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Short communication

Development of pit organs and behaviour during early ontogeny of the Chinese sturgeon (*Acipenser sinensis*)

By L. X. Li[1], Y. Chai[1,2,3], Q. W. Wei[1] and C. X. Xie[3]

[1]*Key Laboratory of Freshwater Fish Germplasm Resources and Biotechnology, the Ministry of Agriculture of PRC, Yangtze River Research Institute, Chinese Academy of Fishery Sciences, Jingzhou, China;* [2]*College of Animal Science Yangtze University, Jingzhou, China;* [3]*College of Fisheries Huazhong Agricultural University, Wuhan, China*

Introduction

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; Qi-wei, 2002), and some research has been done on the developmental aspects of *Acipenser sinensis* (Da-chun et al., 1986; Liu et al., 1999; Xi-hua et al., 2004). Some studies have focussed on photoreceptor cells and visual pigments 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). Research has been on the age-related changes of eye morphology and retina structure during the early development stages of the Chinese sturgeons (Yi et al., 2006). Based on the conclusion, we focused on the structure of pit organs in more detail with microscopy and their behaviour response properties to iron powder, pontil and vitreous stick during early ontogeny. These findings will have practical implications as to the importance of morphological and behavioural cues in adjusting culture techniques and for designing habitats for juveniles at artificial habitats prepared for release and enhancement.

The purpose of this study wasto document the development of the pit organ structure and morphology during early ontogeny of this species, and relate the findings to the behaviour of early ontogenetic stages. Changes of quantity and quality in the pit organs are considered is being adaptive to their feeding requirements, and co-inciding with the ecological shift from surface to benthic habitats.

Materials and methods

Larval fish were obtained by induced spawning in the Yangtze River of Yichang section. Fertilized eggs were incubated at 19 ± 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). Larvae were sacrificed once every 12 h after hatching until 2 dph. Sampling schedule, thereafter, was: once a day (3–12 dph); once every 2 days (12–20 dph); once every 5 days (20–45 dph); and thereafter, in irregular and expanding intervals. The total length (TL) of newly hatched larvae averaged 13.3 mm (n = 10) and reached 301.6 mm (n = 10) at the end of the experiment (140 dph). Samples were fixed in Bouin's solution for more than 24 h before processing. The small individuals (shorter than 50 mm) were preserved directly, while bigger larvae were first anaesthetized, the head removed and preserved separately. The fixed samples were dehydrated in graded ethanol and embedded in paraffin. A total of 20 serial sagittal and transverse sections, 6–7 μm thick, were cut from each block, mounted on glass slides (6–9 serial sections per slide), air dried and stained with haematoxylin-eosin (H-E) for histomorphological studies.

The quantity of pit organs was examined on histological sections under the microscope. The histological sections were divided into five equal sections for further histological analysis. In each section, a stretch of 100 μm was randomly selected to determine the quantity of pit organ.

The length and width of pit organs were measured under the light microscopy on five sections of equal length of each of the studied individuals.

Larvae were cultivated in round tanks (diameter = 1.5 m). Response behaviour was observed in vitreous rectangular tanks ($150 \times 80 \times 60$ cm^3, L × W × H). The depth of water was 30 cm. Iron powder, pontil (r = 2 cm, L = 30 cm) and vitreous sticks (r = 2 cm, L = 30 cm) were put into the water, respectively. The collected larvae were kept in 50 L white plastic buckets. Each bucket contained about 400 larvae. The temperature of the water during the experiment was maintained at 22–24°C. Light conditions followed the natural diurnal cycle. Larval behaviour was observed under these conditions.

All authors contributed equally to this work.

Results

Development of pit organs of Chinese sturgeon

From hatching until dph 6, the pit organ was undifferentiated (Fig. 4a), and not all epithelial cells had been formed yet. The slightly embolic epidermis was first observed at dph 7 (Fig. 4b). At 9 dph (Fig. 4c), the epidermis was obviously embolic. The receptor cells, upper layer, and the supporting cells, lower layer, were present. At 17 dph (Fig. 4d), plenty of receptor cells and the supporting cells were observed which stained deeply. The structures of pit organs were completely developed at 30 dph. With further development of the fish, the quantity of pit organs increased significantly (Fig. 4f). Until 40 dph, the cinquefoil-like pit organs were accumulating close to each other in the rostral area and around the eyes, especially in the ventral rostral part (Fig 3).

