Early development of the Amur sleeper (*Perccottus glenii*, Dybowski, 1877): a remarkable invasive species in Eurasia

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Received: March 2012 Accepted: May 2012

Abstract

To investigate the ontogeny of *Perccottus glenii*, embryonic, larval and juvenile development of *P. glenii* were examined under captive condition. The fertilized eggs with numerous oil droplets were orange-pink in color, prolate spherical in shape and had average length of 3.32±0.14 mm and width of 1.24±0.04 mm. From fertilization to hatching, the ontogenesis of the fish spent more than 200 h, and the process of embryonic development was divided into 25 stages based on the morphological characteristics. The newly hatched larvae, with well-developed swim bladder and pectoral fins, measured 5.07±0.18mm in total length. Initial feeding occurred at day 2 after hatching (AH) and the complete absorption of yolk sphere was observed 3 days after hatching. At day 40 AH, scales and vertical pigment were found to be appear. Scales covered the entire body and all fins were well developed 70 days AH, at which time the juveniles reached the young stage with a total length (TL) of 24.74±4.28 mm. At day 140 AH, The fry with a TL of 29.57±4.65 mm, were morphologically similar to the adults except for size.

Keywords: *Perccottus glenii*, Early development, Invasive species

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[DOR: 20.1001.1.15622916.2012.11.3.12.2]

Introduction

A freshwater fish Amur sleeper, *Perccottus glenii*, is natively found in Russian Far East, north-eastern China and northern North Korea (Mori, 1936; Nikolsky, 1956; Zhu, 1995). The fish can tolerate extreme environment like low temperature and poorly oxygenated water (Terlecki and Pałka, 1999; Reshetnikov, 2003), and has a broad diet spectrum ((Koščo et al., 2008; Grabowska et al., 2009). In case of introduction, the rapid increase of the fish abundance was observed soon (Reshetnikov, 2001; Koščo et al., 2003). In 1912, the fish was first introduced into the European part of Russia. From then on, it was gradually discovered in other European countries (Jurajda et al., 2006; Reshetnikov, 2009; Ćaleta et al., 2011; Covaciu-Marcov et al., 2011). According to Reshetnikov (2009), *P. glenii* could be found outside its native range in 48 regions of the Russian Federation as well as in Latvia, Lithuania, Estonia, Belorussia, Ukraine, Moldova and Kazakhistan. Obviously, *P. glenii* is one of the most widespread alien freshwater fish in Eurasia (Reshetnikov and Ficetola, 2011). Studies revealed that *P. glenii* can depress populations of the native aquatic fauna, and has a negative effect on the native fishes, amphibians, macroinvertebrates and so on (Reshetnikov, 2003; Koščo et al., 2003; Reshetnikov, 2008). In addition, several parasites were also introduced into Europe accompanying the expansion of this species (Košuthová et al., 2004; Nikolic et al., 2007). In spite of all this information, detailed information on the ontogeny of the fish is still scattered and incomplete, except for a brief description of some stages of embryonic and larval development (Kryzhanovskii et al., 1951; Voskoboinikova and Pavlov, 2006).

Materials and methods

The sampling was carried out in Suiling Country (47°14′N, 127°07′E) on May 2010, Heilongjiang Province, northeast China (Fig.1).

Investigation of the ontogenetic development of *P. glenii* may reveal additional systematic characters and shed a new light on the still unsolved problem of the origin of this peculiar fish group (Voskoboinikova and Pavlov, 2006). The aim of this study is to present the full developmental sequence of *P. glenii* from egg to juvenile and to discuss some factors contributing to success invasion of this species.

A total of 48 mature females (18.23±2.16 cm，TL) and 30 males (24.37±3.04 cm，TL) of *P. glenii* were captured by fishing cages and transported to a hatchery located at Huanren Country (41°24′N，125°16′E), Liaoning Province, northeast China (Fig.1). The broodstock were acclimated in 2 cement tanks (3.1×2.5×0.4 m) for 7 days at water temperature of 17-21°C, dissolved oxygen level of 5.60-6.40 mg/L, pH of 8.0-8.6 and

[DOR: 20.1001.1.15622916.2012.11.3.12.2]

ambient photoperiod (12L:12D). The broodstock were fed live loach, *Misgurnus anguillicaudatus* (Cantor), and topmouth gudgeon, *Pseudorasbora parva* (Temminck and Schlegel) (Zaloznykh, 1982), once a day at 17:00. Water supply was well-aerated groundwater and exchanged 30% every day. After acclimatization, each cement tank was provided with red bricks (24×11.5×5.3cm) as spawning substrate.

