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Observation on the Postembryonic Development of *Abbottina rivularis*

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Abstract: *The fertilized eggs of Abbottina rivularis hatched out after 124h at 19~23℃. Postembryonic development stages could have divided into larval stage I, larval stage II and larval stage III. The total length of newly hatched larvae is 3.32 to 4.67mm, and the length of the hair on the head is 0.048 to 0.168mm. The length of the hair on both sides of the body is 0.041 to 0.068 mm. The length of yolk sac is 0.62~ 1.21mm, and the short diameter is 0.26~0.41mm, and disappeared after 24~27h.*

Keywords: *Abbottina rivularis; yolk sac; postembryonic development; pectoral fin*

1. Introduction

Abbottina rivularis belongs to *Abbottina*, *Gobioninae*, *Cyprinidae* of *Cypriniformes*[1]. It is a small benthic fish and likes to live in the sandy bottom with fresh water of high quality [2]. It is common in the middle and lower reaches of the Yangtze River and is a common species [3]. At present, there are few research reports on this fish. The research reports mainly focus on reproductive habits [3-5], mitochondrial genome determination and analysis [6] and muscle nutrition analysis [7]. Although the individual is small, its fecundity is relatively strong. It can be used as a local famous snack, or processed into ready-to-eat food, and can be cultivated as a bait for rare fish. Therefore, it is necessary for us to further research and develop it. In this study, we observed and studied the postembryonic development of *Abbottina rivularis* to understand the characteristics of postembryonic development, to provide theoretical reference for the development, utilization and cultivation of this small benthic fish.

2. Materials and Methods

2.1. Obtainment and Hatching of Fertilized Eggs

In the experiment, the fertilized eggs of the *Abbottina rivularis* used were collected from a small water body in Taizhou, Jiangsu Province. The collected fertilized eggs incubated in still water indoors. The water for hatching comes from the water source of the fertilized eggs collection site. When the water temperature was 19-23℃, the fertilized eggs hatched after 124 hours. The experimental fish was just the fry newly hatched out. The total length of larvae was 3.32-4.67mm.

2.2. Sampling and Observation of Specimens

Take 30 synchronously hatched *Abbottina rivularis*, and then observe the postembryonic development of the larvae with Motic BA210 microscope, and record the main characteristics and time of development in detail. At the same time, use Motic Images Plus 2.0 image software to take micrographs and measure them. The newly hatched larvae were observed every 2 hours, and then take photos and measure their total length, the length of their eyes, the short and long diameters of yolk sac, the diameter of anterior otolith and posterior otolith, and the number of pectoral fins. After 5 days, observe them every 2 days.

3. RESULT

3.1. Egg Hatching

The eggs of *Abbottina rivularis* have a certain viscosity, but the viscosity is not strong. After it lays eggs, there are a large number of fine sand particles adhering to the egg membrane (Fig. 1), but with a little vibration, the attached sand can fall off. When the water temperature is 19-23℃, the fertilized eggs hatch after 124 hours. When the fry hatched, the egg membrane is semicircular (Fig.2). The animal pole dissolved and the fry broke through the membrane and got out. The egg membrane and the sand attached to it will be dissolved and disappear gradually with time.

The yolk sac absorption time of the hatched larvae (Fig. 3) is very short, so the time of endogenous nutrition stage is also relatively short; the characteristics if transformation from endogenous nutrition stage to exogenous nutrition stage are not obvious; after the exogenous nutrition stage comes, the food they feed on is not specific is not specific. At the same time, the occurrence of its swim bladder is lagging behind, and there is no obvious characteristic of its development as that of conventional fish for us to refer to. For the convenience of description, we divide the postembryonic development into three stages: larval stage I, larval stage II and larval stage III.

