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Research Article

Postembryonic Ontogeny of Lake Sturgeon Acipenser Fulvescens with Quantitative Data of Forebrain Growth

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Abstract

External morphological changes along lake sturgeon postembryonic development from hatching to year-0 juvenile were described. We also provided data for the growth of the olfactory bulb and telencephalon during the period based on micrographs of confocal imaging. By the time of hatching, yolk-sac larvae were approximately 1 cm in total length with many features corresponding to the pharyngula period of zebrafish embryos. Larval Stage started with the beginning of exogenous feeding when fish snout was flattened and specialized into triangular-shaped rostrum. By then, the larvae were approximately 2 cm in in total length and the forebrain was differentiated into the telencephalon and diencephalon. Sturgeon larvae transformed their eel-like body into spindle-shaped trunk armored with five rows of scutes at late Larval Stage. Juvenile Stage, when fish were over 4 cm in total length, began with the complete transformation of the ray-fin tail and disappearance of fin folds. At 7-8 cm in total length, pigment distributions resembled those in adults. In early Juvenile Stage, the olfactory bulb reduced it's widthwise expansion pace while maintained longitudinal growth. Anatomical features of the olfactory bulb stabilized in fish larger than 5.5 cm. Quantitative data indicated steady longitudinal growth of the telencephalon during the period examined while an increase in width slowed down after fish reached 5.5-6.5 cm. The data suggest that the forebrain development is most active in fish smaller than 5.5 cm. This time period included two vulnerable phases in which fish transformed themselves into new developmental stages. Our study has provided valuable information for future research and fisheries of lake sturgeon.

INTRODUCTION

Sturgeons are a group of primitive actinopterygian or ray-finned fish species. Their phylogenetic history can be dated back to 200 million years ago of the early Jurassic period [1,2]. They represent an early stage in evolution and are close to the bifurcation of the tetrapod lineage. Their exceptionally slow pace in evolution and unique phylogenetic position has received considerable attention for scientific research.

Among over 27 sturgeon species around the world, lake sturgeon (Acipenser fulvescens) is a typical North American temperate freshwater species. Lake sturgeon inhabits large river and lake systems primarily in the Mississippi River, Hudson Bay and Great Lakes basins [3]. It represents an important biological component of fishes in the Great Lakes. Once flourished in the Great Lakes, lake sturgeon had high economic and recreational values. From 1879 to 1900, the commercial catch of lake sturgeon in the Great Lakes averaged over 4 million pounds per year [4]. However, due to overfishing, habitat loss, dam constructions, and pollution, lake sturgeon populations have been greatly reduced or extirpated since 1900. In the 1990's, lake sturgeon populations were estimated at 1% of historical levels [5]. Today, few healthy populations remain [3,6]. Rehabilitation of self-sustaining populations of lake sturgeon in the Great Lakes has been a top priority for fishery communities throughout the Great Lakes.

In this report, we describe our observations of postembryonic development of lake sturgeon to provide information for future scientific research and fisheries. Following the development of lake sturgeon, we describe changes of exterior morphology of lake sturgeon from hatching to year-0 juveniles and provide data for the growth of the forebrain. Changes of the exterior morphology were correlated with the developmental time lines and the information is more applicable to fishery activity. A

detailed developmental process of the central nervous system will be reported elsewhere.

MATERIALS AND METHODS

Specimens and morphological observations under stereoscopic microscopes

Lake sturgeon used in the study originated from Rainy River, Ontario, Canada. Eggs and milts were collected from the mating pairs of lake sturgeon. The eggs was fertilized on site and transported to the Genoa National Fish Hatchery, Wisconsin, USA for rearing. The specimens were randomly collected from a large pool of fish and fixed by 4% paraformaldehyde in phosphate buffered saline (PBS, pH = 7.4). After an overnight fixation, the specimens were washed three times in PBS and then stored in the PBS containing 0.02% NaN3 at 4°C. Fish total body length described here was measured under this condition.

The specimens were inspected under stereoscopic microscopes. The bright-field images were photographed with a Nikon Digital Sight DS-Fi1 high-definition color camera (Nikon Co., Tokyo, Japan) and processed using the Adobe Photoshop software.

Whole mount immunohistochemistry

We measured growth of the olfactory bulb and the telencephalon using confocal micrographs obtained from specimens processed with whole mount immunohistochemistry [7]. This process increased visibility of the brain under confocal microscopes. For immunohistochemistry, the specimens originally stored in PBS containing 0.02% NaN3 were fixed again in 4% paraformaldehyde overnight. Before they were incubated in the pre-chilled acetone (-20°C) at room temperature for 30 minutes to increase permeability of the tissue, they were rinsed with PBS three times for 30 minutes each followed by MillQ water for 5 minutes. After acetone treatment, specimens were washed in water and PBS for 5 minutes each, in PBDT (PBS containing 1% DMSO and 0.1% Tween-20) for three times at 10 minutes each, and then incubated in blocking solution (5% normal donkey serum/3% bovine serum albumin/0.3% triton X-100) for 2 hours at room temperature to block non-specific binding. Afterwards, specimens were incubated in the mouse monoclonal anti-SV2 primary antibody (1:4000, the Developmental Studies Hybridoma Bank, IA, USA) for 5 hours at room temperature and then overnight at 4°C on a shaker. The next day, the specimens were washed four times with PBDT for a total of 2 hours before incubation in the secondary antibody Alexa 568 anti-mouse serum (1:500, Life Technologies, NY, USA) in a time schedule the same as the primary antibody incubation. After rinsing off nonspecific binding of the secondary antibody, the specimens were dehydrated by gradual transfer to 100% methanol and then stored in a mixture of benzyl-alcohol and benzyl benzoate (1:3, v/v, BABB) at 4oC for examination by microscopes. BABB is a clearing solution that facilitates structural examinations under microscopes. The SV2 antibody specifically recognizes synaptic vesicle protein 2, the integral membrane glycoproteins of synaptic vesicles, in neurons and is often used as a marker for neuronal processes [8,9].

