Study on Embryo Development in Sterlet (*Acipenser Ruthenus*) Reared in a Recirculating Aquaculture System

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Abstract
Sterlet (*Acipenser ruthenus*) is a sturgeon that hold an important place in aquaculture, being a species that can be raised both outdoors in fishponds and indoors in recirculating aquaculture systems (RAS). Knowledge of this species' biology has enabled the development of breeding technology in RAS; however, it is not yet fully optimized, and certain aspects of the technological process still require improvement. One of these is related to the artificial reproduction in order to produce the sterlet larvae. The aim of this study was to highlight the particularities of the embryo development in the sterlet during incubation, the studied embryos being obtained from sterlet breeders raised exclusively in the RAS. Eggs and sperm were collected during the 2024 breeding season, following the stimulation of ovulation and spermiation with pituitary suspension prepared from carp pituitary glands. After fertilization, the eggs were incubated at an average temperature of 17.3°C in a Zuger jar. Embry development was monitored by taking at regular time intervals 20 - 30 eggs from the incubator and studying them with a stereo magnifier. Embryo development was staged as follows: the zygote period (0-2h 15′), the cleavage period (2.5-9 h), the blastula period (9-17 h), the gastrula period (17-32 h), the neurula period (32 -44 h), the organogenesis (44-100 h) and the hatching period (100-124 h). The main features observed were recorded, photographed and described.

Keywords: sterlet, embryo, embryo development, RAS

1. Introduction

Sturgeons are fish considered true "living fossils," given their evolutionary history spanning over 200 million years [1]. However, in contrast to their ancient lineage, the technologies for their intensive/highly intensive rearing in recirculating aquaculture systems (RAS) are relatively recent [2, 3, 4]. These technologies have been developed primarily to supply the market with sturgeon meat and caviar - highly valued products with substantial consumer demand in the fishery sector [5, 4].

The expansion of RAS farms has significantly contributed to the stabilization of sturgeon aquaculture, providing an alternative to wild-caught fish and reducing pressure on natural stocks [5, 6]. Consequently, sturgeon species, most of which are critically endangered and facing extinction, have been removed from their natural habitats and introduced into controlled environments that meet their biological requirements. As a result, the majority of sturgeon-derived products currently available on the market originate from aquaculture, thereby contributing to the conservation of wild sturgeon stocks [7, 3].

Sterlet (*Acipenser ruthenus*) is a species particularly well suited for rearing in recirculating aquaculture systems [7, 8] due to its relatively small size and early sexual maturation, which allows it to reach reproductive maturity and produce caviar within a period of 3–4 years [3, 4]. Despite its suitability for intensive aquaculture, several aspects of sterlet farming in RAS require

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further optimization, including broodstock nutrition, water quality management, and reproduction protocols [3, 9, 6].

One critical aspect pertains to artificial reproduction for the production of sterlet fry, either for stocking aquaculture systems or for restocking natural waters. In this context, advancements in controlled reproduction techniques are essential to ensure high fertilization rates, embryo and larval survival [10, 5]. This issue has been a constant research focus in recent years. This aspect is even more relevant given that the sterlet broodstock used in the study were exclusively reared in recirculating aquaculture systems and fed solely with formulated pelleted feed [6, 4].

The objective of this study was to highlight the specific characteristics of sterlet embryo development during the incubation period, the embryos studied being originated from sterlet broodstock reared exclusively in a RAS at Didactical Station from the University of Life Science from Timișoara.

2. Materials and methods

The study was conducted during the sterlet (*Acipenser ruthenus*) reproductive season in the spring of 2024 at the Pădurea Verde Fish Farm, Didactical Station from the University of Life Sciences from Timișoara.

The fish and rearing conditions. Three sterlet males and females respectively, five years old, were used in this study being reared entirely in indoor tanks within a recirculating aquaculture system (RAS). The RAS included 18 m³ tanks equipped with mechanical and biological filters, two recirculation pumps for full water turnover every hour, and a blower providing aeration to both tanks and filters. The broodstock were fed exclusively with 6 mm pelleted feed formulated for sturgeon (SUPREME 10, Alltech Coppens).

Artificial reproduction followed a protocol developed and refined over the years at the farm.

Maturation status of the oocytes was assessed in late February through oocyte biopsies examined under a binocular microscope. Females with yolk-filled oocytes and an eccentrically located germinal vesicle were selected for reproduction.

