The Ontogeny of the Retina of Chinese Sturgeon

(*Acipenser sinensis*)

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Abstract

Chinese sturgeon (*Acipenser sinensis* Gray) is anadromous species. They were declared as endangered species in China. Vision is an important cue in larval survival to capture prey. Thereafter, we examined the development of the retina structure during ontogenesis, the density of single cones, the ganglion cells and the nuclei of the outer nuclear layer of newly hatched larvae, covering different developmental stages ranging from hatching (13.3-13.6 mm TL) to 180 days post-hatching (dph) (290.7-301.6 mm TL). At hatching, larvae exhibited an undifferentiated retina. The retina of 3 dph (16.0-16.9 mm TL) had single cones (SC) with high density. The rods appeared at 9 dph (24.8-25.5 mm TL), and it is assumed that at this time the visual system was developed completely. However, the larvae did not show apparent retinomotor responses until 25 dph (42.7-44.1 mm TL). The density of single cones (SC) and ganglion cells (G) decreased with proceeding development, while the density of the rods (R) gradually increased. The ratio of nuclei of the outer nuclear layer to ganglion cells (ON/G) per 100 µm increased, and so did the ratio of nuclei of the outer nuclear layer to single cones (ON/SC). The investigation showed that the structure of the retina changed rapidly from 9 to 17 dph, and many became fully functional at 25 dph when retinomotor responses occurred. This is the transitional period of the ontogenetic development of the visual system. These changes in the retina of Chinese sturgeon are adaptive to their feeding requirements, and co-inciding with the ecological shift from surface to benthic habitats.

Keywords: Chinese Sturgeon; Ontogeny; Retina; Ontogeny

Introduction

Eyes are among the major sensory organs in fish, detecting photic stimuli, and forming images of the environment. They show a wide range of structural adaptations to the visual environment. In most species, vision is considered as the dominant sensibility stimulus during the larval period. It is essential for feeding, orientation, schooling and avoidance of potential predators (Olla et al., 1995). In Chinese sturgeon, the larvae (0-9 days post-hatching = dph) are living in surface waters before they shift to bottom life. During this interim period, light and vision seems to be crucial. Research has so far intensively focussed on artificial propagation, habitat ecology, diseases and nutrition (Kynard Boyd et al, 1995; Wei Qi-wei, 1998; Zhang Si-ming et al, 1999; Wei Qi-wei, 2002), and some research has been done on the developmental aspects of *A.sinensis* (Lu Da-chun et al, 1986; Liu Ling, 1999; Chen Xi-hua et al, 2004), while there is limited information on the development of specific morphological and functional features such as the retina during early ontogeny. Some studies have focussed on photoreceptor cells and visual pigments in young and adult *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 eye morphology and retina structure during the early developmental stages of Chinese sturgeon.

The purpose of this study was, thereafter, to determine the development of the retina structure and eye morphology during early ontogeny of this species, and relate the findings to the behavior at different developmental stages. These findings will have practical implications as to the importance of morphological and behavioral cues in adjusting culture techniques and for designing habitats for bottom settling juveniles at artificial habitats prepared for release and enhancement.

Material and methods

Larval fish were obtained by induced spawning of female Chinese sturgeon in the Yangtze River of Yichang section. Fertilized eggs were incubated at 19±1℃, and hatched larvae were kept in similar tanks during the experimental period within a temperature range of 19-22℃. Larvae were hatched on October 19th, 2003, and this day was recorded as day 0 post-hatching. Individuals were held separately in light (5 individuals) and in the dark (5 individuals). Adaptation was allowed before processing for 1 hr. Larvae were sacrificed once every 12 hrs after hatching until 2 dph. Sampling schedule, thereafter, was: once a day (3 to 12 dph); once every 2 days (12 to 20 dph); once every 5 days (20 to 45 dph); and thereafter, in irregular and expanding intervals. The total length (TL) of newly hatched larvae that were6 months old averaged 13.3 mm (n=10) and reached 301.6 mm (n=10) at the end of the experiment. Samples were fixed in Bouin's solution for more than 24 hrs before processing. The small individuals (shorter than 50 mm) were preserved directly, while bigger larvae were first anaesthetized, the eyes 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, 6-7μm 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.

