Growth and Survival Rate of Russian Sturgeon (*Acipenser gueldenstaedtii*) Larvae from Fertilized Eggs to Artificial Feeding

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Abstract

Fertilized eggs of Russian sturgeon (*Acipenser gueldenstaedtii*) were brought from the Krasnodar Research Institute of Fisheries in 12.01.2001 to the University of Istanbul, Fisheries Faculty, Sapanca Inland Water Fish Culture Research and Applied Station on January 13 th. 5 kg fertilized eggs were put on 13 plates (size of each plate; 65 x 40 x 15 cm). Fertilized eggs were incubated in waving system (model designed according to modified Yushenko apparatus) at 14-15°C by means of water paddles continuously in action. The mass hatching was completed in 7 days after fertilization. The larvae were taken from the moving plates and were put to the fibreglass rectangular tanks (2.9 x 0.2 x 0.4 m size). From 8 to 11 days post-hatching, the larvae were fed by *Artemia nauplius* five times a day. At this period, the gill filaments were clearly red and covered by the operculum, and the anal fins were shaped. Between 12-16 days post-hatching larvae had been fed with both *Artemia* and *tubifex*. Between 17-18 days post-hatching, the larvae fed with *artemia, tubifex* and artificial diets. The *tubifex* were minced before they were given to the larvae. 19-32 days post-hatching, *tubifex* and artificial diets were used. At the end of the feeding trial, sturgeon larvae had reached to 12 cm of length and 5.25 g of weight. During the 33-75 days, the larvae were fed by only commercial trout diets five times a day (containing 52% protein, 14% lipid and 13% ash; BioAqua, Turkey). The artificial granule feed size increased from 80 µm to 1200 µm parallel to the growth of the larvae size. At the end of 75 days, the survival rate of Russian sturgeon was 27%. During the first 7 days of incubation, mortality rate of fertilized eggs was approximately 69% of the total number.

Keywords: Russian sturgeon, survival rate, growth, fertilized egg, larva.

Introduction

Sturgeons (Acipenseriformes) are one of the most ancient groups of the Osteichthyes with 25 species distributed in the temperate waters of the Northern Hemisphere, Eurasia and North America (Birstein, 1993).

Environmental pressures, such as water pollution, dam construction and development of adjacent watersheds for irrigation purposes, which have been responsible for the loss of spawning grounds, has threatened populations for some time (Gisbert and Williot, 2002).

Aquaculture of sturgeon can help in the conservation of wild populations declined through restocking and by providing a consistent supply without exploiting wild population. Sterlet (*A. ruthenus*) and Siberian sturgeon (*A. baerii*) were successfully reproduced and the technology to breed *A. baerii* was fully established by the USSR in the 1970s and later exported to other countries (e.g. France, USA, Italy, Japan, Germany and Poland) (Chebanov and Billard, 2001). The main species used in aquaculture production worldwide are white (*A. transmontanus*) Siberian (*A. baerii*), Russian sturgeons (*A. gueldenstaedtii*), sterlet and bester (Mims *et al*., 2002). In 1999, the dominant species in Western Europe were: the white sturgeon in Italy (46%), the Siberian sturgeon in France, Germany, Italy or Spain (35%), the Adriatic sturgeon *(A, naccarii)* in Italy and Spain (13%) and the Russian sturgeon at low level in various countries. In Italy, the hybrid *A. naccarii x A. baerii* is produced for growing and the pure species *A. naccarii* for restocking. Embryos, which were the progeny of a single pair of Russian sturgeon (*A. gueldenstaedtii*) were incubated in zug jars at 16°C and investigated hatching time and post-hatch growth in Russian sturgeon (Nathanailides *et al*., 2002).

In recent studies, results of rearing trials with Siberian sturgeon, white sturgeon and Italian sturgeon have demonstrated that artificial larval diets can be successfully used for intensive commercial culture of several sturgeon species from the onset of exogenous feeding (Gisbert and Williot, 1997).

The objective of the present paper is to report the first production of sturgeon, which fertilized eggs imported from Russia to Turkey because of juvenile restocking in natural environment. Although Russian sturgeon culture and feeding by artificial and live feeds is well known in nearby countries such as Russia and Iran, description of growth conditions of Russian sturgeon larvae and fingerlings was the first time for Turkey. Also, this study was the first sturgeon hatchery trial that was made by Istanbul University of Trout hatchery system was reconstructed so that Russian sturgeon eggs can be hatched. In one part of this project, Russian sturgeon fish were restocked in the Black Sea in 75 days. The other part of this study was to evaluate growth and survival rate of Russian sturgeon larvae up to 40 days post-hatching in relation offered live feed and commercial diets.