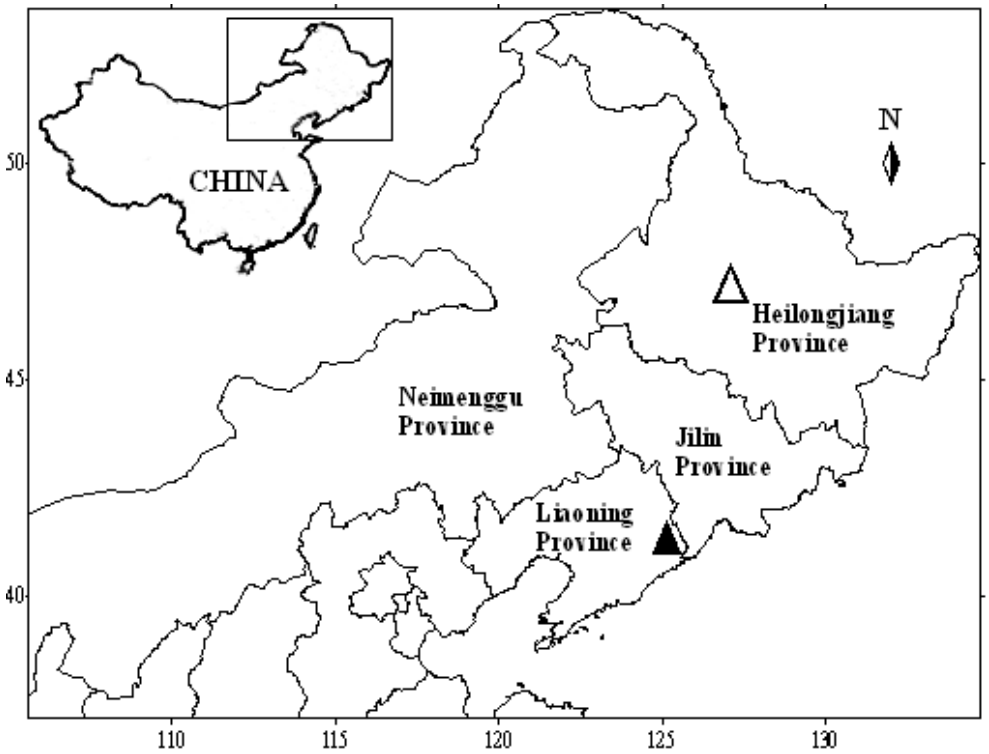

Figure1: Map showing sampling site (△) and hatchery location (▲)

The eggs together with the spawning substrate were removed immediately after spawning and transferred into another cement tank without broodstock for incubation. A total of about 200 larvae were reared in a plastic case (50×35×40cm), and fed zooplankton, insect larvae and tubificidae (Sinelnikov, 1976) at 8:00 once a day. Tilapia larvae with a TL of 10.12±0.12 mm (mean ± SD) (n=13) were fed 30 days after hatching (AH). Eggs and the larvae were maintained under conditions given above. A total number of 30 eggs were sampled randomly by scraping with a scalpel at each stage. Embryonic development was observed every 15 min until the gastrula stage and then at an hourly intervals. Time was recorded when 60% of the embryos were developed to attain cleavage, blastula, gastrula, organogenesis, embryo, pre-hatching and larval stage clearly. Samples of 20 larvae were taken from the plastic case at 12 h intervals during the first 2 days AH, at 24 hour intervals from day 3 to day 5, and every 5 or 10 days thereafter until the end of rearing. Live eggs and larvae were placed into petri dish and observed under a stereomicroscope (Olympus). The main morphological and functional features of each development

[DOR: 20.1001.1.15622916.2012.11.3.12.2]

stage were photographed by a digital camera (Olympus FE-320). In addition, the eggs, egg yolks and larvae were also measured using a stage micrometer (minimum scale: 0.01mm) and an image analysis system (Adobe Photoshop 7.0). The development stage was described as the previously studies (Kimmel, et al., 1995; Iwamatsu, 2004), and approximately 2400 images were recorded and analyzed in this experiment.