Ovulation and spermiation were induced using intramuscular injections of common carp pituitary extract - 2 mg/kg for males and 4 mg/kg for females [11, 12] - administered in two doses: 10% initially (D1) and the remaining 90% (D2) after 12 hours, into the thickest part of the body.

Gamete collection and fertilization. Around 24 hours after the second hormone injection, females were monitored for oocyte release. The first ovulation was observed 29 hours post-injection, on March 7, 2024. The milt was collected from the males using a sterile 3 mL Pasteur pipette, sperm was drawn directly from the spermiduct to avoid urine contamination. A semi-dry fertilization method was used [9].

To remove egg adhesiveness mechanical method was carried out, using a mixture of talcum (0.5 kg), whole milk (1 L), and water (7 L). After thorough rinsing to remove the mixture, the oocytes were ready for incubation.

Incubation was carried out in Zuger jars where the water temperature was kept averaging 17.3°C (range: 16.5–17.7°C). The water quality was monitored daily with a Hanna multiparameter device, remaining within optimal ranges for sterlet.

Embryo development was tracked by sampling 20–30 oocytes at intervals of 1–3 hours early on (the first 24 hours), and 4–5 hours later, with observations made at a binocular magnifying glass, recorded and described accordingly.

Biometric measurements were taken from ten newly hatched, anesthetized larvae using a binocular microscope and micrometer, assessing total and preanal length, body height, and body weight. Body weight was measured with an analytical balance, and results were statistically processed in Excel, reported as mean ± standard error.

3. Results and discussion

Monitoring the embryo development of sterlet (*Acipenser ruthenus*), at an average temperature of 17.3°C, allowed the identification of the main characteristics of their embryogenesis, as well as the sequential stages of key developmental events from fertilization to hatching.

Zygote Period (Fertilized Egg) (0–2h 15')

Both before and after fertilization, until the first cleavage occurs, the eggs remain in the zygote stage. Initially, no perivitelline space is visible, as the chorion is closely adhered to the yolk. However, within a few minutes, the eggs begin to hydrate, making the perivitelline space distinguishable. As hydration progresses, the eggs significantly increase in volume.

The germinal vesicle can be observed in an eccentric position, marking the location of the animal pole. This pole appears darker in color, with a distinct white ring at its base. The majority of the eggs initially align with the animal-vegetal axis in a horizontal orientation (Fig. 1). However, by the end of this period, they gradually reorient, positioning the animal pole upward.

Fig.1. Sterlet (*Acipenser ruthenus*) eggs during the zygote stage

Cleavage period (2.5–9 h p.f.)

The first cleavage was observed at approximately 2 hours and 15 minutes post-fertilization (p.f.), and by 3 hours p.f., approximately 95% of the analyzed eggs in our study exhibited cleavage (Fig. 2). The time interval between the first and second cleavage (Fig. 3) was approximately one hour.

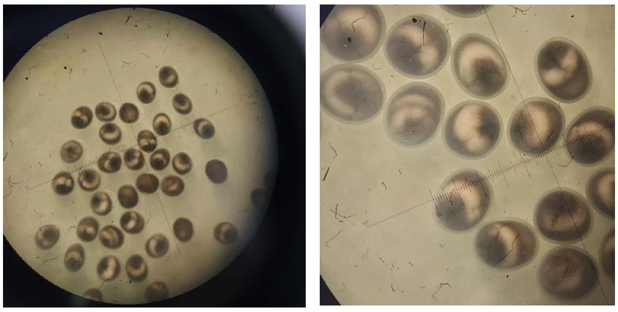

Fig.2. Sterlet (*Acipenser ruthenus*) eggs at 3 hours post-fertilization in the 2-cell stage, cleavage period

The cleavage furrows formed during the first two cleavages were restricted to the animal pole. However, starting from the third cleavage, corresponding to the 8-cell stage (5 hours post-fertilization, p.f.), cleavage extended toward the vegetal pole (Fig.4). From this point onward, a distinctive feature of sterlet and sturgeon embryo development becomes evident, characterized by unequal holoblastic division, where smaller blastomeres (micromeres) form in the animal region, while larger blastomeres (macromeres) develop in the vegetal region or hemisphere. In contrast to sturgeons, in most teleost fish, the initial cell division follows a discoidal meroblastic pattern, occurring exclusively at the animal pole, where the blastodisc is formed.

