial semen” for this use or stored in cold storage for next use. Sprinkle the “artificial semen” prepared in advance on the extruded eggs, and use hard chicken feathers to keep stirring, so that the sperm eggs can be better combined with fertilization. The preparation of artificial semen is the core of this technology. The spermary of the Yellow River bullhead was ground into a thick slurry, which was diluted 8-fold with our self-developed spermatogenetic preservation solution. After the dilution, 10ml tubes were placed on the tube rack and stored in the refrigerator at 4°C for cold storage [7-9].

The quality of the prepared “artificial semen” can be detected by Cyto FLEX flow cytometry and analyzed by SCSA method [1], that is, sperm chromosome breakage index. After SCSA detection of the preserved 4h, 96h and 144h “artificial semen”, the rupture index (AT) data was obtained as follows:

$At1 = 6.37 \div (39.03 + 6.37) = 0.14$

$At2 = 11.28 \div (40.65 + 11.28) = 0.21$

$At3 = 9.48 \div (23.23 + 9.48) = 0.29$

We repeatedly carried out experimental tests on the “artificial semen”. The analysis shows that the spermatozoa which has DNA

breakage index (AT) ≤ 0.15 are vigorous and can be used as the source of spermatozoa for artificial insemination, while the spermatozoa which has DNA breakage index (AT) ≥ 0.3 tend to collapse and are no longer used as the source of artificial insemination.

Incubation and Emergence

Incubation

The fertilized eggs are incubated in the incubating frames, and every four incubating frames are placed in one incubation tank, with 10,000 fertilized eggs in each frame. The incubation tank adopts the method of micro running water and aerating, and the water is thoroughly changed once a day. The incubation water temperature is kept above 20°C, preferably between 22 and 24°C. Keeping the microflow state during incubation, dis-solved oxygen is above 6.0 mg/liter, and at the outlet end, the filter should be constantly brushed to avoid blockage. When incubating for about 35 hours, the formalin solution should be sprinkled into the incubator of fertilized eggs to 100ppm to prevent the occurrence of mold. When the incubation temperature is 20-23 ℃, the incubation time from fertilization to membrane production is 60-70 hours. When the incubation temperature is 24-25 ℃, the incubation time is 40-50 hours, and the incubation accumulated temperature is in the range of 1440-1630 degrees per hour.

Emergence

When all the larvae hatched, the larvae with the newly hatched membrane gathered in the four corners of the hatching frame, which were only about 6.0mm in length, tadpole like in shape, with large yolk sac and nutrition, weak and tender constitution, and unable to swim freely. After 5 to 7 days of temporary rearing, the total length of the larvae can reach 1.0cm, the yolk sac shrinks, and the larval fish begin to feed. At this time, fry breeding can be carried out. However, the water should be clean, the dissolved oxygen should be sufficient, and direct sunlight should be avoided.

Fish fry Breeding

Fry breeding is mainly to grow the larval fish into 2.5 to 3.5cm summer fry, which can choose to be carried out in cement pool and pond. Pond breeding is introduced in this paper.

Pond Disinfection

Choose a fishpond with good water quality and sufficient water source and no pollution, with an area of 400m^2. Disinfection must be carried out on sunny days. 2 to 3 days before stocking, 2kg of chlorine dioxide is dissolved and sprinkled in the whole pool. After 4 days of disinfection, add the water to 60cm - 70cm to enrich the water quality and increase the biological bait in the water [10-11].

Stocking Density

50,000-60,000 splashes are put into the pond of 300 square meters, and a certain number of summer fry or species of silver carp and bighead carp are put into the pond. The ratio of silver carp and bighead carp is 3:1 to regulate water quality [12].

Feeding

The feeding technology combining the lower pond of hypertrophic water (mass breeding of zooplankton) with artificial feeding was adopted [13-15]. Under the fishpond after feeding on zooplankton cultured, it can hold about 5 days or so. Artificial feeding and starting from the fifth day, pour soy milk on the shore in the next 5 days, four times a day, every time 4 kg, add ice fresh halogen insect at the same time. And in up to 10 days start splashing, soyabean milk and powder mixture four times a day, 6 kg each time, add a bowl of powder into each barrel and mix well. After feeding for up to 15 days, powder can be completely sprinkled four times a day. Of course, it is also necessary to adjust the feeding amount flexibly according to the weather changes and the feeding situation of the fry. When feeding, appropriate microbial agents such as photosynthetic bacteria can be added as feed additives, on the one hand, can prevent water deterioration, but also can enhance the disease resistance rate of fish body; Strengthen water quality management during the cultivation period by regularly replenishing fresh water.