Confocal imaging and quantitative analysis of the forebrain

Confocal micrographs were acquired from a Zeiss LSM 510 confocal laser-scanning microscope using the LSM 5 Pascal software (Zeiss Inc., NY, USA). The whole mount micrographs were obtained when the specimens were laid inside a custom-made glass chamber filled with BABB. The micrographs of sturgeon head were acquired using a low magnification objective. The length and width of the olfactory bulb and the telencephalon along the fish development were measured using the Adobe Photoshop software by pixel units and then converted to millimeters based on calibration. Figure 6a and 6b show the positions used in measurements. Each data point shown in Figure 6c and 6d were an average from five fish and presented as mean ± SE. One way ANOVA was used for statistical analysis.

RESULTS

Yolk-sac Stage

Lake sturgeon eggs were obtained from mating pairs at Rainy River, Ontario, Canada. After artificial fertilization, lake sturgeon was raised in the Genoa National Fish Hatchery, Wisconsin, USA. Lake sturgeon eggs typically start to hatch in the second week from fertilization when water temperature ranges from 13 to 15 °C. Water temperature has a significant impact for the process of embryo development. Lake sturgeon eggs maintain circular shapes until hatching since they have tough chorions. Laying on the top of yolk ball, embryos circle around the yolk when their bodies grow. When approaching hatching, the trunk of embryo elongates so that the tail tip can reach over the head. This period is equivalent to embryonic Stage 32-35 of Russian sturgeon (Acipenser gueldenstaedti colchicus) and shovelnose sturgeon (Scaphirhynchus platorynchus) according to the descriptions [10,11]. Our observations of lake sturgeon development began at this period [Figure 1a]. In this report, we describe the morphology of lake sturgeon from hatching to 0-year juveniles. We divide this period of growth into three stages: Yolk-sac Stage, Larval Stage and Juvenile Stage. Inspection of morphological changes over the development leads us to conclude that morphological changes along the development are more closely associated with the fish body length than the age, consistent with reports for other fish species [12]. Studies have shown that the time of hatching is not a reliable stage index for development since hatching of siblings in a single developing batch may vary and last for days [11,13]. Therefore, our description of lake sturgeon staging is primarily based on anatomical land markers and the size of fish.

Lake sturgeon embryos at the time close to hatching are 0.8-0.9 cm in total length. Most exterior features of lake sturgeon at this time resemble the developmental progress of the pharyngula period in zebrafish embryos (Danio rerio) [13]. The head remains downward but its anterior portion has moved away from the yolk ball (Figure 1a). The posterior trunk and tail do not attach to the yolk ball as well while the tip of the tail slightly curves downward. In the next few days, the main changes occur in the morphology of the tail and fin folds in addition to increasing in thebody length.

Figure 1a shows an embryo of 0.8 cm in total length. At this time, morphology of the tail and fin folds resembles with Stage