Results

Egg development and hatching

The fish spawned from 5 to 9 am, peak in the morning hours. The female laid a clutch of eggs on the red brick, and the male guarded them until they hatched. The newly laid eggs, with orange-pink in color and a bundle of attaching filaments, were spherical, demersal and had length of 1.41±0.02 mm and a width of 1.14±0.04mm (n=16). Under the microscope perivitelline space and oil droplets were very easy to be recognized (Fig. 2A). After spawning, eggs were held together in clumps by a tuft of long attaching filaments on the surface of each egg.

The morphological changes during development were shown in Fig.2. Immediately after fertilization, the eggs absorbed water and acquired a prolate spherical shape with an average 3.32±0.14 mm in length and 1.24±0.04 mm in width (n=18). The lens-shaped blastodisc appeared at 6 min after fertilization (AF) (Fig. 2B). At 32 minutes AF, the cleavage furrow arose near the animal pole and divided the blastodisc into tow blastomeres of equal size (Fig.2C). The second cleavage was meridional at the right angle to the tow blastomeres of the first cleavage and gave rise to four blastomeres of equal size (Fig.2D).The third cleavage was similar to that in the first cleavage and resulted in eight blastometes (Fig.2E). A 16-cell stage was observed at 2:35 h AF (Fig.2F). The egg took 2:57 h to reach 32-cell stage, 3:28 h to reach 64-cell stage and 3:59 h to reach 128-cell stage (Fig.2G, H,I). As successive cleavages occurred, it was difficult to precisely trace the other symmetrical cleavages. Morula, high blastula and flat blastula developed in that order at 5:20 h, 7:39 h and 8:35 h, respectively (Fig.2J, K, L). Epiboly began 14:55 h AF, forming a cap-like structure when the blastomeres covered about 25% of the yolk sphere (Fig.2M). Mid-gastrula stage was observed at 17:52 h AF when the blastomers covered about 50% of the yolk sphere (Fig.2N). At 20:44 h AF, the blastomeres covered 90% of the yolk sphere and a yolk-plug was formed (Fig.2O).

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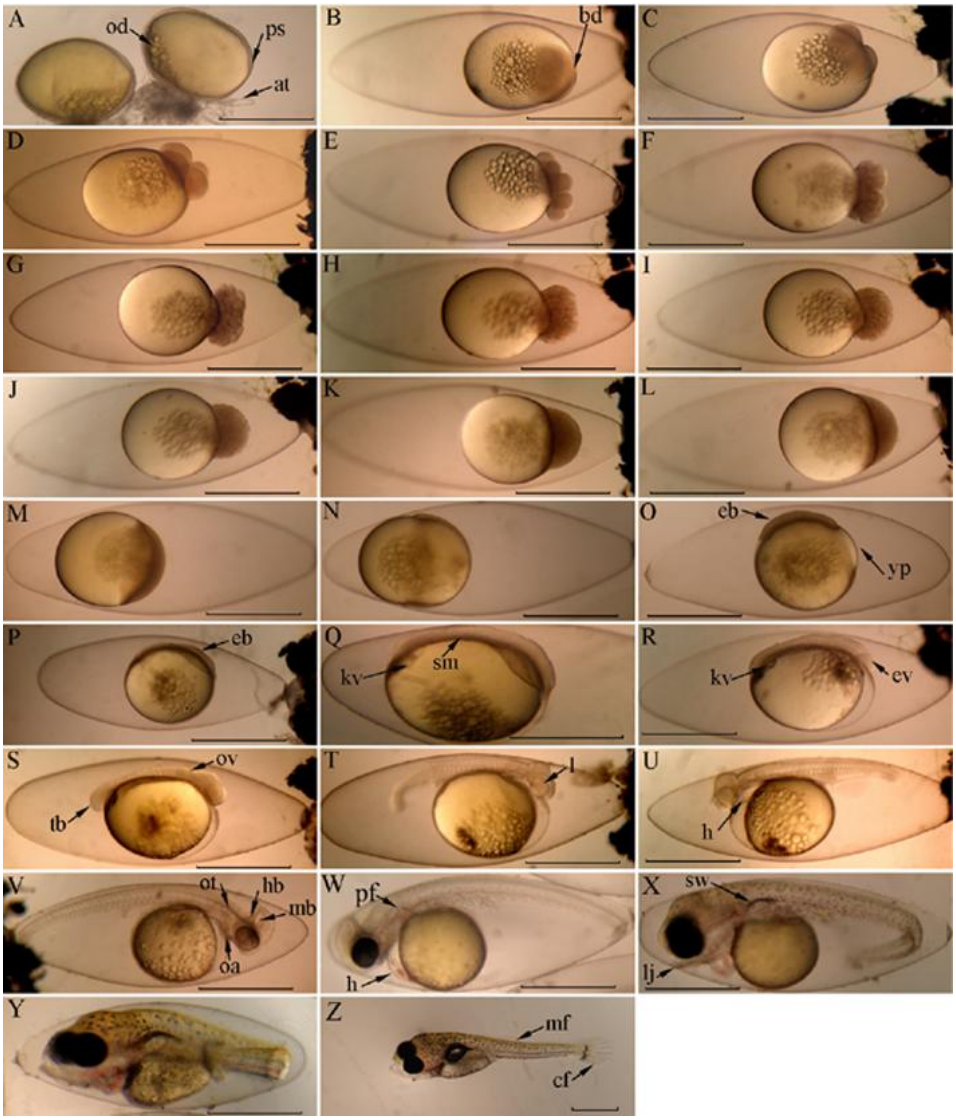