Larvae were cultivated in round tanks (1.5 μm in diameter), covered partly by a board, dividing the tanks into light and dark sections. Larval behaviour was observed under these two conditions.

Lens diameter was measured by light microscopy after removed. The quantity of single cones, ganglion cells and nuclei of the outer nuclear layer was examined under the microscope. The whole retina tissue was divided into 5 equal sections for further histological analysis. In each section, a stretch section of 100 μm was randomly selected to determine the quantity of single cones, ganglion cells and nuclei of the outer nuclear layer (including the nuclei of rods).

The minimum separable angle (α) was calculated from the formula,

$$\sin\alpha = (1 / f)\ [0.1\ (1 + 0.25) \times 2 / n^{1/2}],\ f = 2.55\ r$$

Where f was the focal length of lens, r was the radius of the lens and n was the quantity of single cones per 0.01 mm^2 of the retina.

The length of pigment layer (P) and visual cell layer (V) was measured under light microscopy on 5 sections of equal length. The pigment index (PI) was determined by the equation PI = P/V, where P was the farthest length from the retina brink to the pigment, while V was the length from the brink to the outer border membrane.

Results

At hatching, the retina was undifferentiated in larvae, and not all cells had formed yet. Pigments were first observed at 2 dph (14.1-15.2 mm TL), and these distributed as a thin layer. Pigment epithelium cells scarcely distributed, and the structure was irregular while being stained slightly only.

At 3 dph, the visual cell layer and the outer nuclear layer appeared. There were plenty of single cones in the lighted photophase retina. Four layers were formed in the retina at 4 dph: (1) pigment epithelium, (2) the visual cells layer (containing only single cones), (3) the outer nuclear layer (containing only single cones nuclei) and (4) the ganglion cell layer. At 9 dph, each layer of the retina was present, and the order of cell structures was similar to that of common teleost. The noticeable changes in the retina were either the increase or decrease in diameter and density of rods, cones and ganglion cells. On day 30 post-hatching (54.8-60.1 mm TL), all retina structures completely developed.

In the dark-exposed retina, pigment distributed out of the visual cells layer between 2 and 9 dph, and did not relapse to the pigment cell layer. There was no obvious retinomotory response at this stage. In the dark-exposed retina (10 dph, 25.2-26.1 mm TL), pigment showed partly relapse to the pigment cell layer, thus at this stage retinomotory responses were observed. At 19 dph, pigment relapse was complete, concentrating out of the visual cell layer, showing apparent retinomotory responses to changes in light intensively.

High density of single cones were distinguished from 3 dph onward and stained slightly. At 9 dph, plenty of rods were observed which were stained deeply. With the development of fish, the density of single cones decreased while that of rods increased, and finally double cones were absent.

The outer nuclear layer consisted of two types of nuclei of photoreceptor cells, rods and single cones. At 3 dph, the outer nuclear layer formed, only consisting of single cone nuclei, which were ovoid and stained deeply. The outer nuclear layer consisted of rods nuclei correspondingly, ovoid but smaller and stained less deeply. At 30 dph, the outer nuclear layer developed completely, the nuclei of rods were predominant.

From hatching to 7 dph (23.6-24.3 mm), the inner nuclear layer was not stratified, but differentiated into 1-2 layers of horizontal, amacrine and bipolar cells at 8 dph (24.0-25.0 mm TL).

At early stage the layer of ganglion cells was faintly thick, consisting of numerous long and round cells, which were stained slightly. As the quantity of cells per unit area decreased, the layer also became thinner towards day 9 post-hatching, and the ganglion cell layer solely contained a single row of spherical cells.