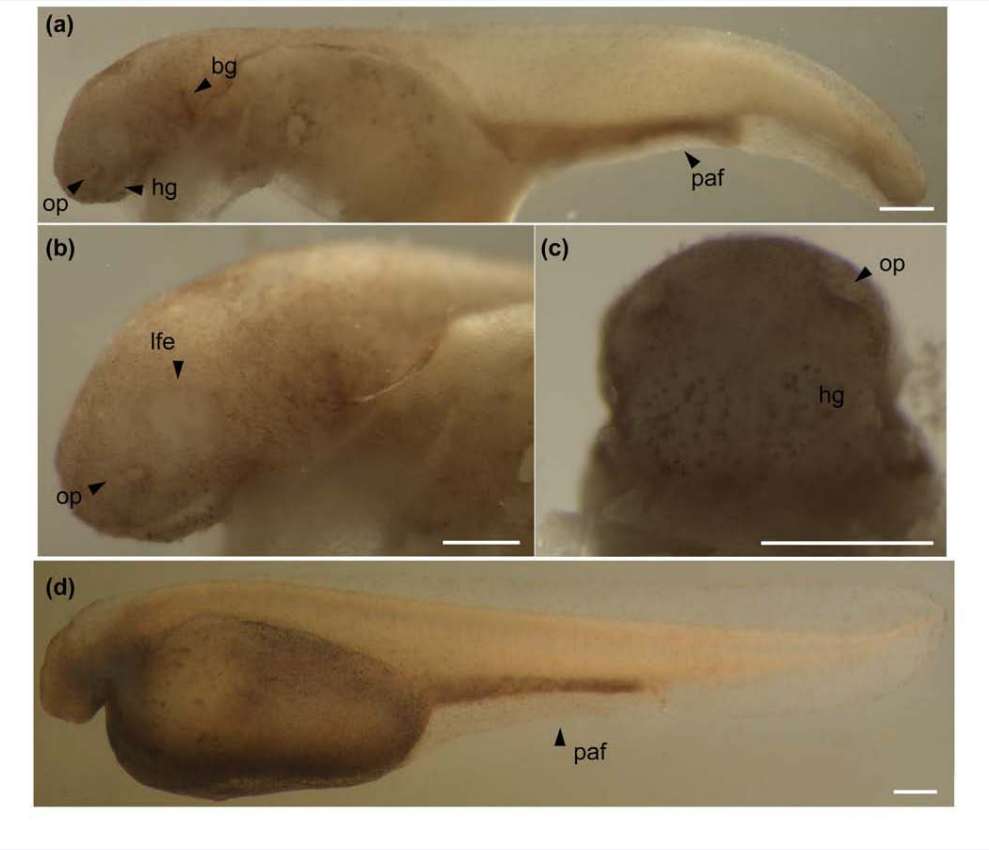

Figure 1 External views of yolk-sac larvae close to hatching. (a) to (c) were obtained from an embryo prior to hatching. (a) is the side view of a larva. The yolk was removed during sample collection. (b) is a larger view of the head region, and (c) is the front view of the head. (d) is the side view of a hatched larva of 1 cm long. bg, branchial grooves; lfe, the location of future eye; hg, hatching gland; op, olfactory pit; paf, preanal fin fold. Bar = 0.5 mm.

32 Russian sturgeon embryos [11]. The fin folds surrounding the trunk are thin lines that extend to the tail. The lower blade of the caudal fin fold is slightly expanded, which makes it easier to spot than the upper blade. The membranous fin folds are opaque, differing from older yolk-sac larvae. Although sparse fine pigments scatter throughout the entire body, there is no aggregation in any particular region of the body. The whole body is pale but the yolk sac and areas for the gut rudiment are more brownish in color than the trunk. Unlike adult lake sturgeon, the head of yolk-sac larvae at this time is round and similar to other fish species [Figure 1]. Olfactory pits are present at the front of the snout. The olfactory pit deepens gradually throughout this stage to form an enlarged sac that opens outside as a round aperture. Pigmentation around the nasal opening is denser than other areas. The olfactory epithelium lining at the bottom of the sacs is pigmented. Posterior to the olfactory pit, there is a barely visible circular shadow, which makes up the location of the eye. At this time, however, eyes are is covered by the epidermis although the retina and lens rudiments are formed. Posterior to the location of the eye is the pharyngeal raphe where pairs of branchial grooves are outlined. Ventral of the head and below the olfactory pits, there are two slight swelling structures that represent hatching glands. Massive hatching starts within next few days.

Newly hatched lake sturgeon reaches approximately 1 cm in length. Sparse pigments are visible around the eye regions. From late phase of embryos to hatching, the most significant external changes are the tail and fin folds: the tail vertebrates straight out from the downward bent (Figure 1a and 1d]; fin folds expand and become more translucent; and the preanal fin fold becomes discernible.

In two more days, fish may reach 1.2-1.3 cm in total length (Figure 2a to 2c). Fish skin and the membranous fin folds become translucent and inner organs of the digestive system are visible. The whole body is more brownish than before. A close inspection reveals that small and brownish melanocytes are sparsely distributed all over the body. Small and oval shaped pectoral fin rudiments are evident. The posterior trunk and entire tail are completely straightened out. Under the translucent skin, there are numerous muscle segments distributed from head to tail. The skin around the nasal opening accumulates into folds. Eye cups appear brown in color. Posterior to the eyes, auditory vesicles are visible through the translucent skin. Translucent barbel rudiments are found at the ventral of the head, anterior to the mouth. Translucent cartilages develop in the upper and lower jaws. Opercular folds and rudiments of gill leaflets emerge. At this phase, the yolk remains large in cylindrical shape. The intestinal rudiment behind the yolk appears dark brown in color. The posterior yolk is absorbed earlier than the anterior in next a few days [14]. This phase resembles Stage 38 prelarvae of Russian sturgeon and the pectoral-fin stages of zebrafish [11,13].

Sturgeon larvae grow to approximately 1.7 cm in total length in the next four days (Figure 3a to 3d). Whole body pigmentation darkens further. Melanocytes spread all over the surface of the body and along the muscle segments. The lower part of the body, below the midline, accumulates more melanocytes than the upper body (Figure 3b). In a few more days, pigmentation becomes stronger and forms a dark band starting from the tip of the snout to the end of tail (Figure 3a). In addition, eye pigmentation darkens and the olfactory pits appear dark brownish since the olfactory epithelium in the nasal sac is heavily pigmented. There are skin folds around the nasal opening. Considerable amount of pigments also accumulate at the caudal fin folds, more at the dorsal lobe than the ventral (pointed by black arrows in Figure 3a). Anterior to the dense pigmentation at the tail fin folds, there are dark spotty pigments aligned along the ventral lobe. The fin membrane broadens further and contains fiber-like textures, except for the one third of the thickness of outer circle. The preanal fin fold has separated from the anal fin fold by a deep groove. Tiny pelvic fin rudiments emerge and the dorsal fin rudiment becomes visible.