Figure2: Embryonic development of *P. glenii* at a temperature of 17-21 ºC

attaching filament (at), blastodisc (bd), caudal fin (cf), embryonic body (eb), eye vesicle(ev), heart(h), hind-brain (hb), Kuffer's vesicle(kv), lens(l), lower jaw(lj), mid-brain (mb), membranous fin(mf), operculum anlage(oa), oil droplet(od), otoish (ot), otic vesicle(ov), pectoral fin(pf), perivitelline space(ps), swim bladder(sb), tail bud (tb), yolk-plug (yp). Scale bars indicate 1 mm.

Embryonic body was recognized at 22:07 h AF. During this stage, the blastomeres nearly covered the whole yolk and the head rudiment formed. In addition, a cell mass in the front of the head rudiment exhibited a beak-like structure (Fig.2P). Following completion of epiboly, embryo reached the 4-somite stage at 25:20 h AF and Kupffer's vesicles were noticeable (Fig.2Q). A pair of eye vesicle appeared at 27:17 h AF, and the embryo was in the 6-somite stage (Fig.2R). At 33:50 h AF, tail bud started to separate

[DOR: 20.1001.1.15622916.2012.11.3.12.2]

from the yolk sphere and otic vesicles formed (Fig.2S). Embryo exhibited active movement at 41:45 h AF with the expansion of head and the formation of lenses (Fig.2T), followed by the tube-shaped heart appearance on the yolk sphere and behind the head at 48:24 h AF (Fig.2U). At 76:13 h AF, the eye started to develop brown pigment, and some organs, such as otolith, operculum anlage, mid-brain and hind-brain, were easily distinguishable. Meanwhile, the first heartbeat (about 130 per minute) was observed (Fig.2V). Continuously, the pectoral fin, as well as the dark eye, was easily observed at 91:58 h AF when the melanophores began to appear on the anterior part of the head and the ventral part of body, and the heart was divided into tow chambers: an atrium and a ventricle (Fig.2W). At 130:29 h AF, both low jaw and caudal fin were recognized, and the star-shaped melanophores scattered on the whole body. In addition, the swim bladder was recognized above the yolk sphere (Fig.2X). The embryo took 194:59 h to reach the pre-hatching stage, at which time the larva tried to escape from its egg capsule by moving itself frequently. Moreover, the eye, operculum and pectoral fin actively moved and the yolk sphere became oval in shape (Fig.2Y). Hatching occurred 204:06 h AF when the entire body was orange-yellowish in color and the eyes were deeply pigmented. The newly hatched larvae with a mean total length (TL) of 5.07±0.08mm (n=18), had membranous fin fold and the caudal fin rays, and stayed on the bottom without significant movement (Fig. 2Z).

Larval and juvenile development

The morphological changes of larvae and juveniles were illustrated in Fig.3. 12 h AH, the spleen was recognized as a small reddish globule below the swim bladder in the larvae with a TL of 5.13±0.12 mm (n=14). The yolk sphere became cylindrical and occupied about 1/4 of the body length, with pigmentation throughout the head, trunk and yolk sphere. Black melanophores on the tail region were easily observed (Fig.3A).