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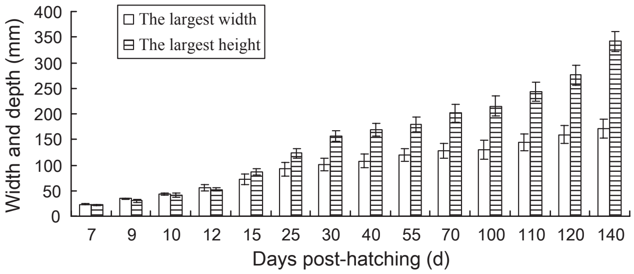

Fig. 1. Changes in width and depth of pit organs of the Chinese sturgeon larvae raised at temperatures of 22–24°C. Columns represent mean values; Bars indicate standard deviation; n = 10 (n = number of samples counted per day); The total length (TL) of newly hatched larvae averaged 13.3 mm (n = 10) and reached 301.6 mm (n = 10) at the end of the experiment (140 dph)

Changes of the length and width of pit organs

With the development of larvae, both height and width of the pit organ increased rapidly. At early stages, from 7 to 12 dph, the largest width was slightly greater than the largest height. In contrast, from 15 dph onward the largest height distinctly increased more rapidly than the largest width. Figure 1 illustrates the increase of the largest width and height between 7 and 140 dph.

Changes of the quantity of pit organs of Chinese sturgeon

Up to 15 dph, the quantity of pit organs in ventral part of the rostral region was approximately the same as that on two sides around the eye pit. The quantity of pit organ in the ventral region of the rostral increased initially fast while the numbers along thei two sides of the rostral and around the eye pit remained largely unchanged until 40 dph. Figure 2 illustrates the developmental tendency of the quantity of pit organs during ontogeny between 7 and 140 dph.

Receptor response

At 9 dph receptor cells are well developed (Fig. 4c), and larvae displayed biting and sucking behaviour to the ferrous plates belonging to the heaters placed on the bottom of the tank. With the development of larvae, it seemed that many should frightening reactions, speeding up and trying to swim away. In contrast, early larvae yet insensible to the exposure were seens to reamina calm, occasionally sucking slightly on or having contact with the vitreous stick while swimming normally.

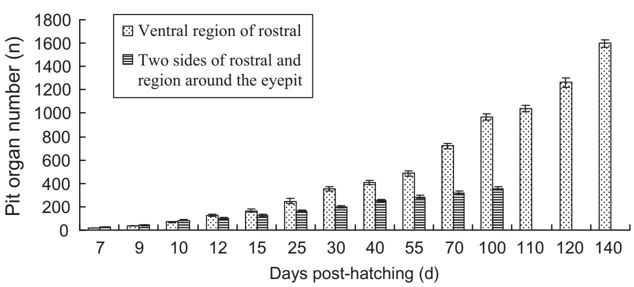

Fig. 2. Changes in quantity of pit organ of Chinese sturgeon raised at temperature of 22–24°C. Columns are means; Bars indicate Standard deviation; n = 10 (n = number of samples counted per day) [The total length (TL) of newly hatched larvae averaged 13.3 mm (n = 10) and reached 301.6 mm (n = 10) at the end of the experiment 140 dph.]

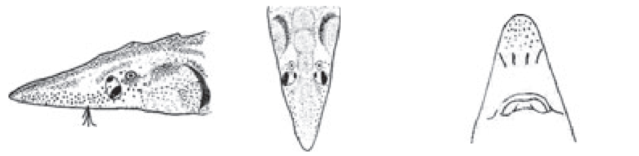

Fig. 3. Anatomical sketch to depict the limits for each of the larvae body regions carrying pit organs

Discussion

Development of pit organs

At the time pit organs were fully developed (Fig. 4), larvae had reached the beginning of exogenous feeding (9–10 dph). Some of the larvae did start to feed on zooplankton. There was a marked quantitative difference between the pit organs in two region, the ventral region of the rostral and along the two sides of the rostral as well as the region around the eye pit. The number of pit organs in the ventral region of the rostral was significantly higher here than along the other regions. It appears that the changes in number of pit organs reflects the adaptation to the ecological shift from pelagic to a benthic life style.