In larvae of 1.7 cm in total length, cartilages in the head remain translucent. Thus, the ventral view of the head exhibits a reflection of the structures seen from the dorsal view (Figure 3b and 3c). Barbel rudiments are elongated. Two rows of gills are seen as they extend beyond the lower edge of the operculum. Fish at this stage have a significantly bigger belly than that in 2 cm fish due to the residual yolk in the digestive system (Figure 3a and 4a). A notable feature at this time is the melanin plug in the spiral hindgut due to development of spiral valves in the distal intestine and accumulation of pigment granules. It is considered that the melanin plug is discharged with the feces when fish begin exogenous feeding [14-16]. Organs in the abdominal cavity are visible through the translucent skin. Due to the distribution of pyloric appendages, the fish belly has an asymmetric look since one side is more smooth than the other. The liver seen from the right covers a large area of the belly and the piece on the left side is over a portion of pyloric appendages (Figure 3a and 3b). There is an interesting structural organization at this period if fish are viewed from the ventral. The heart, stomach, pylorus and the proximal intestine form a chain of knots (Figure 3d). The gall bladder is located at the right side of the abdomen. The abdominal contents are no longer visible exteriorly in larger fish when muscles and connective tissues are developed to enwrap the digestive system (Figure 4d).

At this time, the ventral portion of the head becomes flat. The width of the head is now larger than the depth, although the snout remains round and lacking protrusion (Figure 3a and 3b). In the next a few days, fish undergo considerable morphological transformation towards Laval Stage: the median recess of the upper lip smoothes out and the rostrum becomes triangular shaped. By then, the head of larval sturgeon clearly differs from that in other fish species (Figure 4a and 4d).

Larval Stage

The exterior morphology of larvae at approximately 2 cm in total length shows a few remarkable differences compared to younger ones. The rostrum is clearly triangular shaped and becomes flat at the dorsal-ventral axis (Figure 4a). Larvae at this

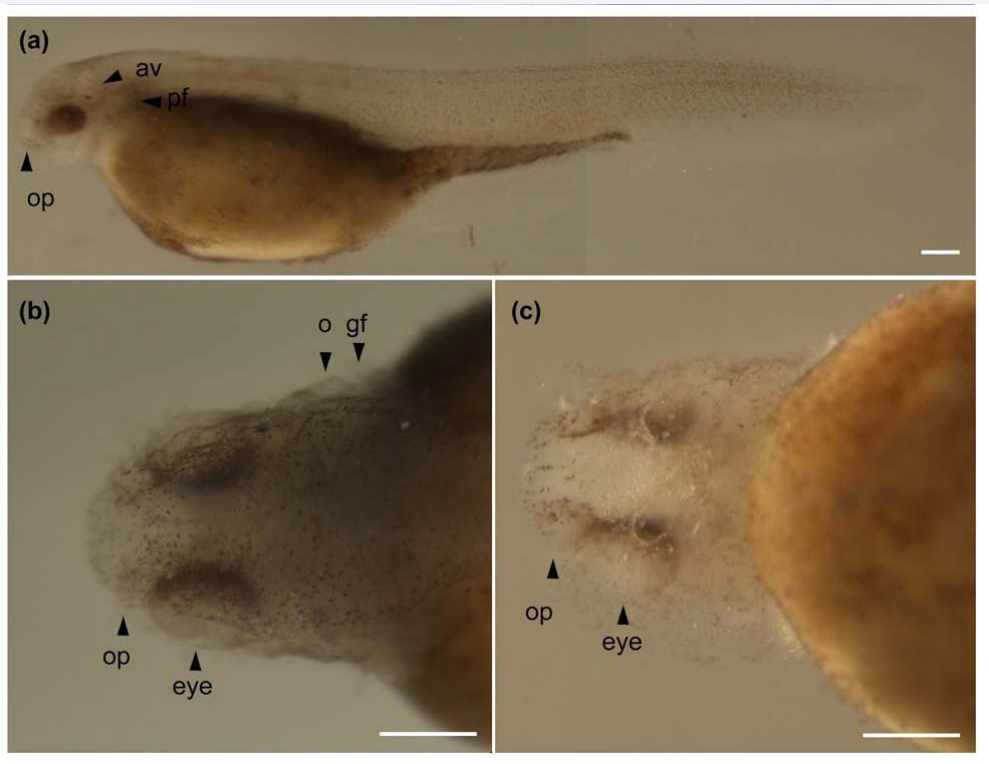

Figure 2 External views of yolk-sac larvae. Micrographs were obtained from a larva of 1.2 cm. They are the side view (d), dorsal view (d) and the ventral view (f). av, auditory vesicles; gf, gill filaments; o, operculum, op, olfactory pit; pf, pectoral fin;. Bar = 0.5 mm.

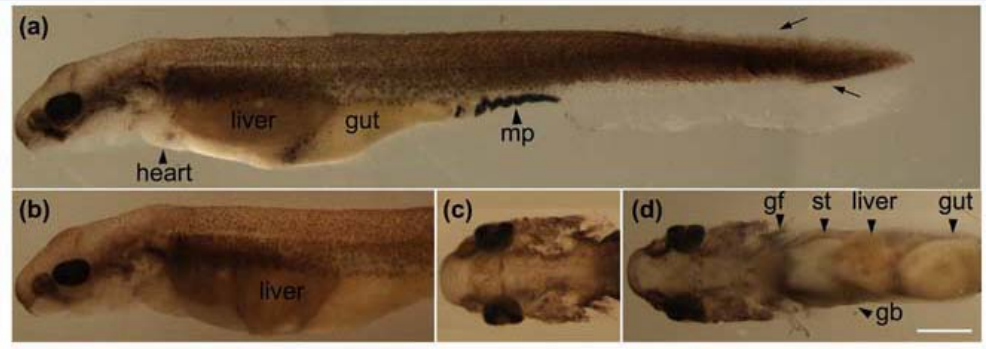

Figure 3 External views of a yolk-sac larva of 1.7 cm. (a) is the view from the right-side of the larva. Two black arrows point to pigmentation at the base of the dorsal and ventral fin folds. Images were photographed from the right-side (a), and the left-side (b), and from the dorsal (c) and the ventral of the head (d). gb; gall bladder; gf, gill filaments; mp, melanin plug; st, stomach. Bar = 1 mm.