Fig1. *Fertilized eggs*

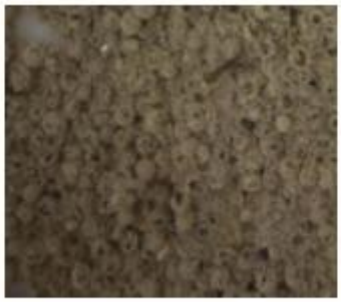

Fig2. *Egg membrane after fry hatching*

Fig3. *Newly hatched fry*

3.2. Larval Stage I

The larval stage I of *Abbottina rivularis* refers to the endogenous nutrition period from hatching of fry to the disappearance of yolk sac. The total length of the newly hatched larval is 3.32-4.67mm. The color of the body is transparent. The heartbeat and blood flow are clearly visible. On the head, dense sensory mounds and sensory hairs are distributed from the snout to the gill cover on both sides, from the upper jaw to the top of the head and from the lower jaw to the pectoral fin (Fig. 4), and the length is 0.048-0.168mm. There are sensory mounds in each sarcomere on both sides of the body, and on most of the sensory mounds, there are sensory hairs, while on a few of sensory mounds, there is no sensory hair (Fig. 5). From the pectoral fin to the base of the caudal fin, the sensory mounds are arranged in two rows along the spine, and the length is 0.041-0.068 mm (Table 1). The notochord and sarcomere are clear; the pectoral fin has soft fin and there are branches, and the maximum length is 0.768-0.948 mm, and there is no differentiation occuring on the dorsal fin, caudal fin and anal fin. The yolk sac is larger, the long diameter is 0.62-1.21mm, and the short diameter is 0.26-0.41mm. The yolk sac of *Abbottina rivularis* disappears after 24-27 h (Table 2) (Fig. 6), and during this period, the fry moves in the bottom of the water.

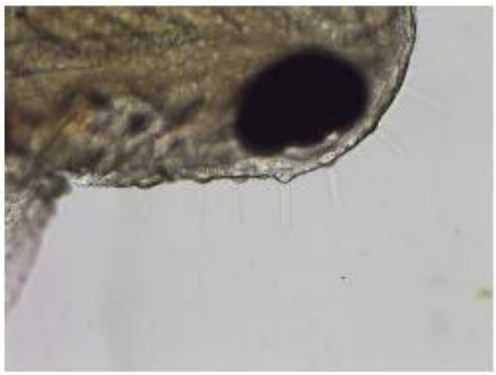

Fig4. *Sensory mound and sensory hair of the head*

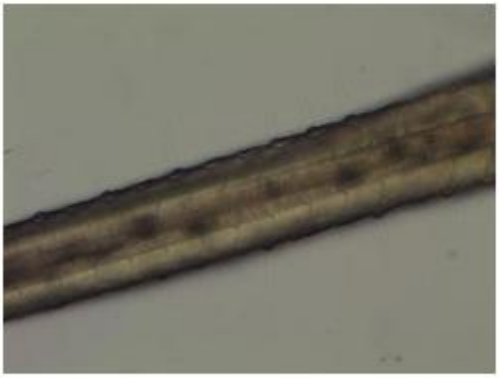

Fig5. *Sensory mounds and hairs on both sides of the body*

Fig6. *The changing process of yolk sac*

Note: *A. hatching for 0 h; B. hatching for 6 h; C. hatching for 12 h; D. hatching for 18 h; E. hatching for 24 h; F. hatching for 30 h; G. hatching for 36 h*

Table1. *Determination table of sensory hair and pectoral fin characters of Abbottina rivularis fry*

Length of pectoral fin (mm)	Length of sensory hairs on the head (mm)	Length of sensory hairs on body sides (mm)	Hatching time (d)
0.588	0.252	0.072	0
0.540	0.216	0.072	0
0.564	0.156	0.060	0
0.528	0.204	0.084	0
0.576	0.228	0.084	0
0.804	0.132	0.084	5
0.792	0.096	0.096	5
0.78	0.156	0.084	5
0.816	0.096	0.072	5
0.756	0.132	0.096	5
0.936	0.090	0.072	10
0.960	0.095	0.084	10
0.936	0.085	0.072	10
0.948	0.090	0.096	10
0.960	0.076	0.096	10
1.212	0.142	0.066	15
1.116	0.133	0.042	15
1.104	0.104	0.057	15
1.200	0.109	0.057	15
1.164	0.104	0.071	15

