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Yolk-sac absorption and point of no return in Chinese sturgeon *Acipenser sinensis* larvae

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Summary

This study aimed at determining the point of no return (PNR) for Chinese sturgeon when in transferring fromyolk absorption to first external feeding of Chinese sturgeon larvae, and their growth in relation to the PNR. During the experimental period temperature range from 22 to 24°C, the yolk-sac volume of the newly hatched larvae was 52.3 mm^3 becoming distinctly reduced during the period from 4 dph (days post hatching) to 7 dph and most critically until 10 dph. Mixed feeding of larvae was initiated 24 h before the yolk-sac was fully exhausted. The initial feeding rate was reached with a maximum of 73.7% of the larvae at 13 dph with a range of 9 to 17 dph. When start-feeding was conducted at 15 dph, the initial feeding rate was as low as 37.2% of the test larvae. Thereafter the rate declined further and the point of no return (PNR) was assumed to have been reached on the 14th day after hatching.

Introduction

In general, most fish species with high fecundity and no parental care experience high mortality during early life history stages. The high mortality is attributed partly to the reproductive strategy (match-mismatch theory) leading to starvation and predation, but also to other stressors such as disease, environmental deterioration and other sublethal factors, reducing the population size at early stages.

The point of no return (PNR), also called ''irreversible starvation'', was first defined for herring larvae by Blaxter and Hempel (1963). It was the threshold point to which larvae can endure starvation at the end of the yolk sac phase without internal food. The PNR describes the tolerance of larvae to starvation, after which the larvae are predestined to die regardless of subsequent food availability.

A number of related research about changes in the morphology of larvae have been observed during starvation. Shelbourne (1957) assessed the status of sea-caught plaice (*Pleuronectes platessa*) larvae in good and poor plankton patches. Blaxter (1971) related the condition factor (dry mass multiplied by negatively third-order squares of the length) of sea-caught herring (*Clupea harengus*) larvae to the condition factor of herring known periods of starvation in the aquarium. Ehrlich et al. (1976) used a more sophisticated measures of such as the condition factor, the pectoral angle (the angle of the ventral surface of the body at the insertion of the pectoral fin) during periods of experimental starvation of herring and plaice larvae. Theilacker and Dorsey (1980) summarized experiments on marine fish larvae and recorded the time to reach the PNR for post-yolk-sac larvae of 12 species. McGurk (1984) reported the time from fertilization to irreversible starvation for Pacific herring larvae reared at different temperatures and compared these periods with those of 25 species of pelagic marine fish larvae. The time to reach the PNR from hatching for herring and plaice larvae has been reported by Blaxter and Ehrlich (1974) and Ehrlich et al. (1976), for herring (*Clupea harengus*) from three races, Clyde, Baltic and North Seas, cod (*Gadus morhus*) and flounder (*Platichthys flesus*) by Yin and Blaxter (1986, 1987), for marbled flounder (*Pseudopleuronectes yokohamae*) by Zhou et al. (1998), for red sea bream (*Pagrosomus major*) and Japanese founder (*Paralichthys olivaceus*) by Bao et al. (1998), for halfbeak (*Hyporhamphus sajori*) by Wan et al. (2003) and for tonguefish (*Cynoglossus semilaevis*) by Zhuang et al. (2005).

Research has so far intensively focussed on controlled propagation, habitat ecology, diseases and nutrition (Kynard et al., 1995; Wei et al., 1998; Zhang et al., 1999; Wei, 2002), and some research has been done on the developmental aspects of *A. sinensis* (Lu et al., 1986; Liu et al., 1999; Chen et al., 2004; Chai et al., 2006), while there is limited information on the development of behaviour such as feeding during early ontogeny. Some studies have focussed on eyesight and correlative behaviour in young and adult specimens of *A. baerii* (Govardovskii et al., 1991), *A. transmontanus* (Sillman et al., 1990; Loew and Sillman, 1993), *A. gueldenstaedtii* and *A. stellatus* (Baburina, 1956), but to date, no studies have considered the age-related changes of yolk-sac absorption, initial feeding rate and mortality during the early developmental stages of the Chinese sturgeons. This is the objection of the pristined study.