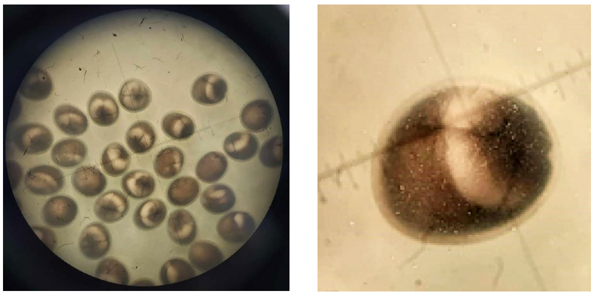

Fig.3. Sterlet (*Acipenser ruthenus*) eggs at 4 hours post-fertilization in the 4-cell stage, cleavage period

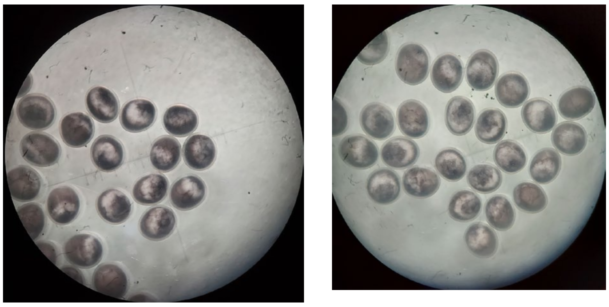

Fig.4. Sterlet (*Acipenser ruthenus*) eggs at 6 (left) – 7 (right) hours post-fertilization in the 32-64 cell stage, cleavage period

As time progresses, cleavage continues, and the resulting blastomeres become asymmetrical both between the two hemispheres (with smaller blastomeres at the animal pole) and within the same hemisphere. Thus, starting from the 16-cell stage, the blastomeres in the animal region become progressively smaller towards the pole and larger laterally. In general, cleavage results in an irregular division pattern in both hemispheres.

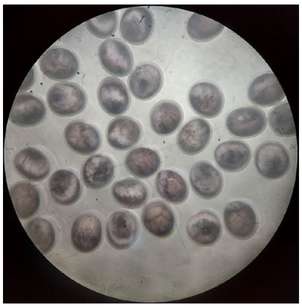

Fig.5. Sterlet (*Acipenser ruthenus*) eggs at 8 hours post-fertilization at the end of the cleavage period (original)

Blastula Period (9–17 h p.f.)

The blastula period is characterized by the continuation of cleavage in both hemispheres, maintaining the irregular division pattern, particularly in the vegetal hemisphere (Fig. 6). In the vegetal hemisphere, smaller blastomeres are observed near the equator, while larger ones are located toward the pole. In the animal hemisphere, blastomeres become less distinguishable, and at approximately 9 hours post-fertilization (p.f.), a cleavage cavity (blastocoel) forms at the animal pole. This structure is relatively easy to observe under a binocular microscope as a darker spot, becoming increasingly visible after 2–3 hours. Toward the end of the blastula period (16–17 hours p.f.), the initially smooth surface of the animal hemisphere transforms into a dome with distinct white margins (Fig. 7).

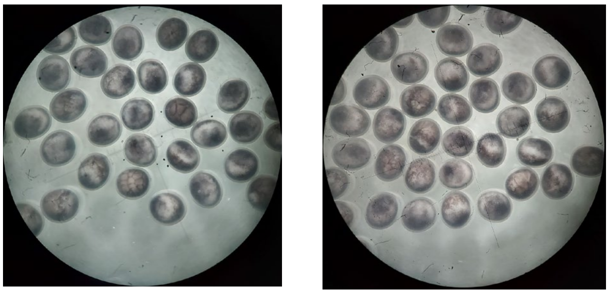

Fig.6. Sterlet (*Acipenser ruthenus*) eggs at 9 (left) – 10 (right) hours post-fertilization at the beginning of the blastula period (original)

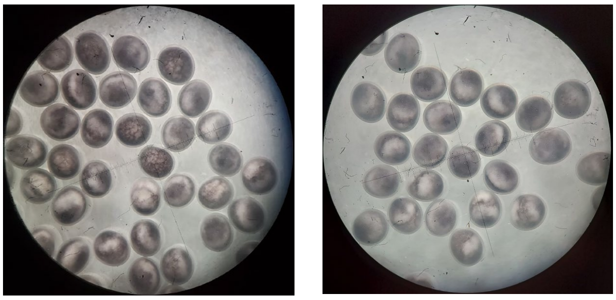

Fig.7. Sterlet (*Acipenser ruthenus*) eggs at 14 (left) – 17 (right) hours post-fertilization at the end of the blastula period (original)

The ***gastrula period (17–32 h p.f.)*** is characterized by continued cell division and epiboly, a process in which the proliferating cells of the animal hemisphere gradually envelop the vegetal hemisphere. The dome structure exhibits a pigmented upper region and a lighter-colored base, which progressively advances, reducing the vegetal hemisphere. The blastomeres in the vegetal hemisphere vary in size, with larger ones positioned toward the pole and smaller ones arranged near the equator.