Materials and Methods

Russian sturgeon fertilized eggs (*A. gueldenstaedtii*) where of fertilization was occurred in 08.01.2001 were brought from the Krasnodar Research Institute of Fisheries on 12.01.2001 to the University of Istanbul, Faculty of Fisheries of the Sapanca Inland Water Fish Culture Research and Applied Station on January 13^{th}. Due to the some incomplete documents, the fertilized eggs had to be kept (around 35 hours) at Istanbul Airport. 5 kg fertilized eggs (around 200,000 eggs) were put on 13 plates (approximately 385 g each plate; 65 x 40 x 15 cm). The water level on the eggs was 5 cm in plates. The fungal infection was isolated from fertilization during the embryonic and early larval development and used malachite green was used for treatment (5 mg/L; 5 ppm solution of malachite green for 10 min) (Williot, 1990).

Fertilized eggs were incubated in waving system: this model was designed according to modified Yushenko apparatus (Williot, 1990) at 14-15°C by means of water paddles continuously in action. After 7 days, the larvae hatched. The larvae were taken from the moving plates and were put to the 9 fibreglass rectangular tanks. Size of each experimental tank is 2.9 x 0.6 x 0.4 m (0.7 m^3). The water level was about 15-20 cm depth (water volume of tank, 0.2 m^3) and 1-2 hours after, it was recorded that the some larvae began to swim actively. Initial densities of larvae were 25 larvae/L. First feeding of larvae started 8-11 days post-hatching with *Artemia nauplii* (size of eggs 200 µm-300 µm, INVE) five times a day at 9:30 am, 11:30 am, 13:30 pm, 15:30 pm, 17:30 pm. Initial feeding was started with 250 g *Artemia nauplius* per meal in all tanks. During the feeding regime larvae were fed by *Artemia nauplii, tubifex* (initially, two times a day) and artificial diets. *Daphnia* were given one time adding into daily diets on 17^{th}, 25^{th} and 26^{th} days post-hatching. The Russian sturgeon larvae were fed with just artificial feeds after 33 days post-hatching.

The initial artificial feeding rate was 15% of total body weight per day. This feeding rate was reduced to 10% when fish were fed from 15 days post-hatching to 30 days end then 5% at the end of the trial (Gisbert and Williot, 1997). After 33 days, larvae started to feed with granules of 800-1200 µm diameter artificial diets according to the larvae size. Feeds were delivered to each tank by hand during the day. One hour after feeding, the tanks were cleaned, except over night. To prevent fungal growth, the tank bottoms were carefully cleaned with a dry rot, always taking care of not stressing the larvae too much. All experimental tanks were fed with a commercial trout started diet containing 52% protein, 14% lipid and 13% ash (BioAqua, Turkey).

Because of cannibalism, Russian sturgeon larvae were graded at 18^{th} and 36^{th} days post-hatching due to slight size differentiation of larvae. Tanks were covered with shadow in corner of the tanks because of the photo tactic behaviours. Larvae were exposed to a 12 h light-dark photoperiod using overhead fluorescent lights.

Flow-through culture unit and ground water were used for incubation and post-larvae of Russian sturgeon. The water was supplied from heating and aerated system. The water temperature, dissolved oxygen (DO), pH and flow rate were 14-15°C for fertilized eggs, 18-22°C for larvae, 7-9 mg/L, 7.5 and 3 L/min. for fertilized eggs, 5 L/min. for larvae, respectively. Wet weight of larvae was measured by using a laboratory scale (0.01 mg). The condition factor (K= $(W/TL^3)*100$) and specific growth rate (SGR) was calculated using the following formula; SGR ($\%day^{-1}$) = $100x(\ln W_t-\ln W_0)/t$ where W_t and W_o represent final and initial mean body weights and t is the growing period in days (Gisbert and Williot, 1997). Total length (TL) was defined to be the distance from the tip of the snout to end of the upper lobe of the tail. Food conversion ratio (FCR) was calculated: FCR= Total feed intake (kg)/weight gain (kg). Each day, dead larvae were removed out of the tanks and recorded. Percent mortality was determined by hand-counting all dead fish at the periods. Growth, SGR, condition factor, and mortality between experimental tanks on different periods were tested to analysis of variance (Zar, 1974).