Table2. *Changes of the long diameter of yolk sac in 24 hours*

Hatching time (h)	The long diameter of yolk sac (mm)
0	0.804
2	0.636
4	0.696
6	0.564
8	1.332
10	1.308
12	1.092

14	1.128
16	1.128
18	1.056
20	1.032
22	0.960
24	1.068

3.3. Larval Stage II

Larval stage II refers to the mixed nutrition period in which the yolk sac is about to disappear, the mouth of the fry has been opened and there is simultaneous existence of both endogenous and exogenous nutrition stages, and the time is 8-12 hours. The larvae begin to ingest food, and organs such as intestine and liver gradually differentiate, but they are not perfect. At this time, the total length of the larvae is 4.78-5.18mm, and there are soft fin branches in the pectoral fin. From the third day of hatching, the end of the soft fin rays begins to expand, and the maximum number of branches is 16-18 sticks. The sensory hairs on the head are shorter than those at the beginning of the hatching, and the length is 0.11-0.13mm. However, the sensory hairs on both sides of the body are longer than those at the beginning of the hatching, and the length is 0.071-0.095mm. On the 7th day after hatching, above the foregut, a red oval liver appears (Fig. 7). During this period, the fry moves in the bottom of the water, especially likes to move in the water plants.

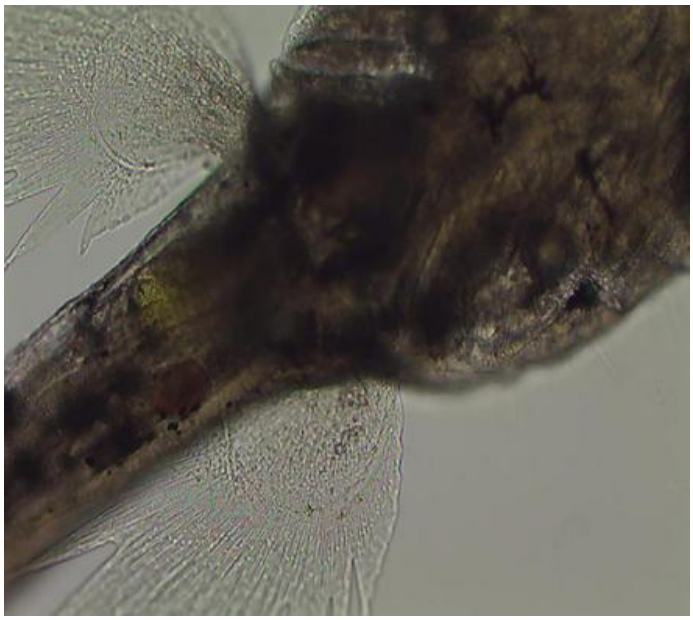

Fig7. *The red dot is the liver*

3.4. Larval Stage III

Larval stage III refers to that, the fry has completely entered the stage of parenteral nutrition. At this time, the larval body is transparent, scales begin to form (scales develop from head to chest and from back to abdomen in turn), and the fin ray of each fin initially forms. The digestive system is well developed, and there are many melanin's on the body surface. The fin where branch fin ray first appears is caudal fin, and then pectoral fin (Fig. 9), dorsal fin, abdominal fin and anal fin. The swim bladder of *Abbottina rivularis* did not appear until 20 days after hatching, and when the swim bladder was inflated, the sensory hairs on both sides of the body began to disappear gradually, and the disappearance time was 70-75 days. The order of disappearance was from the body side to the head, and on the body side, the order of disappearance was from the front to the back. The sensory mound and sensory hair on the tail disappeared last. The disappearance order of sensory mound and sensory hair was from top to bottom and from snout to gill cover in turn, and the sensory mound and sensory hair disappeared last were the sensory mound and sensory hair on gill cap.