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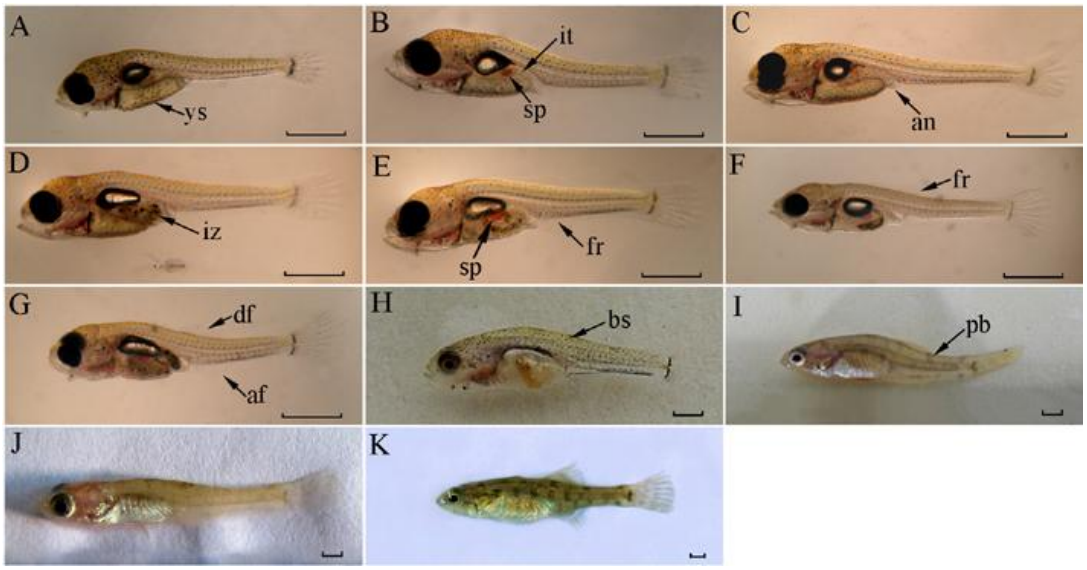

Figure 3: Larval and juvenile development of *P. glenii*

anus(an); anal fin(af); black spots (bs); dorsal fin (df); fin rays (fr); indigested zooplankton (iz); intestine (it); pigment bands (pb); spleen(sp); yolk sphere (ys). Scale bars indicate 1mm.

One day AH, the intestine was straight to slightly curved in anterior portion, and the anus was well developed. The total length (TL) of the larvae increased to 5.36±0.33 mm (n=17), while the length and width of the yolk sphere decreased to 1.04±0.02 and 0.32±0.06 mm (n=17), respectively (Fig.3B).

36 hrs AH some larvae exhibited vigorous movements, and immediately reacted to the external stimulation (Fig. 3C). The larvae were observed to attack rotifer or cladocera 2 day AH, in spite of the presence of a noticeable yolk sphere. The intestine, filled with indigested rotifer or cladocera, started to move. The melanophores started to decrease in number and become less conspicuous on the body (Fig. 3D). At day 3 AH, the yolk sphere was completely absorbed and the dorsal fin began to differentiate. In addition, the rays in anal fin were easily observed (Fig. 3E). When the larva was 4 days old, the dorsal fin rays were well developed accompanying the differentiation of membranous fins, and the spleen became non-distinct (Fig. 3F). Five days AH, the larva exhibited voracious feeding behaviors with an elongated swim bladder. Moreover, the lens started to become somewhat visible under stereomicroscope (Fig.3G). In 15-day-old larvae, the swim bladder became silvery colored and gut content was no longer visible. Saddlebrown pigmentation started to appear on the eye, except for the area of lens. Black spots nearly scattered on the whole body (Fig.3H). Scales could be found on the intermediate area between anus and caudal fin 40 days AH. Vertical pigment bands on the whole body become distinct. During this stage, the larvae with yellow-brown in color, mainly fed on insect larvae and tubificidae

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(Fig.3I). The entire body was covered by scales and all fins were well developed 70 days AH, at which time the larvae were reached the young stage with a TL of 24.74±4.28 mm (n=20) (Fig.3J). At day 140 AH, the characteristic dark bands on both sides were easily observed. The fry with a TL of 29.57±4.65 mm (n=20), slender in shape and brownish in color, were morphologically similar to the adults except for their sizes (Fig.3K).