Results

Feeding Regime of Sturgeon Larvae and Development Stages

The first embryo to hatch often did not yet have a pigmented spot in the eyes. 7 days later from fertilization, hatching occurred when the larvae broke free of egg membranes, usually tail first. The yolk appeared ovoid and granular. Each eye could be distinguished by small, distinct pigment spot. At the stage of mass hatching, the tail at this stage was still protocercal. 3 days post hatching the mouth opening was apparent. The larvae were taken from the moving plates and were brought to the fibreglass tanks. In this period, the top of the tanks was covered with shade and thus protected from excessive light 4-5 days post hatching; mouth opening had broken through. The pigmentation of larvae was markedly stronger, yolk-sac disappeared, external gill filaments and barbells were clearly visible in day light, dorsal and anal fin were segregated. Phototactic behaviour was observed.

6-7 days post-hatching, pigmentation increased, the larvae had quite a dark colors. Powder feeds were given for improving smell and taste sense of larvae.

During the first feeding, the water temperature was raised slowly to 17-18°C. On the 8th days post-hatching 90% of fish larvae had depleted their endogenous reserve, and by day 9, yolk reserves were completely exhausted in all tanks. 8-11 days post-hatching, larvae started active (exogenous) feeding by *Artemia nauplius.* It was recorded that the larvae began to swim actively, schooling behaviour disappeared, and that the majority of the larvae were distributed at the bottom of the tank while some larvae swam along the tank walls. *Artemia nauplii* were observed inside the stomach. Larvae feeding regime for 75 days of trial was showed at Figure 1.

Between 12-16 days post-hatching, larvae were fed by *Artemia, tubifex* (two times a day). 17-18 days post-hatching, the larvae were fed with *Artemia*, *tubifex* and artificial feeds. The *tubifex* were minced before they were given to the larvae. 19-32 days post-hatching, *tubifex* and artificial diets were used.

The water temperature was increased to 20-22°C in this period. Just *Daphnia* were given on 17th, 25th and 26th days post-hatching by adding to the normal diets. After one month, appearance of larvae traits and metamorphosis were completed. The dorsal, lateral and ventral rows of scutes developed. During 33-75 days, the fry were fed only with commercial diets five times a day. Juveniles were divided into 2 groups in hatchery on day 36 post-hatching. The first group was 0.5-0.8 g of larvae and they were fed with 800 µm size of granule diets. The second group was 0.8-1.5 g of larvae and they were fed with 1200 µm size of granule diets. 45 days later, the water level was increased to 30 cm and around 2500 juvenile sturgeons were put in each tank.

Growth Performance of Sturgeon Larvae (Growth Rate, Condition Factor and Specific Growth Rate)

No significant differences in final weight and length were detected amongst the experimental tanks. Growth rate was more rapid among larvae fed after 22 days compared to the first 7-14 (endogenous feeding period). Because of this rapid growth rate, grading of fish size and moving ability of fish. 41 days post-hatching, the larvae reached to 1.18±0.36 g of weight and 5.5±0.32 cm of length. In this period, sturgeon larvae were fed with artificial feeds. Figure 2, shows the growth of Russian sturgeon larvae as weight (g) and length during for 75 days.

During the rearing period of Russian sturgeon larvae, K values decreased dramatically from 1.63 to 0.3. At the beginning of the first week, embryonic period of eggs, K did not calculate. During the second period (14 days), the specific growth rate of fish was 12.45. SGR considerably decreased to 6.83 compared with 3rd period (22 days) and then increased to 13.26 again. After 41 days, SGR of larvae decreased again (Table 1).

Stocking Density and Food Conversion Ratios

Approximately 385 g of fertilized Russian sturgeon egg was put on the plate of incubator, which was 0.013 m^3 of volume (30 g eggs/L). During the hatching and endogenous feeding stages, 61,878 and 60,878 of pre-larvae individuals were transferred to 9 tanks (each of 0.2 m^3), respectively. At the first feeding stage, 56,850 of larvae were put to 11 tanks

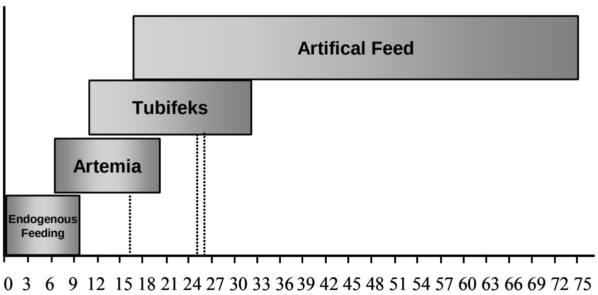

Figure 1. Feeding regime of the Russian sturgeon larvae.