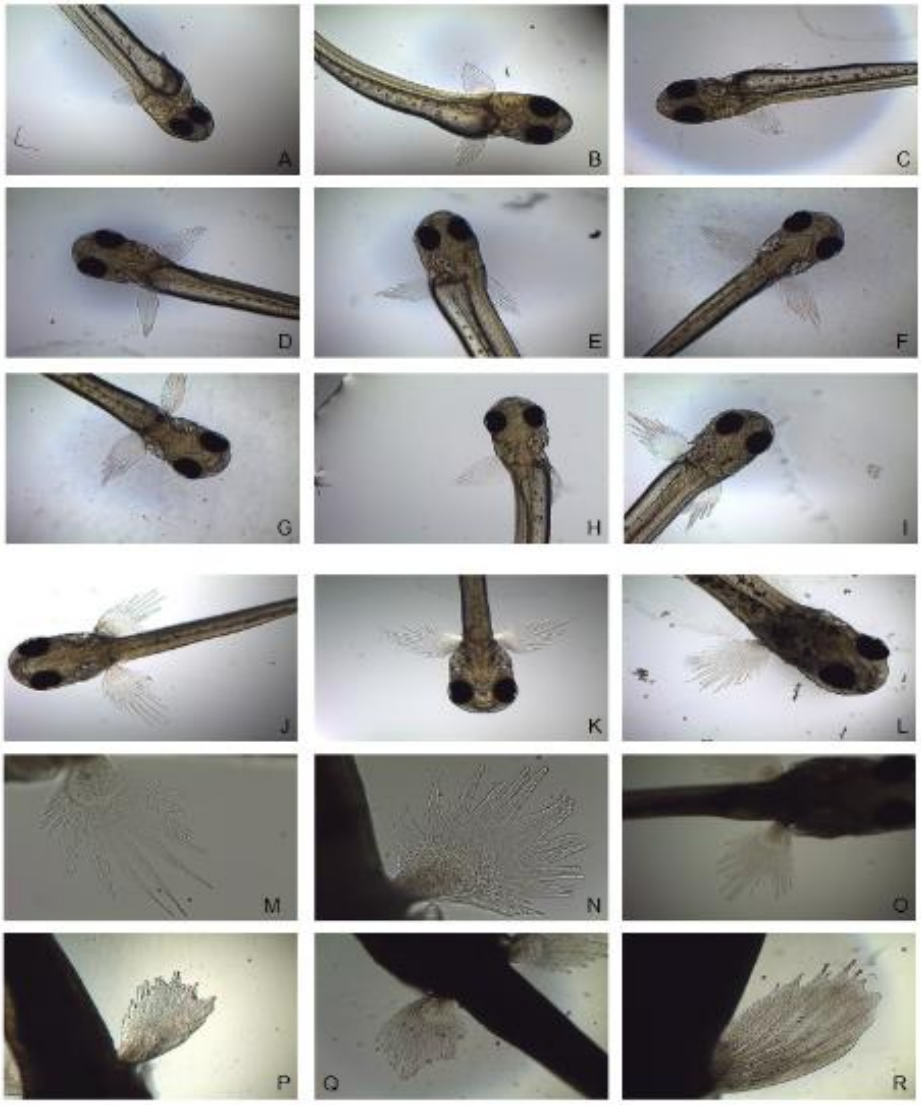

Fig8. *Morphological changes of pectoral fin*

A.0 h after hatching, B : 6.5 h after hatching, C: 13 h after hatching, D: 19.5 h after hatching, E: 26 h after hatching, F: 32.5 h after hatching, G: 39 h after hatching, H: 45.5 h after hatching, I: 52 h after hatching, J: 58.5 h after hatching, K: 65 h after hatching, L : 72 h after hatching, M : 15 d after hatching, N : 19 d after hatching, O : 23 d after hatching, P : 27 d after hatching, Q : 30 d after hatching, R : 33 d after hatching

4. DISCUSSION

4.1. Morphological Changes of Yolk Sac

The yolk sac that has just hatched is round in front and cylindrical in back. In still water, larvae lie on the side at the bottom of the water or move by swinging their tails in a large extent. At this time, the energy consumed by the movement comes from the yolk sac, which is called endogenous nutrition period. 20-27 hours after hatching, endogenous nutrition gradually transforms into exogenous nutrition, and the front of yolk sac becomes smaller and cylindrical, and the larvae move in the middle and upper layers of water. During this period, there is also a mixed nutrition period with simultaneous existence of both endogenous and exogenous nutrition stages, and the time is 28-75 hours after hatching. The mixed nutrition period is an evolutionary mechanism to provide sufficient energy for the larvae before they can find the suitable food. If the larvae reach the initial feeding stage, but received no exogenous nutrition, its will enter the starvation stage [6]. During this period, a large number of larvae die. The lack of food organisms in nature is one of the reasons for the high mortality of fish in the early development stage [7]. In the artificial feeding experiment of *Clupea pallasi* that the highest mortality rate of early larvae is due to low feed density [8]. The survival rate of larvae in the later stage mainly depends on whether the feed supply is timely and whether the larvae adapt to its utilization [9]. Through the study