The quantity of single cones, ganglion cells and nuclei of the outer nuclear layer

With the development of larvae, the density of single cone cells (SC) decreased rapidly before 17 dph (33.6-34.6mm), and slowed down after that. Finally the density reached the lowest and remained stable. On the contrary, the density of nuclei of the outer nuclear layer increased rapidly between 9 and

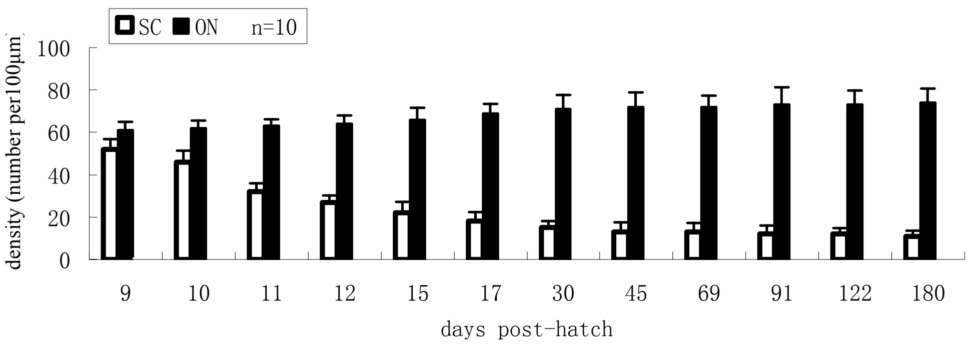


Fig.1 The mean number of single cones (SC) and nuclei of the outer nuclear layer (ON) per 100 µm unit length in the retinal cross section of Chinese sturgeon larvae between 9 and 180 days post-hatching. Bars indicate Standard deviation; n = number of samples counted

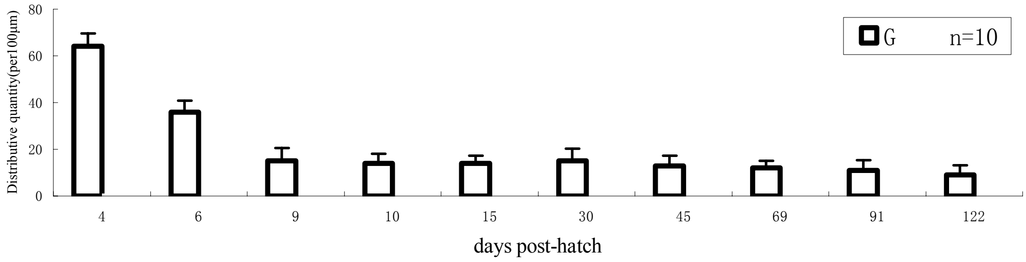


Fig.2 The mean number of ganglion cells (G) per 100 μm unit length in the retinal cross section of Chinese sturgeon larvae between day 4 and 122 post-hatching. Bars = Standard deviation; n = number of samples counted

17 dph. Figure 1 illustrates that single cones decreased and rods and nuclei of the outer nuclear layer increased, and the inclination between 9 and 17 dph was significant. Fig. 2 illustrates the declining tendency of the density ganglion cells with ontogenetic age. And between 4 and 9 with little changes, thereafter.

The ratio of nuclei of the outer layer to single cones and to ganglion cells

Both the ratio of nuclei of the outer nuclear layer to single cones (ON/C) and the ratio of nuclei of outer layers to ganglion cells (ON/G) increased simultaneously with ontogenetic development (Fig.3) and this became particularly obvious from day 9 post hatch where ON/C ratio was initially 1.2, but steadily increased about 91-122 dph. This illustrates that the single cones were still predominant. At 17 dph, ON/C ratio was 3.8. Here we observe that rods were predominant, taking the place of single cones. ON/G ratio represented the degree of retina network converging. However, the changes were statistically insignificant.

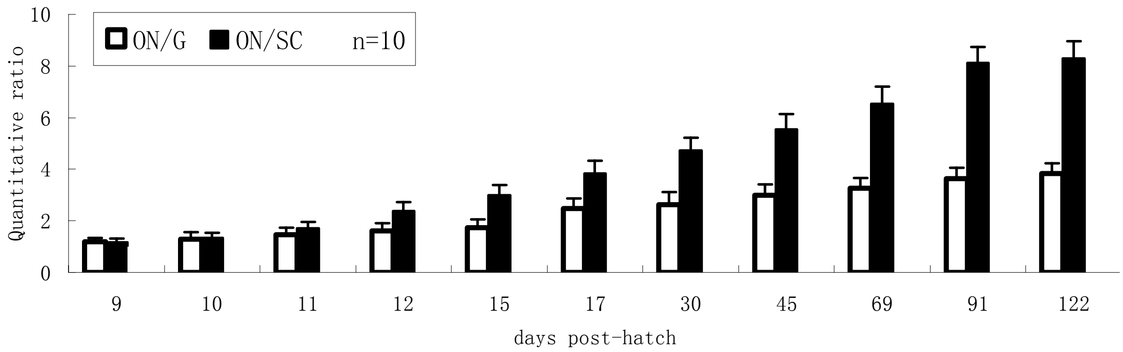
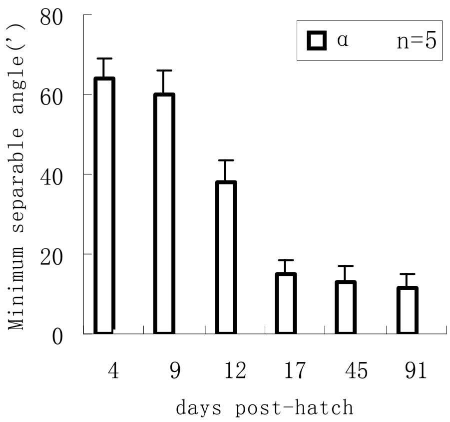