In most eggs, by 26 hours post-fertilization (p.f.), epiboly reaches approximately 80–90%, and by 29 hours p.f., the blastopore becomes distinctly visible. Initially appearing as a small pigmented spot at the vegetal pole, the blastopore gradually elongates and takes on a slit-like appearance, marking the onset of neurulation.

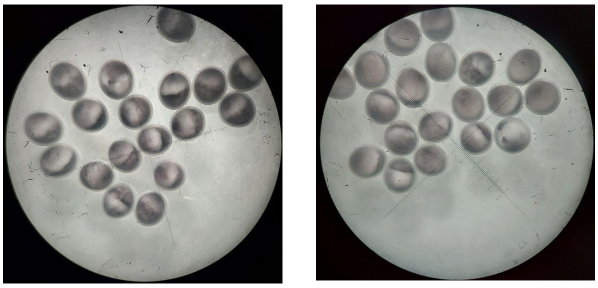

Fig.8. Sterlet (*Acipenser ruthenus*) eggs at 20 (left) – 23 (right) hours post-fertilization at the beginning of the gastrula period (original)

In most eggs, epiboly reaches approximately 40–50% at 20 hours p.f., increasing to 60–70% by 23 hours p.f.

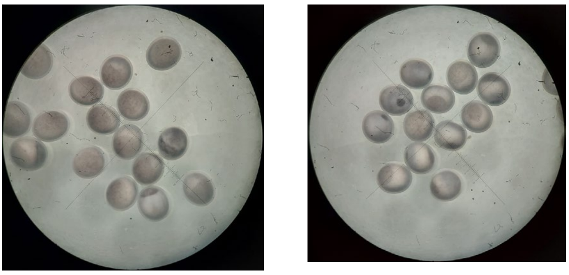

Fig.9. Sterlet (*Acipenser ruthenus*) eggs at 26 (left) – 29 (right) hours post-fertilization during the gastrula period.

In most eggs, by 26 hours post-fertilization (p.f.), epiboly reaches approximately 80–90%, and by 29 hours p.f., the blastopore becomes well-defined and highly visible.

Neurula Period (32–44 h p.f.)

Neurulation begins with the elongation of the blastopore at 32 hours p.f., forming a slit-like structure (Fig. 10), which marks the emergence of the neural groove. Subsequently, the neural plate becomes distinguishable. The neural plate gradually thickens and expands in the head region, while at the opposite end, the pronephros rudiments start to develop.

As development progresses, a significant elevation of the neural folds is observed, and the pronephros becomes increasingly prominent, rising alongside the neural folds. Toward the end of the neurula period, the neural tube is nearly completely closed, and the pronephros is positioned almost perpendicular to the neural tube. The tail region continues to thicken substantially as development advances, though it remains attached to the yolk sac membrane.

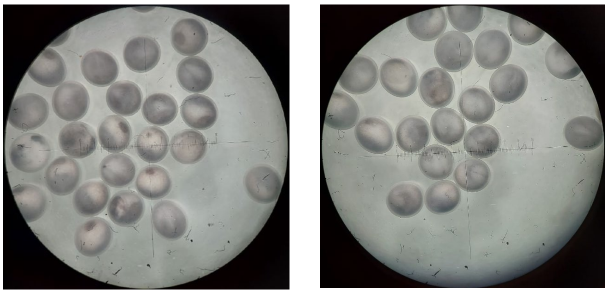

Fig.10. Sturgeon eggs at 32 (left) – 35 (right) hours post-fertilization at the onset of the neurula stage

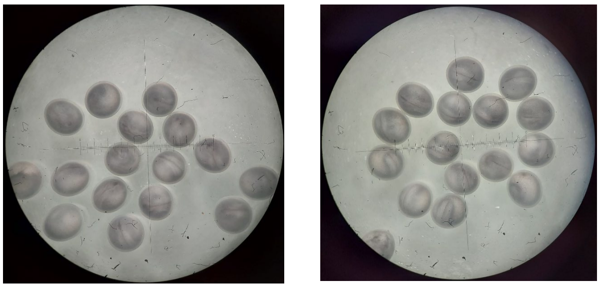

Fig.11. Sturgeon eggs at 40 (left) – 44 (right) hours post-fertilization at the end of the neurula stage

Organogenesis Period (44–100 h p.f.)