on the feeding behavior of larvae, it has been speculated that the distribution of plankton is affected by the Langmuir circulation, resulting in the change of bait, which may be the reason for the decrease of fish catch of anchovy in southern Peru [10]. Therefore, the key factor determining the survival rate of larval stage is to provide suitable and sufficient food at this time. The yolk sac disappears completely, the larvae open their mouth and begin to feed, and the development of organs gradually improve, and they live in the bottom of the water body, and only when they need to feed, they start to move in the upper and middle layers of the water body. (Table 3)

Table3. *Yolk sac disappearance time and individual size of different larvae*

Type	Water temperature (℃)	Hatching days	Total length (mm)	Yolk sac	Data source
Abbottina rivularis	19～23	1	3.32	Disappear	This study
Carassius auratus	17～19	8	6.8	Disappear	*Yangtze River fish*, 1976
Parabramis pekinensis	21.4～24.4	8	6.9	Disappear	*Yangtze River fish*, 1976
Xenocypris davidi Bleeker	20	5	7.0	Disappear	*Yangtze River fish*, 1976
Ophiocephalus argus	22～26.5	5	7.4～7.5	Disappear	*Yangtze River fish*, 1976

4.2. Relationship Between Sensory Hair and Body Length and Swim Bladder

After the fish is hatching out, on both sides of the body, the number of sensory hairs from the base of pectoral fin to the base of caudal fin increases with the increase of body length. For most individuals, when the number of sensory hairs reaches 33-35, sensory hairs stop increasing; after a period of time, the number of sensory hairs remains unchanged until the appearance of the swim bladder, and when the swim bladder is inflated, the number of sensory hairs decreases gradually with the increase of full length, and disappear after 70-75 days.

4.3. Fin Development

Fin is the main organ for fish to adapt to water movement and maintain body balance. In the postembryonic stage. The developmental order of each fin is as follows: caudal fin > pectoral fin > dorsal fin > abdominal fin > anal fin. The newly hatched larvae lie on their side on the bottom of the water and have little movement ability, because the fins have not yet formed, but they can move for a short distance with the help of the swing of caudal peduncle and caudal fin. In the observation of postembryonic development of *Pagrus major* [11], *Channa argus* [12] and *Oplegnathus fasciatus* [13], it is found that the fin membranes of pectoral fin and caudal fin of 2-day-old (or 3-day-old) larvae had certain movement ability, which can promote and maintain body balance. The transformation from endogenous nutrition to exogenous nutrition is a major turning point in the early life of fish, and the development of pectoral fin provides morphological guarantee for them. The caudal fin has the function of promoting the fish's movement and changing its direction, and it is the first to segment and differentiate into soft ray; the development of the soft ray of the abdominal fin is relatively late (15 days old), and its function is to help the fish maintain balance in the water. The development of the soft ray of dorsal fin is similar to the anal fin in time and shape. Dorsal fin and anal fin can help fish keep balance and improve their swimming and feeding abilities.

5. CONCLUSION

At 19-23 ℃, the hatching time of fertilized eggs of *Abbottina rivularis* is only 124h; at 22-23 ℃, the yolk sac disappears in 24-27 hours; before and after the fry is hatched out, sensory mounds and sensory hairs can be seen in the head and side of the body, and they gradually disappear. At the beginning of hatching out, the pectoral fin appears as lobular, and then the fin becomes longer and thicker, showing palmate shape, and then the fin is shortened, and then the fin membrane is formed and connected into a whole, and the shape is consistent with the shape of adult fish's pectoral fin.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors."

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