Divided Pools

After 20 days of cultivation, it can grow into 2.5-3.5 cm summer seedlings with a survival rate of about 70%. After stopping eating and pulling the net, the next stage of feeding will be carried out in different pools to avoid the influence of excessive density on growth. After the ponds are divided, the No.0 artificial compound bait can be used for domestication and feeding. The bait particle size is gradually adjusted along with that growth of the fish body. When the body length of the fish reaches 10-12cm, it can be used as the fish species for proliferation and release or adult culture.

Proliferation and Release of the Yellow River Bullhead

Guxian Reservoir Proliferation and Release

Guxian Reservoir is located in the middle reaches of Luohe River, the main tributary of the Yellow River. The dam is located in Guxian Town, Luoning County, and the water surface spans Luoning County and Lushi County. The maximum height of the dam is 125 meters, the crest elevation is 553 meters, the total storage capacity is 1,175 million cubic meters, and the water surface area is 21,800 mu. It belongs to deep water canyon reservoir. The surface water temperature of the reservoir is 10-20°C on average all the year round. The water depth of 20 meters below the water temperature of 8-24°C is rich in cold water resources, water quality is clear and transparent, few suspended matters, to meet the national second-class drinking water standards. The Yellow River bullhead used to be an important economic fish in the Yellow River. With the change of ecological environment and other reasons, the resources decline sharply. According to the survey, anglers can occasionally catch one fish.

In 2006, Lushi County Agriculture Bureau and Lushi County Public Security Bureau Xujiawan police station in the joint law enforcement of the seizure of electric fishing in the upper reaches

of the Luohe River, the Yellow River bullhead hit 80 kg, a total of 520 tails, which shows that at that time still maintain a certain number of resources. Then every year in the Yellow River bullhead breeding season in May and June, fishermen can catch 5-30 fish a day from the upstream of the Guxian reservoir. On May 19, 2020, in order to effectively protect the wild resources of the Yellow River bullhead, and arouse the awareness of the whole society to protect rare fish resources in the Yellow River, Joint fisheries research institute of henan province agriculture and rural areas in henan province hall, department of henan province, luoyang Sanmenxia bureau of agriculture and rural areas, agriculture and rural areas bureau, Luoning county people's government, Guxian county administration of reservoir, henan daily in so county reservoir terminal for the Yellow River to discharge Chang proliferation ceremony, a total of 15 cm of the Yellow River bullhead species 20000.

Proliferation and Discharge of The Main Channel of The Yellow River

According to the survey, in the 1970s, the Yellow River fishing often caught hundreds of catties, the Yellow River bullhead (cow tail), but now it is rarely caught, occasionally a few can be caught. In order to increase the main channel of the Yellow River the Yellow River bullhead wild resources and speed up the conservation of aquatic organisms in the Yellow River, on September 17th, 2021, we launched a proliferation and release activity in Changyuan section of the lower reaches of the Yellow River, with the participation of Henan Science and Technology Department, Henan Agriculture and Rural Department, Henan Academy of Agricultural Sciences, Changyuan Municipal People's Government, Henan Daily and other units. A total of 20000 the Yellow River bullheads with 13cm diameter and 200 parents with 20-30cm diameter were released in this activity, which were labeled with PIT.

Implement the Yellow River Fishing Ban and Increase the Conservation of Aquatic Organisms in The Yellow River

Proliferation of the Yellow River discharge is an effective way to increase the aquatic biological resources effectively, but other measures are also important, such as preventing water pollution, optimization of reservoir scheduling to prevent the river cutoff, combating poison fish electrical behavior, the implementation of the Yellow River banning fishing, etc. There are necessary measures for water conservation and ecological restoration of aquatic creatures. In recent years, several measures have been taken before, but the Yellow River fishing has just begun, and not enough. Since 2018, the Yellow River has been closed to fishing. The mainstream of the Yellow River, three main rivers and lakes and 13 main tributaries are closed to fishing from April 1 to June 30 every year. The fishing ban on the Yellow River was strengthened in 2022, with the annual fishing ban in the head waters of the Yellow River and key upstream waters from April 1, 2022, to December 31, 2025.

The fishing ban on the Ningxia section of the Yellow River from April 1 to June 30 to July 31 was extended for the first time in history. As a matter of fact, February to March is the spawning period for the Yellow River Leuciscinae fish in the Luo River. A large number of fish swim in the shallow water and are caught by fishermen, which damages the resources of the Yellow River Leuciscinae fish. Therefore, the fishing ban should be extended to February 1 from July 31, and a 10-year fishing ban should be carried out when the time is right. It is hoped that through the breakthrough in the artificial breeding technology of rare fish, the proliferation and release, coupled with the environmental control, fishing administration and the 10 years ban on fishing, pseudobagrus hwanghoensisand other rare fish resources will be restored and the aquatic biodiversity of the Yellow River will be restored, making the Yellow River really become a happy river that benefits the people.

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