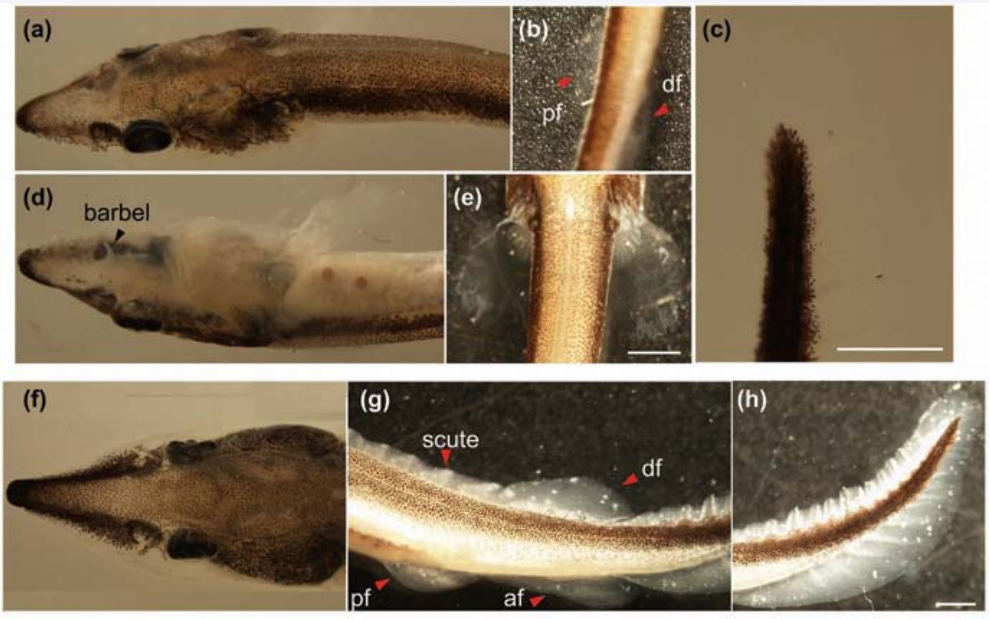

Figure 4 Images from larval lake sturgeon. (a) to (e) were obtained from a 2.0 cm sturgeon. They are the dorsal view (a) and ventral view (d) of the head region. (b) shows the dorsal and pelvic fin folds. (c) shows the tail and caudal fin folds and (e) is the dorsal view of the pectoral fins. (f) To (h) were photographed from a 3.2 cm fish. (f) is the dorsal view of the head, (g) is a micrograph of the intermediate and caudal trunk showing the morphology of several fin folds, while the tail and caudal fin folds are shown in (h). af, anal fin folds; df, dorsal fin folds, pf, pelvic fin folds. Bar = 1 mm. Images (a, b, d, e) were acquired at the same magnification. Images (f, g, h) have the same scales.

stage are heavily pigmented, especially at the lower portion of the trunk below the lateral line. This band of pigmentation extends from the rostrum to tail. However, it lacks melanocytes at the ventral of the body. When fish grow bigger, the skin of the ventral body becomes shiny silver in color. In the head region, more pigments are deposited to the olfactory epithelium. The opening of olfactory pit is divided into two lobes by a membranous slit. On the other hand, the pigments in the caudal fin folds are no longer noticeable, which makes the caudal peduncle slender-looking (Figure 4c). Dorsal and ventral fin folds are constricted. Barely visible, the spiny structures of fin-ray supports emerge at the base of the dorsal fin. Pelvic fins are noteworthy. Lobes of the pectoral fins broaden and reach to the abdomen (Figure 4e). The anterior edges of the pectoral fins touch the gill filaments. Spiny fin-ray supports are apparent at the base of the translucent fin lobes in pectoral fins. In these larvae, the digestive system is no longer visible exteriorly from the side view due to the development of muscles and connective tissues. However, they have not reached the centroventral abdomen, leaving a translucent slit that allows observation of the digestive system in the belly. From the translucent slit at the ventral abdomen, it is observed that the melanin plug is still present in many fish at this time. Among 65 fish in the same collection, 72% of them contain pigment granules in the intestine. In a few fish, the melanin plug becomes short and may be be evacuated at any minute. In some fish, the melanin plug becomes light, indicating loss of a portion of pigment granules. In these fish, the food intakes may be too few to completely push the pigment granules in the intestine. Typically, there are a few oil droplets on the fish belly at this phase.

Larvae at approximately 3.2 cm in total length appear more

developed and more or less resemble juveniles (Figure 4f to 4h). The rostrum is further elongated and the ventral trunk completely flattened. The caudal peduncle bends upwards, which begins the heterocercal tail morphology of lake sturgeon. Although dorsal and ventral fin folds, including preanal fin folds, have not completely retracted, caudal fin folds are separated from them by deep cuts. Fin-ray supports are visible at both dorsal and ventral lobes of the caudal fin folds. Scute rudiments appear along the base of the dorsal fin fold (Figure 4g). Unlike scales in modern fish species, fish in Acipenseriformes have shell-shaped bony plates, scutes or "armors", over their body.