The development of the most important organs begins during neurulation and continues until hatching.

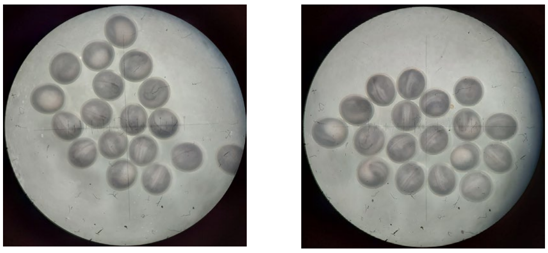

Fig.12. Sturgeon eggs at 47 (left) – 50 (right) hours post-fertilization

The embryos become increasingly well-defined, with the emergence of somites, which are metameric formations along the length of the embryo (Fig. 12). The cephalic region becomes rounded, and the rudimentary eyes start to form, initially appearing as two small pigment spots. Additionally, the primary pronephros wings are distinctly outlined (Fig. 13).

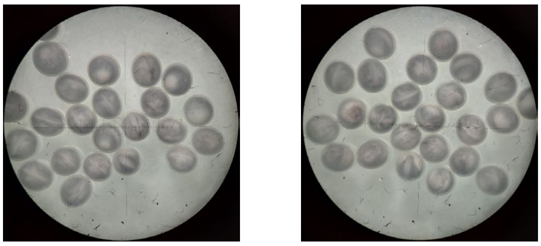

Fig.13. Sturgeon eggs at 53 (left) – 57 (right) hours post-fertilization

Beginning at 57 hours post-fertilization, the flattened tail gradually starts to detach from the yolk sac. Subsequently, the head region thickens and begins to separate from the yolk (Figs. 13 and 14). As a result of the detachment of the tail and head from the yolk sac, the embryo gains mobility, with the first embryo movements within the egg being observed at 70 hours post-fertilization (Fig. 15). At the same time, the heartbeat became clearly visible.

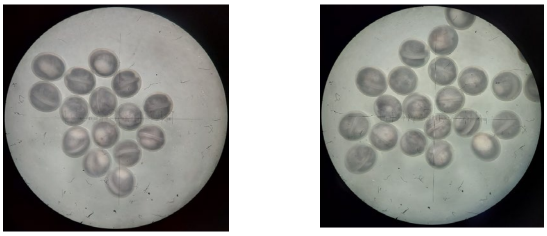

Fig.14. Sturgeon eggs at 63 (left) – 67 (right) hours post-fertilization

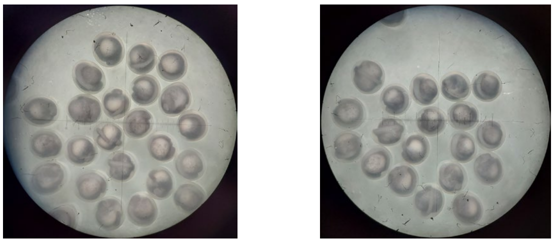

Fig.15. Sturgeon eggs at 70 (left) – 73 (right) hours post-fertilization

Development continued with a more pronounced elevation and elongation of the embryo. Thus, at 73 hours post-fertilization, the embryo covered approximately 90% of the yolk sac perimeter (Fig. 15). After an additional 10–15 hours, the tail reached the head, completely encircling the yolk sac (Fig. 16). During this period, the rudimentary fin buds became visible in the caudal region. As development progressed, the head took on a triangular shape, with the eyes becoming more distinct. The olfactory organs also began to take shape.