Pit organ development and feeding

Pit organs were distributed in the cluster in venter region of rostral and two sides of rostral and region around the eyepit. The density of pit organs in venter region of the rostral is much higher than in the other regions. Larvae do not complete their development of the visual until 9–10 dph and this is in line with the differentiation of other sensual organs, especially pit organs, and the digestive syestem (Investigation group for fishery resource in Sichuan Province, 1988). Vision appears to be needed during first feeding of zooplankton by responding to feed very near the rostral effectively (Loew and Sillman, 1993). There is no obvious approach response to feed (Yi et al., 2006). There is also a biting and sucking response to weak electric stimulus. The signals being released from organism (Xu-fang, 1996). Chinese sturgeon may have a delicate electro-sensibility to direct feed. This observation is consistent with our describing. The ferrous powder could release weak electric stimulation because larvae showed biting and sucking behavior. The pontil could release stronger electric stimulation than ferrous power, causing an evident response. As soon as other sense organs have completed their differentiation, Chinese sturgeon still kept this feeding behavior finding food through touching rather than pursuing visually, and this might be related to their long rostral.

Characteristics of pit organs in aquaculture production

Usually Chinese sturgeon females spawned in autumn. Optimal water temperature for larvae in controlled cultivation is at 18–22°C. It is very important for larvae to overwinter in captivity before being released in rehabilitation programmes. During this period, water temperature must be raised at least to 18°C. Sometimes water temperature was controlled by metal heaters in small fish container. This method has now been eliminated because larvae would show obvious response electric fields near metal plates, becoming frightened by electro-stimulation released from the metals. The pit organ seems to be involved in this process. The information gained in

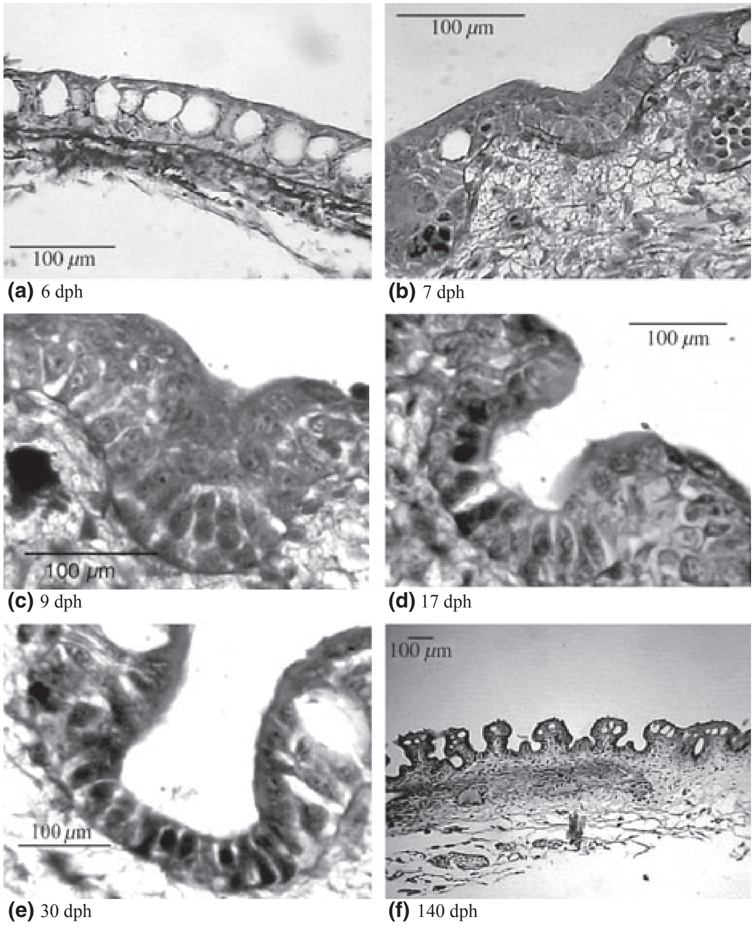

Fig. 4. The morphological features of pit organs of Chinese sturgeon (*Acipenser sinensis*) at different developmental stages (in days post-hatching = dph) reared at 22–24°C: (a) larva at 6 dph, showing the top skin cell layers with no pit organ yet visible; (b) larva at 7 dph = initial indentation to form a pit organ; (c) larva at 9 dph, showing the receptor cells (upper layer) and the supporting cells (lower layer) of a pit organ. The epidermis of pit organ is obviously embolic. (d) larva at 17 dph, showing plenty of receptor cells and supporting cells stained deeply of pit organ; (e) larva at 30 dph, depicting the structures of the completely developed pit organ; (f) larva at 140 dph, showing plenty of developed pit organs in the ventral rostral

this study has helped to improve culture methods for larval and juvenile sturgeons.

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Author's address: Qiwei Wei, Yangtze River Fisheries Research Institute, Chinese Academy of Fisheries Science, No. 41 Jianghan Road, Shashi District, Jingzhou City, Hubei Province 434000, China.
E-mail: weiqw@yfi.ac.cn