When fish grow beyond 3.5 cm in total length, their dorsal row of scutes is formed. The dorsal and anal fins, and the dorsal lobe of the caudal fin reduce translucence as more materials were deposited to these regions. Fin-ray supports appear at the base of the anal fin. Although largely reduced, preanal fin folds remains.

Lake sturgeon between 3 to 4 cm in total length may be considered a transition period from larvae to juveniles. During this period, the membranous fin folds, including preanal fin folds, gradually disappear. Ray-fins form since textures of the dorsal, anal, pectoral and pelvic fins became similar to those in juveniles. The dorsal lobe of the caudal fin is largely reduced to a narrow band but the ventral lobe of the caudal fin remains large and semi-translucent. In addition, body scutes are completed. However, the pigment distribution shows similar patterns as in Larval Stage.

Juvenile Stage

Their exterior body shows a clear resemblance to adults when sturgeon fish are larger than 4.0 cm (Figure 5), which takes more than 40 days from fertilization. By then, the fin folds disappeared completely and the caudal is a miniature kind of that in adults. Lobes of other fins are enlarged. Lobes of pectoral and pelvic fins have faint pigments at the beginning while more pigments are deposited to distal regions of the lobes when fish grow (Figure 5c and 5d). The tip of the rostrum is narrowed and curved up dorsally. The body is spindle-shaped but the anterior half is flattened at the ventral. In the middle trunk, the pigments, initially concentrated to the areas below the lateral line in a dark band running along the body, now show regional aggregations and gradually move towards the dorsal trunk. Five rows of scutes, one on the dorsal, two on central-lateral and two on ventral-lateral, are clearly visible at this time as the scutes protruded. The scutes start at the very beginning of the trunk. The dorsal scutes end before the base of the dorsal fin. The center-lateral scutes run along the central line of the body and end with the trunk. Two lines of scutes at the ventral-lateral of the body stop just before the pelvic fins.

Fish reach 7-8 cm in total length a month later (two months after fertilization). When lake sturgeon juveniles grow further, the scutes become prominent as they are larger while their edge appears jagged. Aggregated pigments gradually migrate toward the upper part of the trunk and eventually reach the dorsal scutes when lake sturgeon is approximately 8 cm in total length. This far, the dark band of pigmentation seen in Larval Stage has turned into a few regional spots: one on the middle of the trunk, one on the upper portion of the body just below the dorsal fin, and pigments in the tail and the snout. Additionally, pigments are also deposited to lobes of the pectoral and pelvic fins, and the dorsal, anal and caudal fins. Normally, there are condensed pigments at the base of fins (Figure 5d).

Growth of the olfactory bulb and telencephalon along the development

In this study, we have measured the length and the width of the olfactory bulb and the telencephalon along the development to learn from the quantitative prospect of the forebrain development (Figure 6). We measured sizes of the olfactory bulb and telencephalon based on the dorsal views of confocal micrographs to the head in fish larger than 2 cm since, by then, the sturgeon brain have compartmentalized into five vehicles from previously three. The forebrain have subdivided into the telencephalon and diencephalon. The midbrain does not subdivide further but rather forms into the rectum mesencephalon while the hindbrain subdivides into the metencephalon and myelencephalon. From the dorsal views, diencephalon structures are largely unseen, except for a pair of habenulae that sit right before the mesencephalon (Figure 6a and 6b).

The positions of measurement for the olfactory bulb are shown in Figure 6a and 6b. Central axes were used for the olfactory bulb measurement. The length of the telencephalon was measured along the central line and the biggest distance from right to left for the width. Figure 6a and 6b are the dorsal views of the brain from a 4 cm and a 6 cm lake sturgeon, respectively. The gross morphology of the olfactory bulb and the telencephalon shows differences between lake sturgeon at these two periods.

The olfactory bulb begins to differentiate from the rostral

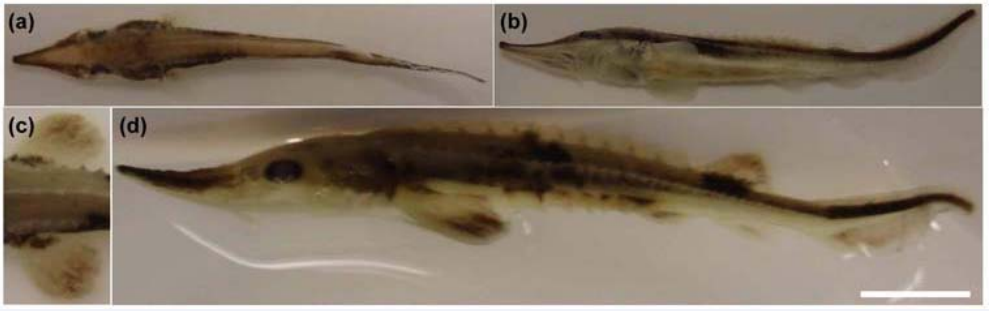

Figure 5 Exterior morphology of juvenile lake sturgeon. (a) to (b) were acquired from a 4.3 cm sturgeon. They are the dorsal view (a) and ventral view (b) of the fish. (c) is the dorsal view of the pectoral fins in a 6.5 cm fish. (d) is the side view of an 8 cm juvenile. Bar = 1 cm.