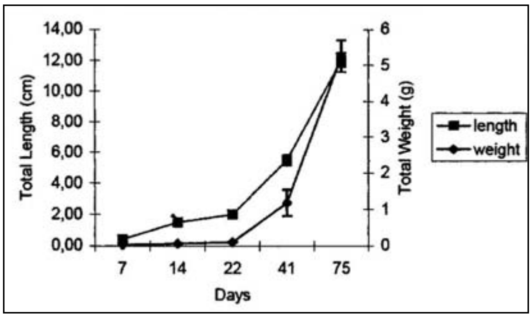

Figure 2. Growth in length (cm) and weight (g) of Russian sturgeon larvae.

Table 1. Growth, total length (cm)/body weight (g), condition factor (K) and specific growth rate (SGR) of Russian sturgeon larvae during the 75 days

Rearing days	Total Length (cm)	Live Weight (g)	K	SGR(%day^{-1})
7	0.40 (±0.04)	0.023(±0.001)	-	-
14	1.50(±0.15)	0.055(±0.001)	1.63	12.45
22	2.00(±0.11)	0.095(±0.008)	1.19	6.83
41	5.50(±0.32)	1.18(±0.36)	0.71	13.26
75	12.00(±0.43)	5.25(±0.44)	0.30	4.39

and then during the mixed feeding stage (live feeds and artificial feeds), 55,250 of larvae were separated into 22 tanks (each of 0.3 m^3). Sturgeon fish was sorted into 2 groups in the hatchery on day 36 post-hatching according to fish size. The first group was 0.5-0.8 g and the second group was 0.8-1.5 g of weight. At the end of 75 days, around 2,500 juveniles of Russian sturgeons were put into place in each experimental tank (Table 2). Calculated food conversion ratios (FCR) of sturgeon for the first, mixed and exogenous feeding stages were 1.15, 1.7 and 1.0, respectively. From the first feeding to the artificial feeding stage (about 25 days), sturgeon larvae consumed around 6.25 kg of *Artemia* and around 110 kg of mixed feed (*Artemia, tubifex, dafnia*, artificial feeds).

Survival Rate of Russian sturgeon

Survival rate fertilized eggs and pre-larvae of Russian sturgeon was significantly affected by fungal infection during eggs transportation and incubation period including first week after fertilization. Fertilized eggs had to be kept in transport boxes in airport because of the official procedure. During the first 7 days after fertilization, mortality rate of fertilized eggs was 69% of the total number (Figure 3). On day 8 and day 14 after fertilization (endogenous feeding stage), mortality rate decreased due to the fungal infection treatment. Mortality started to increase again between 14-22 days at the first feeding and the mixed feeding periods. The mortality rate was affected by cannibalism and feeding competition among the fish larvae in this period. After 22 days, mortality quickly decreased from 6.6 to 2.8%. At the end of the rearing period (75 days) Russian sturgeon larvae survival rate was 27%. But survival rates on day 22, 41 and 75 were 93.4%, 97.2% and 97.1%, respectively (Figure 3).

Discussion

The time taken for incubation to be completed is essentially temperature-related and also varies between species. Thus, time varies between 5-6 days at 16°C for sterlet, 7 days at 16°C for the white sturgeon, 16 days at 10-15°C for Siberian sturgeon (Williot, 1990). The mean water temperature during the rearing of pre-larvae of *A. gueldenstaedtii colchicus* was 18.6°C (Dettlaff *et al.*, 1993). Nathanailides *et al.* (2002) reported that water temperature was 16°C for Russian sturgeon eggs and 19°C for larvae. Optimal pH of the incubation water should be neutral to slightly alkaline 6.5-8.5 The oxygen level in the water during this period must be maintained above 5 mg/L (80% of saturation level) (Dettlaff *et al.*, 1993; Mims *et al.*, 2002).