Discussion

The fertilized eggs of *P. glenii* are orange-pink, prolate spherical and adhesive, which generally agree with Kryzhanovskii et al.(1951). Meanwhile, these features are more or less similar to those of the other Odontobutidae, including *Odontobutis. Potamophila* (Xie and Sun, 1996), *Micropercops swinhonis* (Iwata et al., 2001), *Odontobutis yaluens is* (Wang et al.,2007) and *Odontobutis.obscura*(Zhang and Zheng, 2008). The eggs from Amur basin, as reported by Kryzhanovskii et al. (1951), with a size of 3.8 mm in length and 1.3 mm in width, are slightly larger than those obtained in our experiment. This difference may be attributed to the existence of different strains, distinct broodstock feeding and dissimilarities in broodstock size and age. Previous studies on reproductive strategy of *P. glenii* (Kryzhanovskii et al., 1951; Voskoboinikova and Pavlov, 2006; Grabowska et al., 2011) showed that the laid eggs were guarded by the male. In this study we also found the same event, but the parental care could effectively prevent the laid eggs from being damaged. Therefore, this spawning behavior is important to increase offspring survival in an invasive water body.

From fertilization to hatching, the ontogenesis of *P. glenii* spend more than 200 hrs at 17-21^{o}C. Xie and Sun (1996) reported the incubation time of 501 hrs at a temperature range of 11.6-24.0^{o}C in *O. potamophila*. Zhang and Zheng (2008) found that the embryonic development of *O.obscura* lasted 528 h at 22^{o}C. Regarding *M. swinhonis*(Iwata et al., 2001), the time required for hatching was 12 days at a temperature of 18-23^{o}C. These variations in hatching time suggest that sensitivity of developmental processes to temperature varies even among related fishes (Yamazaki et al., 2003).

The larval developmental sequence described here generally agrees with Voskoboinikova and Pavlov (2006), except that the timing of events we observed was slightly shorter (Table1). These differences are probably due to the incubation temperature since the decrease of the developmental time with higher temperature is known for many fish species (Blaxter, 1969). In addition, some environmental factors (diet composition, alkalinity, pH) not reported in papers could have affected the timing of developmental events. The larvae of *P. glenii* opened mouth at day 2 AH, the similar case was found in *O. potamophila*

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(Günther) by Xie and Sun (1996).Obviously, This feeding behavior should be related with the functioning of some important organs such as caudal fin, pectoral fin, swim bladder, jaw and so on. On the other hand, the behavior of feeding almost immediately after hatching could be useful to increase survival rate in wild. In our observation, the TL of one-day-old larva was 5.36±0.33 mm (n=17), which was smaller than that (6.0±0.3 mm) reported by Voskoboinikova and Pavlov (2006). This difference could be related to the size of eggs. According to Bagarinao and Chua (1986), the eggs showed positively relationship between egg diameter and larval length and weight at hatching.

Table 1: Comparisons on larval development of *P. glenii* between our study and previous work

Events	Time (days after hatching)	
	Voskoboinikova & Pavlov (2006) *	Present study
Appearance of caudal fin rays	0	1
Mouth opening	2	2
Complete resorption of yolk sphere	4	3
Appearance of scales	45	40

*the incubation temperature ranged from 12 to 26 °C.

As in the previous study (Kryzhanovskii et al., 1951), the swim bladder is filled with gas before hatching. Our observation revealed that the inflation of swim bladder appear at the pre-hatching stage, and the fish has no the planktonic stage at early stages of larval development. Similar phenomena were also found in *O. obscura obscura* (Iwata et al., 1988) and *O. potamophila* (Günther) (Xie and Sun, 1996). As described in medaka *O. latipes* by Iwamatsu (2004), the spleen (also called red gland) in *P. glenii* larva, firstly appeared below the swim bladder 12 h AH. Voskoboinikova and Pavlov (2006) noted that the gas filling of swim bladder is associated with the functioning of the spleen. In fact, the spleen appeared after the inflation of swim bladder in our observation. Therefore, we tend to consider the spleen in *P. glenii* larva to be an erythropoietic organ and a storage of erythrocytes like in rainbow trout (Lane, 1979; Randall and Daxboeck, 1982). Considering there is little information available on the development and function of fish spleen (Zapata et al., 2006), more research is needed to well understand its function in *P. glenii* larva, as well as its contribution to tolerate

[DOR: 20.1001.1.15622916.2012.11.3.12.2]

poorly oxygenated water in adult.

Acknowledgements

We are grateful to Dr. Reshetnikov A N (the Russian Academy of Sciences, Russia) for sharing his literatures and critical reading of the manuscript. Thanks are also due to Dr. Grabowska J (University of Lodz, Poland) and Dr. Pavlov D A (Moscow State University, Russia) for bringing relevant literatures. This study was supported by a grant (No.2008203001) from Department of Science & Technology of Liaoning Province, China.

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