Materials and methods

Egg collection

Larval fish were obtained by induced spawning of Chinese sturgeon females in the Yangtze River of Yichang section. Fertilized eggs were incubated at 22 ± 1°C, and hatched larvae were kept in similar tanks during the experimental period within a temperature range of 22–24°C. Larvae hatched on October 5, 2008, and this day was recorded as 0 day post-hatching (dph).

Method of cultivation

The collected Chinese sturgeon larvae were kept in six white plastic buckets (V = 50 L). The larvae were divided into two

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groups, one was the control group (n = 3) and the other one was the food deprivation group (n = 3). Each bucket contained about 400 larvae. The condition of the larvae was checked every day at 08:00, and one third of the water exchanged. New feed was added six times every day, at 04:00, 08:00, 12:00, 16:00, 20:00 and 0:00. The starvation group was not fed. The temperature of the water during the experiment was maintained 22–24°C. Light conditions followed the natural diurnal cycle.

Yolk-sac volume

The yolk-sac volume was calculated from the formula,

$$V = (4/3) \cdot \pi \cdot (R/2) \cdot (r/2)^2$$

R was length-diameter of the yolk-sac.
r was short-diameter of the yolk-sac.

Initial feeding rate

The feeding rate of larvae was obtained by a series of feeding experiments. From the eighth day after hatching, the control groups were fed with fairy shrimp (about 30 ind ml^{-1}). After 2 h, 10 larvae in each bucket were randomly taken out and anaesthetized (60% alcohol) for immediate microscopic observation of gut contents. The feeding individuals were those with fairy shrimp in there alimentary canals. The feeding rate is defined as the percentage of feeding individual of the observed larvae. The feeding rate was obtained every day from the eighth day after hatching. And then the highest feeding rate was obtained by a series of feeding experiments. At the same time, from the eighth day after hatching, more than 30 larvae were sampled randomly from the starvation groups (10 larvae or so in each bucket, there were three replicates.) every day at 8:00, and transferred into a 400 ml beaker with a water temperature of 22–24°C. The larvae were fed with fairy shrimp (about 30 ind ml^{-1}). After 2h the larvae were taken out and anaesthetized (60% alcohol) for immediate microscopic observation of gut contents. The initial feeding rate is defined as the percentage of feeding individuals of the observed larvae.

Determination of PNR

The PNR was determined by a series of feeding experiments. Initial feeding rate of the larvae in the starvation groups was measured every day, and the highest feeding rate was determined in the control groups. When the first feeding rate in the starvation groups dropped to half of the highest feeding rate, the time of PNR is determined (Blaxter and Hempel, 1963).

Results

Mixed feeding stage

Chinese sturgeon larvae started to feed on exogenous on the eighth day after hatching at a water temperature range range from 22 to 24°C. The yolk-sac was fully absorbed on the 10 dph. The duration of the mixed feeding stage of Chinese sturgeon larvae was a little more than 2 days.

Yolk-sac absorption

Temperature range from 22 to 24, the mean yolk-sac volume of the larvae was about 52.3 mm^3 on the first day after hatching.

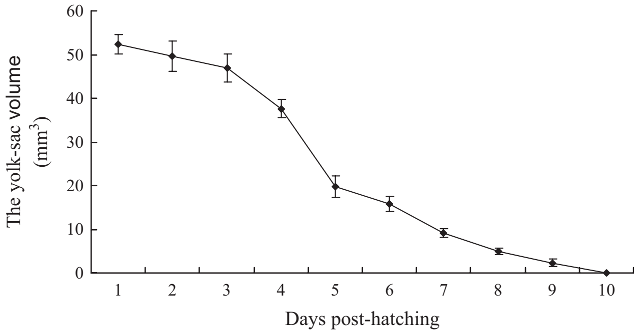

Fig. 1. Changes in yolk-sac volume of Chinese sturgeon larvae. Columns are means; Bars indicate Standard deviation; n = 10

The volume decreased rapidly between 3 and 6 dph and slowed down after that. Finally the yolk-sac disappeared on 10 dph. Figure 1 illustrates the declining tendency of the volume of the yolk-sac of Chinses sturgeon larvae with ontogenetic age.