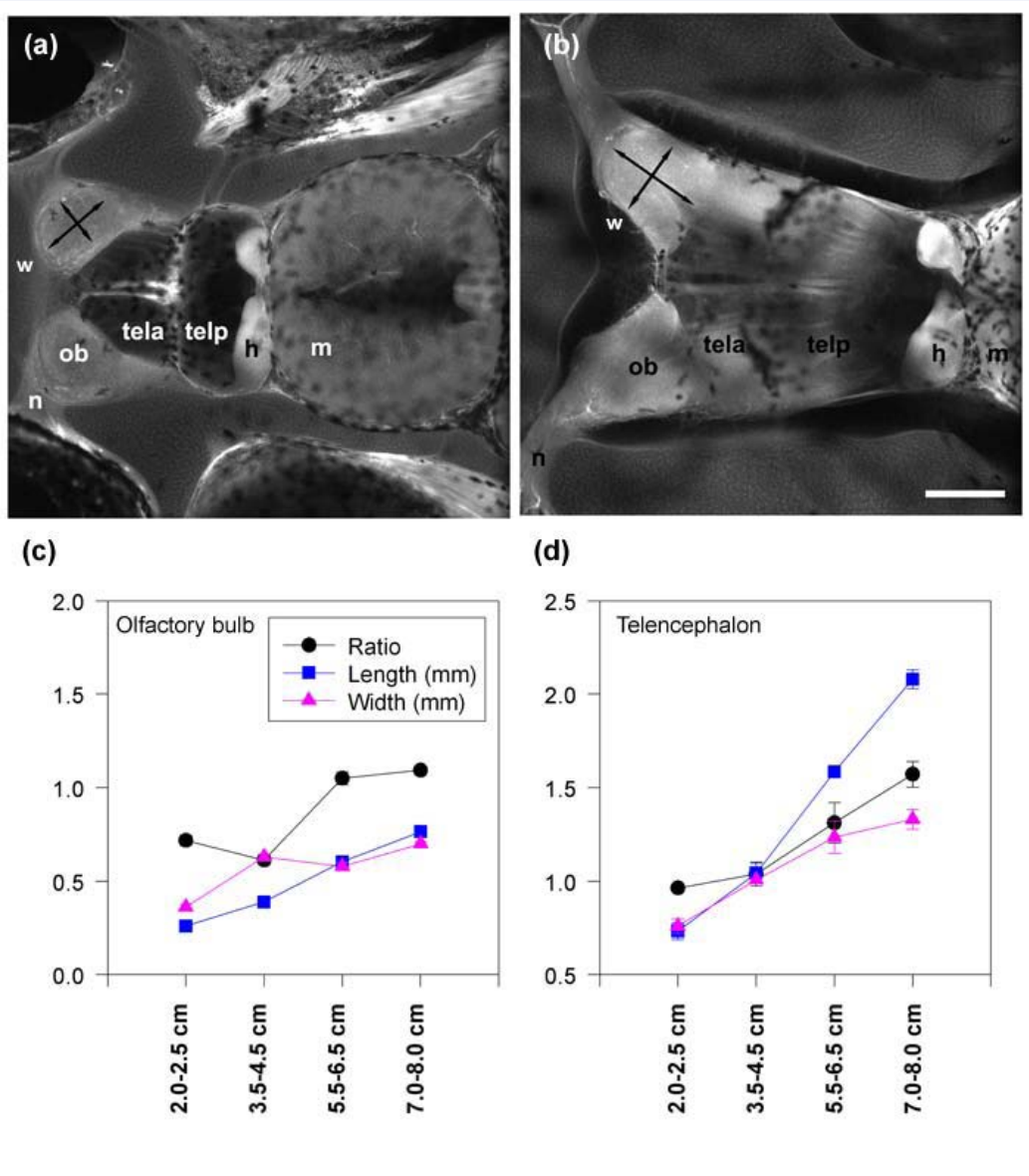

Figure 6 Quantitative analysis of the growth of olfactory bulb and telencephalon in the period of Larval Stage and early Juvenile Stage. Measurements were based on dorsal views of confocal imaging to the heads. Axes for measurements of the olfactory bulb are indicated in (a) and (b), in which the axis pointed to w' is for the width. Micrographs of (a) was from a 4 cm fish and (b) from a 6 cm individual. The data for olfactory bulb growth are shown in (c) and telencephalon in (d). The data shown are mean±SE. h, habenula; m, mesencephalon; n, olfactory nerve; ob, olfactory bulb; tela, anterior telencephalon; telp, posterior telencephalon. Bar = 400 μm.

forebrain as two football-shaped structures when sturgeon is approximately 1.7 cm in total length and then turned into to a bread-shaped protrusion at each side of the telencephalon (Figure 6a). In sturgeon ranged 2.0-2.5 cm, the bulb length is shorter than the width ($p < 0.001$). The averaged length is 0.26±0.014 mm and the width is 0.36±0.014 mm. The growth of the olfactory bulb starts with fast expansion in width. In fish ranged 3.5-4.5 cm, the average width of the olfactory bulb reaches 0.63±0.016 mm, almost twice as large as that in the previous phase ($p < 0.001$, Figure 6c). Although in a slower pace, the length of the olfactory bulb has also increased and reached 0.39±0.009 mm, significantly larger than that in the previous phase ($p < 0.001$). Longitudinal growth then catches up. In fish ranged 5.5-6.5 cm in total length the length of the olfactory bulb increases to 0.60±0.015 mm ($p < 0.001$) whereas the width of the olfactory bulb stays approximately the same as before ($p > 0.05$). Later on, the length and the width grow at a similar pace. In fish ranged 7.0-8.0 cm, both parameters are significantly bigger than those in fish of 5.5-6.5 cm ($p < 0.001$). The shape of the olfactory bulb is stabilized with the length slightly bigger than the width on average.