In our hatchery, the water conditions were similar to those reported in the literature. Russian sturgeon larvae are mainly produced in open

Table 2. Stocking density and food conversion ratio of Russian sturgeon during the experiment

Rearing days	Density individual/L	Total Fish Weight	Total Feed (kg)	FCR
Incubation	1200 eggs/L	5 kg eggs		
Hatching	35 larvae/L	1.4 kg pre-larvae	-	-
Endogenous feeding	34 larvae/L	3.3 kg pre-larvae	-	-
First feeding	26 larvae/L	5.4 kg post-larvae	6.25 (artemia)	1.15
Mixed feeding (live+artificial feeds)	8 larvae/L	65 kg post-larvae	110 (artemia, tubifex, dafnia, artificial feeds)	1.7
Exogenous feeding/Releasing	8 juveniles/L	282 kg juveniles	277.5 (artificial feeds)	1.0

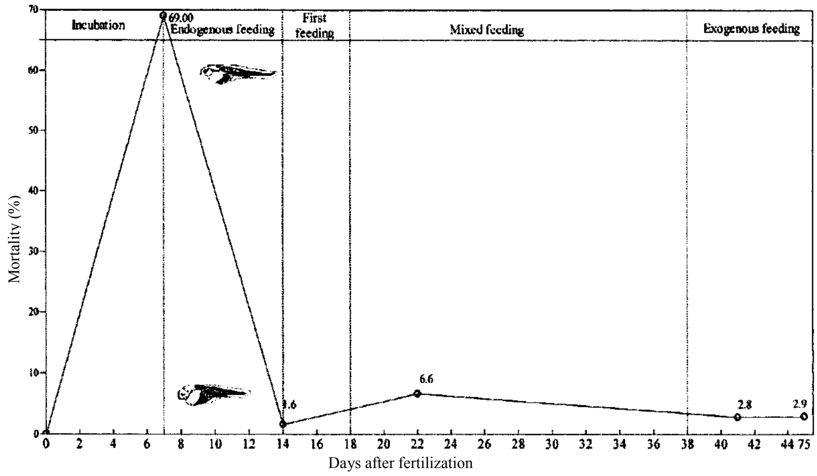

Figure 3. Mortality rate of Russian sturgeon fertilized eggs and larvae during the rearing period of 75 days.

freshwater flown systems. The well water was used during for 75 days. Sturgeon juveniles feed mainly on bottom fauna (invertebrates), bigger species being available at an early age to eat fry and juveniles of fish. Because sturgeons are carnivorous fish, this must be taken into consideration during their culture. Nauplii of *Artemia salina* are well suited for primary nursing of sturgeon fry. Sterlet with a body weight of 1-2 g exhibited fastest growth when fed on *tubifex* and chironomid larvae. Promising food organisms are zooplankton (cladocerans and copepods), larvae of chironomids, tubificiad and enchytraeid worms (Steffens *et al*., 1990).

Traditionally, hatchery-produced sturgeon larvae and fingerlings have been raised on live food organisms e.g. oligochaetes (*Enchytraeus* sp. and *Tubifex* sp.) and zooplanktonic organisms, such as *Daphnia* (*Daphnia* sp. and *Moina* sp.) or *Artemia salina* (Gisbert and Williot, 2002). Dabrowski *et al*. (1985) demonstrated that artificial larval diets could be used successfully for intensive commercial culture of several sturgeon species from the onset of exogenous feeding. These results are similar to those reported for *A. transmontanus* (Hung, 1991). An ideal larval sturgeon feed should be consisted of small (0.5-1.4 mm diameter) soft pellets that sink rapidly to the bottom of a rearing tank and can be easily detected and ingested by larvae (Gisbert and Williot, 2002). Modified salmon diets are used to feed fry dining in the first 3 to 4 weeks. Sturgeon juveniles have been fed brine shrimp nauplii for up to 30 days or until they grow about 2.5 to 3.5 cm long, before weaning them onto commercial starter diets (Mims *et al*., 2002). Laboratory experiments have demonstrated that food odour stimulates feeding responses in sturgeon larvae and juvenile (Kasumyan, 1999).

In this study, *Artemia*, *tubifex*, *daphnia* and then commercial feeds were used during the feeding regime of Russian sturgeon larvae. At the first stage of larvae feeding, we also used powder feeds in order to stimulate the smell and the taste senses of Russian sturgeon larvae.