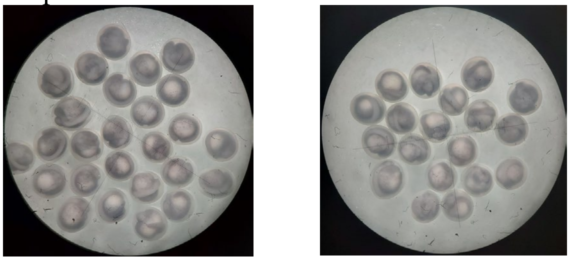

Fig.16. Sturgeon eggs at 79 (left) – 89 (right) hours post-fertilization

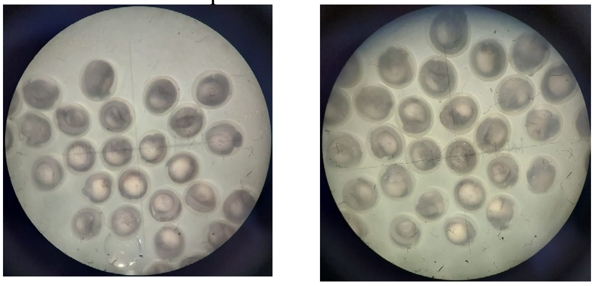

Fig.17. Sturgeon eggs at 95 (left) – 100 (right) hours post-fertilization

Hatching Period (100–124 h p.f.)

At 100 hours post-fertilization, the first embryo was observed breaking through the chorion and hatching shortly thereafter (Fig. 18). Mass hatching was observed 12 hours later, and within 24 hours, the majority of the embryos had hatched. The hatching rate recorded in the studied batch was 87%, which is a very good percentage compared to previous results obtained at the farm level.

Fig.18. Newly hatched sturgeon larvae at 112 hours post-fertilization

The total length of the newly hatched larvae in the studied batch had a mean value of 8.1 ± 0.09 mm. The yolk sac was large, with a preanal length of 5.68 ± 0.14 mm, and a maximum height reaching an average value of 2.20 ± 0.021 mm. The recorded mean body mass was 0.0103 ± 0.0003 g.

The observations made in our study show that the pattern of embryonic development in sterlet (*Acipenser ruthenus*) closely follows the same sequence of stages described for other sturgeon species. This resemblance suggests the existence of a common developmental framework within the Acipenseridae family. The stages we identified and described (from the zygote phase through cleavage, blastula, gastrula, neurula, organogenesis, and finally hatching) are in line with those commonly found in the literature.

A relevant example is provided by the Siberian sturgeon (*Acipenser baerii*), where Park et al. (2013) [13] not only outlined these major stages but went further to describe 30 distinct stages within them. This level of detail reflects a careful and structured approach that offers a valuable point of comparison across sturgeon species.

Similar developmental sequences have been reported in other species [14, 1], supporting the idea that embryogenesis is largely conserved within the genus *Acipenser*. Minor differences do exist, for instance, in the duration of certain stages or the appearance of specific structures, but these tend to be influenced more by environmental factors such as incubation temperature, water quality, or the specific conditions of the reproduction system used.

Recent studies, including our own, help fill in the broader picture of embryonic development in sturgeons. They are also increasingly relevant to modern aquaculture practices, particularly in systems based on water recirculation (RAS). Understanding these developmental phases in more detail is not only valuable from a biological perspective, but also offers practical benefits: fine-tuning incubation conditions, improving broodstock selection, and detecting early signs of developmental abnormalities.

4. Conclusions

1. Based on the data from our study, the embryo development was staged as follows: zygote period (0–2 h 15′), cleavage period (2.5–9 h), blastula period (9–17 h), gastrula period (17–32 h), neurula period (32–44 h), organogenesis (44–100 h), and hatching period (100–124h).

2. After fertilization, eggs hydrate and develop a visible perivitelline space. The animal pole

becomes identifiable by a dark spot surrounded by a white ring, and the eggs gradually reorient with the animal pole facing up.

3. The first cleavage appears around 2 h15’ post fertilization, and by 3 h, nearly all eggs have divided. Early cleavages occur at the animal pole, then progress toward the vegetal pole, showing the typical unequal holoblastic cleavage of sturgeons.
4. By 9 hours post fertilization, a blastocoel forms, marking the start of the blastula stage. Cleavage continues irregularly, and by 17 h, the animal hemisphere takes on a dome-like shape.
5. During gastrulation (17–32h), cells from the animal hemisphere spread over the vegetal pole. By 26 h, about 90% of the yolk is covered, and the blastopore appears, indicating the beginning of neurulation.
6. Between 32–44 h, the neural plate, groove, and folds develop, and the pronephros becomes visible. The neural tube nearly closes, and the tail starts to thicken.
7. Organ development starts to be visible at 44 h post fertilization. Eyes and somites form, the tail detaches from the yolk by 57 h, and the embryo starts to move by 70 h. The heart begins beating, and fin and sensory structures become more distinct.
8. Hatching starts around 100 h post fertilization, with most larvae emerging within 12–24 hours. Freshly hatched larvae measure about 8.1 mm in length and carry a large yolk sac.

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