The length and width of the telencephalon are similar ($p > 0.6$), in 0.7 mm on average, in fish ranged 2.0-2.5 cm [Figure 6d]. The telencephalon grows proportionally at the next a few weeks. When fish reach 3.5-4.5 cm in total length, the size of telencephalon increases to approximately 130% as before [p

< 0.01]. The ratio of length verse width is slightly larger than before but there is no statistical difference between these two phases [1.04±0.064 vs. previously 0.96±0.017, p > 0.2, Figure 6d]. Longitudinal growth of the telencephalon accelerates in the next phase and exceeds the widthwise expansion. The expansion in width clearly slows down in fish bigger than 6.5 cm but longitudinal growth maintains. In fish ranged 7-8 cm, the average length of the telencephalon reaches 2.07±0.050 mm.

DISCUSSIONS

We have described transformation of external morphology of lake sturgeon along the postembryonic development. Following the conventional wisdom we divided the postembryonic development into three stages, Yolk-sac Stage, Larval Stage and Juvenile Stage. By the time of hatching, the yolk-sac larvae were approximately 1 cm in total length. The morphological transformation from late stages of embryos to hatching show many features corresponding to the pharyngula period of zebrafish embryos [13]. At this period, growing of the body, the direction of the tail tip and the development of fin folds are notable progresses. Yolk-sac Stage is a period of substantial organ development. The brain is differentiated into five vesicles. The olfactory pit deepens into a sac-like cavity. Eyes, located anterior the auditory vesicles, are differentiated. Barbels form and elongate. Gill arches and filaments appear. Pectoral and pelvic fins are growing. Notable organogenesis of the digestive system starts with jaw formation. Due to translucence of the belly, organization of the digestive system can be visualized externally. The melanin plug is a prominent feature at the late Yolk-sac Stage. Larval Stage begins with active exogenous feeding. Transformation of the snout into flat and triangular rostrum is the visible anatomic landmark for the beginning of Larval Stage. Disappearance of fin membranes and completion of ray-fin structures of the caudal fin indicate the transition to Juvenile Stage. The caudal fin is the last fin in transformation during the development.

In the development, transition from endogenous feeding to exogenous feeding is extremely critical for survival and growth of fish [17]. The melanin plug provides a visible indicator for this transition phase in various sturgeon species [14-16,18]. The melanin plug appears before complete depletion of the yolk [19]. It exists more than a week under hatchery reared conditions. During this period, the exterior morphology and fish behavioral changes provide clues for appropriate time of feeding [15]. Secretions of a few digestive enzymes are increased at the beginning of active feeding [20]. In lake sturgeon, this transition period occurs in the second week after hatching.

In the Great Lakes, lake sturgeon typically lays eggs from late April to early June. The offspring reaches 10-25 cm in total length by the end of year, which is comparable to the growth of hatchery reared year-0 lake sturgeon [21,22]. Our study shows that lake sturgeon reaches 10-15 cm in 3-4 into juveniles when lake sturgeon is months since fertilization. It is predicted that the third week since fertilization (or the second week since hatching) and the six week since fertilization are two important weeks when fish transform themselves into new developmental stages. It takes approximately 40 days for fertilized eggs to transform into juveniles, reaching more than 4 cm in total length. With another month of growth, juveniles may reach 7-8 cm and exhibit typical lake sturgeon features [Figure 5a].

Since our quantitative data analysis of the olfactory bulb and the telencephalon was made in fish with differentiated telencephalon, these data only covered the information since lake sturgeon entered Larval Stage. From these data, we predict that the fastest growing phase for the olfactory bulb and the telencephalon during the period examined is before fish grow to 5.5 cm in total length. Rapid growth is likely accompanied with high plasticity which may make fish vulnerable to environmental influences. The olfactory bulb and telencephalon are the relay centers of olfactory information process. Plasticity of the olfactory system might provide a time window for olfactory imprinting.

When is the time window of olfactory imprinting and how olfactory imprinting occurs are two critical questions for rehabilitation of native fish species in the Great Lakes [6,23,24]. A major rehabilitation strategy to restore populations of native species in the Great Lakes is stocking. Stocking has effectively increased fish survival in their early life stages but serious challenges remain. Emerging data indicate that hatchery-produced fish have low fitness following release into the wild [25]. Studies show that decades of stocking efforts were unable to form self-sustaining populations of lake trout (Salvelinus namaycush) in the desired areas many years later [26,27]. One speculation for failure of establishing self-sustaining populations from the hatchery-reared fish is that fish are imprinted to a different environment in their early life stages compared to those in the wild, which permanently changed adulthood behavior of hatchery-reared fish and become unfit to the designed areas. Attention is especially needed for lake sturgeon rehabilitation since lake sturgeon has an unusual long life cycle. Under natural conditions, female lake sturgeon does not reach sexual maturity until 18-27 years of age and male lake sturgeon reaches sexual maturity at 12-15 years old [3]. Once reaching sexual maturity, they mate and produce offspring once every 4-9 years. This life cycle increases difficulties in conservation and rehabilitation of lake sturgeon populations in the Great Lakes. Consequently, increasing offspring survival and proper management to ensure that stocking can lead self-sustaining populations in the Great Lakes become the top priorities in lake sturgeon restoration programs. This report has provided the exterior morphology of lake sturgeon from hatching to juveniles. The morphological changes along the development are correlated with the neuronal activity of the central nervous system and the olfactory system through the developmental stages to be reported elsewhere. These data will be useful for management of lake sturgeon resources and rehabilitation.

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