Dabrowski *et al*. (1985) reported that the specific growth rate observed for this species (maximum: 11.7% day^{-1}) fed with *Tubifex* was, however, lower than that of the control (live *tubifex)* in their experiment. Mohler (2000) found that 26 days after feeding, the mean SGR in live artemia feeds ranged between 4.9-11.1% per day. Gisbert and Williot (1997) reported that between 10-22 days of post-hatch of Siberian sturgeon, SGR increased considerably and significant differences were detected between fish offered commercial diets for the first time on day 5, 7, 9 post hatching. Siberian sturgeon eggs, which were incubated at 13-14°C and that of larvae reared at 18°C completed metamorphosis in 20-40 days post-hatching (Gisbert and Williot, 2002). Growth and survival is affected not only by the quality of feed but also by the rate of feeding and the feed amount. Charlon and Bergot (1991) found a significant and positive correlation between the quantity of distributed feed and larval survival, and suggested that feeding rate for Siberian sturgeon larvae should be 20% of fish biomass per day. In this study, growth rate (Figure 2) was more rapid after 22 days as compared to post hatch of the larvae because of its increasing feeding activity and grading larvae size. Metamorphosis of Russian sturgeon larvae completed in one month post-hatching. After 33 days post-hatching period including endogenous feeding stage, the Russian larvae were fed with only commercial trout diets (Figure l, 2). SGRs ranged from 439 to 13.26% per day (Table 1). During the mixed feeding stage, growth rate and mortality rate of larvae were increased because of this cannibalism and passing of larvae from the live food to the artificial feeds. On the other hand, Nathanailides *et al*. (2002) reported that on the third week after hatching, there were large and small size fish and that it was not possible to continue feeding all fish with the same size of dry feed diets. Furthermore, competition between the larger and smaller fish for any food supplied was obvious and too great to be ignored. For this reason, we also sorted 2 groups in hatchery on 36^{th} days post-hatching according to fish size.

Mims *et al*. (2002) reported that there were about 50,000 eggs loaded into McDonald jars (10 l capacity) and than sturgeon fry had been initially stocked at 15-20/L. Therefore, the developing fry should be stocked by weight at not more than 3-5 g/L. Good growth has been observed with salmonid diets, which are the main protein source in fishmeal. Mohler (2000) explained that at initial densities of American Atlantic sturgeon (*A. oxyrinchus oxyrinchus*) of 0.37-2.22 g/L fed a formulated diet (Zeigler) for 28 days exhibited mean percent survival of 87% to 93.3% and had feed conversion factors of 0.50 or less. Sturgeon is an efficient converter of feed. Food conversion ratios (FCR) of 1.0:1.4 are common for juveniles and 1.6:2.0 for adults (Mims *et al*., 2002).

In our study, moving system was used and stocking density of eggs and larvae were higher than that mentioned in literature. The mean FCRs of larvae and juveniles were calculated at first, mixed and exogenous feeding stages as 1.15, 1.7 and 1.0 respectively (Table 2).

Williot (1998) estimated that losses of eggs during incubation were between 11 and 20%. Most losses during gastrulation are from unfertilized, parthenogenetic and polyspermic eggs. These losses depend on the quality of eggs and sperm and unfavorable incubation conditions. The survival of Siberian sturgeon (Lena River population) during the first month of rearing, when fed dry diets, was 81-85% (Dabrowski *et al*., 1985). Williot (1990), reported that during the incubation period there is a 20-50% mortality. Gisbert and Williot (2002) reported that dead larvae during endogenous feeding phase were characterized by severe morphological malformations such as hydrocele in the abdominal cavity, heart tube malformations, thinning and breaking of the yolk-sac walls, twisted body and/or undeveloped tail myomeres. Gisbert (1999) reported that predation and starvation were the main factors of larval mortality during the early life stages. At the beginning of the study, mortality rate was 69% in first week during the incubation periods due to the fungal infection and unfavorable transport conditions. However, mortality rate of eggs and larvae declined in hatchery after the endogenous feeding stage (Figure 3). In fact, if severe fungal infection was not occurred in first week during the incubation period, survival of Russian sturgeon eggs would not reach such a lower value (31%) till the end of the 75 day. The more attention should be paid to the transition from endogenous feeding to mixed feeding, where cannibalism and difficulties in adaptation to a new diet, as well as over or less feeding will increase mortality at the juvenile or fingerling stages.

Sturgeon larvae rearing will assist in restocking to the natural environment, and improving hatchery conditions and techniques in Turkey. So, more government support (for research and investment) should be made for this alternative species in commercial aquaculture. Knowledge on the effect of dietary components on larvae quality will be improved by the use of formulated diets. Future studies will provide us more understanding of sturgeon larvae culture.

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