Fig.3 The quantitative ratio of nuclei of the outer nuclear layer (ON) to cones (SC) and to ganglion cells (G) in the retina of Chinese sturgeon based on numbers of cones cells per 100μm counted; Bars indicate Standard deviation; n = number of samples counted

Minimum separable angle (α)

Minimum separable angle is to measure the degree of vision sensitivity of eyes (see formula provided in Material and methods). A small angle indicates the high degree of precision in vision. The degree of visual sensitivity is determined by the diameter of lens and the capability of the retina differentiation. The latter is determined by the density of photoreceptor cells and the degree these cells are cross-connected. Figure 4 illustrates the changes of minimum separable angle at different developmental stages. The angle decreased from 64.2′ (4 dph) to 11.3′ (91 dph), and changed most rapidly between 9 and 17 dph..



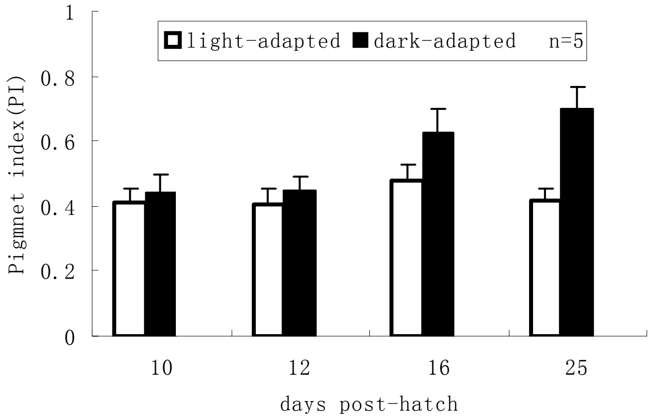


Fig.4 Changes in the minimum separable angles and juveniles (*α*) of the eyes of larvae and juveniles Bars = Standard deviation; n =numbers of observations

Fig.5 Changes in the pigment indices for light and dark adapting retina of larvae Bars = Standard deviation; n = numbers of observations

Retinomotor response

Pigment index (PI) of light-exposed and dark-exposed fish showed slight but non-significant differences before day 12 post hatch. PI was between 0.50 to 0.59 for light exposed fish, and between 0.49 to 0.58 for dark exposed fish. At this stage there were no retinomotor responses observed. At 16 dph, the PI in light and dark exposed fish were 0.63 (0.56-0.70) and 0.48 (0.40-0.54), respectively. These changes illustrated obvious movement of pigment granular cells. At 25 dph, the PI showed rapid changes, with 0.71 (0.61-0.78) in light and 0.44 (0.39-0.47) in dark exposed fish.