Initial feeding rate and PNR

For the starvation group, the first feeding experiments were conducted daily from the ninth day through the eighteenth day. The 15th-d.p.h feeding rate, 37.2%, is near to 50% of the highest feeding rate, 73.7%. Then it is concluded that the PNR of Chinese sturgeon larvae is on the 15th day after hatching (Fig. 2). On the 17th dph, the initial feeding rate decreased to 6.7% and most larvae died (the starvation group).

Discussion

Mixed feeding stage and yolk-sac absorption

During the early development stages of fishes, the period during which both endogenous yolk and exogenous food may be utilized is here called the mixed feeding stage. The observation is consistent with studies on other pelagic fish such as Japanese anchovy larvae. Fukuhara (1983) found that Japanese anchovy larvae begin to feed on the 2 dph, and the yolk-sac was absorbed completely on the 3 dph at 23–25°C. Ruan (1984) reported that at a water temperature below 18°C, most of Japanese anchovy yolk-sac was absorbed on the fourth day, and on the sixth day its yolk-sac disappeared completely. The mixed feeding stage lasted for 3–4 d, which is 2–3 d longer than what was observed in 23.0–24.8°C. Imai and Tanaka (1996) reported that the yolk-sac of Japanese anchovy was absorbed completely in 1.9 d (25°C), 2.2 d (22°C), 2.7 d (20°C) and 4.3 d (16°C). Obviously, the higher the water temperature is, the shorter is the duration of the mixed feeding stage.

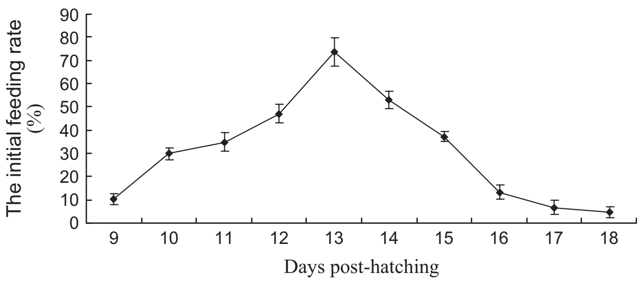

Fig. 2. Changes in initial feeding rates during the period of starvation of early development of Chinese sturgeon larvae. Columns are means; Bars indicate Standard deviation; n = 3

Larvae have to establish their exogenous feeding ability within the mixed feeding stage; otherwise, they will suffer progressive starvation (Blaxter and Hempel, 1963). Extending the mixed feeding stage is beneficial for larvae to accumulate sufficient feeding experience to establish their exogenous feeding ability successfully, to avoid starvation, and thus improve the survival rate. Therefore, the water temperature of the spawning ground is among the key factors affecting the early survival rate of Chinese sturgeon.

PNR and mortality

The yolk-sac of Chinese sturgon larvae disappeared on 10 dph. The PNR is on the 15 dph and the initial feeding rate decreased to 6.7% on the 17th dph, and then most larvae died. This showed that the feeding ability of Chinese sturgeon larvae deprived of food can only be maintained for 7 days or so. It suggests that to survive the larvae should begin exogenous feeding when still endogenous yolk as long as suitable food are available. Therefore, the presence of suitable prey organisms at the right time is of vital importance for the survival, hence recruitment success of Chinese sturgeon. This supports the match-mismatch theory about survival rates of fish larvae. (Cushing, 1990a,b).

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