Discussion

Development of photoreceptor cells

At hatching, the retina of *A.sinensis* larvae lacks well-differentiated photoreceptors. The observation is consistent with studies on other sturgeon species, such as *A. gueldenstaedtii, Huso huso, A.stellatus, A. ruthenus* (Dettlaff et al., 1993), *A. naccarii* (Boglione et al., 1997) as well as some teleost species (Wahl and Mills, 1993). Single cones differentiated at 3 dph, and rods at 9 dph. When rods developed, larvae were at the beginning of exogenous feeding (9-10 dph). Some of the larvae did start to feed on zooplankton. Besides single cones, several larvae also had some rods. Several studies found that many fish possessed only cones at the onset of exogenous feeding (Wei Kai-jian and Zhang Hai-ming, 1996; Wei Kai-jian et al., 1997), except *Anguilla japonica* and other marine species (Blaxter, 1970) which had only rods. Siberian sturgeon (*A. baerii*) larvae possessed cones and rods already before exogenous feeding, and the retina completely differentiated (A. Rodriguez and E. Gisbert, 2002). While two types of photoreceptor cells (rods and single cones) were observed when feeding was started. In contrast with all three types described above, the retina of *A. sinensis* appeared to be different at the onset of feeding. Similar to Agnatha, Elasmobranchii and Coelacanthiformes (Wilhem, 1975), the retinas of the Chinese sturgeon larvae were relatively simple, containing only two morphologically distinct photoreceptors, rods and single cones, while double cones were absent. Retinomotor responses accompanied the submerging of rods (Blaxter, 1986), which protect rods at high light intensity and raise their capacity to sense light at dim light. It appears that the changes in number of rods and single cones of Chinese sturgeon adapts to the ecological shift from pelagic to benthic life style.

Phototactic behavior at different developmental stages

Between 0 and 9 dph, larvae were mainly observed at the surface or freely swimming in the upper layer of the water column. Behavioral studies during this period have been described *A. sinensis* larvae to be positively phototactic, showing preference for white substrates rather than grayish or black backgrounds (Zhuang Ping, 1999). At this stage, photoreceptor cells were comprised of high-density single cones, which easily sensed light stimuli. Thereafter, larvae turned to benthic feeding (9-10 dph), and rods differentiated correspondingly to enhance vision at lower light intensities. Such photoreceptor cells might allow larvae to discriminate between a potential predator and water shadows, thus reduce their vulnerability to predation while enabling larvae to select suitable habitats.

Vision development and feeding

Good vision determines the ability to feed, search, distinguish objects and orient in a three dimensional light environment (Li Da-yong et al., 1994). Chinese sturgeon larvae do not complete their development of the visual organ until on 9-10 dph and this is in line to the differentiation of other sensual organs and the digestive system (Investigation group for fishery resource in Sichuan Province, 1988). Although *A. sinensis* larvae depend on good vision in their initial feeding ability, the vision was relatively poor. The larvae initially feed on zooplankton but never pursued feed directly. Still, vision appears to be partially useful during first feeding to capture zooplankton effectively. (Loew and Sillman, 1993). As soon as other sense organs have completed their differentiation, Chinese sturgeon still kept this feeding strategy, finding food through touching rather than pursuing via sight, and this feeding strategy might be related with their long rostral and mouth structure. The changes observed in the decrease of the separable angle indicated that the degree of vision sensitivity was very important at the first feeding period.

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Fig.6 The development of retina of Chinese sturgeon (*Acipenser sinensis*)

6.1 High density of single cones in light-adapted retina of Chinese sturgeon (*A. sinensis*) at 3 dph (H-E stained). Scale bar μm. 6.2 Each layer structures in light-adapted retina of Chinese sturgeon (*A. sinensis*) at 6 dph (H-E stained). Scale bar μm. 6.3 Each layer structures in light-adapted retina of Chinese sturgeon (*A. sinensis*) at 9 dph (H-E stained). Scale bar μm. 6.4 Ten layer structures in dark-adapted retina of Chinese sturgeon (*A. sinensis*) at 12 dph (H-E stained). Scale bar μm. 6.5 Pigment in out-side of cone cell layer in dark-adapted retina at 12 dph (H-E stained). Scale bar μm. 6.6 Pigment in cone cell layer in dark-adapted retina of Chinese sturgeon (*A. sinensis*) at 25 dph (H-E stained). Scale bar μm. 6.7 Retina structure of light-adapted of Chinese sturgeon (*A. sinensis*) at 3 month old. (H-E stained). Scale bar μm.

6.8 Retina structure of dark-adapted of Chinese sturgeon (*A. sinensis*) at 3 month old. (H-E stained). Scale bar μm

C: cone cell; G: (ganglion cell; IN: internal nuclear layer; ON: outer nuclear layer; P: pigment