

# Research on Embryonic and Postembryonic Development to the Pike (*Esox lucius*)

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## Abstract

The paper presents the results obtained in the artificial breeding process and the stages of embryonic and postembryonic development to the pike (*Esox lucius*), under the conditions of Fish Culture Research and Development Station Nucet, Dâmbovita, Romania. Artificial breeding and embryonic development in pike is carried out in the temperature range of 8-12°C with an optimum of 10°C. Fecundity is variable, and depends on the size of the female, but also on water temperature, food availability, density, inter and intraspecific interactions. The number of eggs per gram is between 104 and 148, depending on the size of the females. The embryonic development period lasts 7-9 days at 11-12°C. The postembryonic development period begins on the first day of hatching, larvae ranging in length from 7.5-9 mm, present a nearly spherical yolk sac. The larval period starts when the transition to exogenous nutrition occurs; the end of this phase is marked by the axial skeleton formation and ossification. The larval period ends when the appearance of the body resembles that of adult fish. The results are essential for knowing the pike requirements and factors that influence growth and development at these stages in order to substantiate technological processes.

**Keywords:** artificial breeding, embryonic development, pike, postembryonic, *Esox lucius*

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## 1. Introduction

Pike (*Esox lucius* L. 1798) is now considered as the second reference species of freshwater fish, with rapid growth. It can reach 50 cm in a year under good conditions [1] or 3-4 years [2] when there is competition.

It is a fish that is very popular for sports anglers and is also of interest for the organoleptic qualities of its meat and roe. An important role in pike feeding has the water temperature. In winter when the water temperature is too cold, pike feeds very little. It is the same when the water temperature exceeds 27°C [3]. Pike can coexist with all freshwater fish species. At the top of the food chain, it is a predator that plays an important role

in structuring fish stocks. The species is considered the reference for cyprinid areas because it is sensitive to the state of the food chain and environmental diversity. Due to its biological characteristics, pike is the preferred species for balancing the fish population in aquatic ecosystems. Factors influencing the reproductive mechanisms are many, of which the most important are the temperature, the access to breeding areas and the presence of favorable support for reproduction. The reproduction period for pike begins in February according to the geographical area [4]. The larvae are adhesive and vegetation are suitable for their attachment to the substrate, with the condition that it is dense, short, placed upright on the bottom and to be at small depth [5]. The optimum depth for breeding in the natural environment is 20 cm to 1 m. One of the major factors that trigger gametogenesis and oocyte maturation is temperature. Pike maturation starts in July - August, continuing until December

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for males (presence of sperm in the testicles) and for females up to reproduction [6].

## 2. Materials and methods

The materials and methods of study have been organized according to the corresponding step in the life cycle of pike. In the years 2018 and 2019, experiments were conducted in the pilot station for artificial reproduction of fish at S.C.D.P. Nucet. This paper presents the results obtained in the artificial reproduction of pike and the stages of embryonic and postembryonic development. Biological material comes from the experimental base of S.C.D.P. Nucet. For reproduction, were selected females aged 2-3 years and males 1-2 years old.

The weight of breeders is 0.8-2.0 kg/ex. The average fertility rate of a female is 25,000 oocytes/kilogram of female body weight. Breeding lots were made up of fish selected in spring, respectively, 50 females and 50 males.

The selected breeders were individually examined to assess the overall morphological aspect, gonad maturation stage and sanitary status.

Pike breeders were stocked separately in small ponds with low surface area and depth for easy intervention and monitoring. The induction of maturation and ovulation was achieved by administering carp pituitary extract (CPE). Hormonal therapy was administered in a single dose to both males and females. The males dose was 1 mg/kg body ♂, and the females was given 3 mg/kg body ♀ [7]. Accordingly, with the weight and sex of each breeder, the doses of hormonal product were calculated and the injection administered at the base of the dorsal fin. After injection, the fish were introduced separately by sex into the maturing ponds, where they were monitored to capture the moment of ovulation triggering and the appearance of the first eggs. After approx. 20-24 hours after the injection, the eggs and sperm were harvested by the abdomen massage. To determine the number of eggs/gram, samples were taken from each female. Fertilization of eggs was done using the dry method. The eggs collected separately from each female were fertilized with sperm from 3-4 males. After fertilization the eggs were incubated on nyal frames (about 50-70 g eggs/frame) in concreted basins of station no. 3. In 2019, the

water temperature during the incubation period and postembryonic development at pike was in the range of 11.5°C-13.5°C. For each moment of development in economically important fish species, experimental temperature limits have been established. The indicator, *degrees-days index* or *degrees-hours index* is calculated by summing the average temperature degrees. It is useful for experimental practice because it differs depending on the water temperature and corresponds to certain stages of the embryo development. Pike larvae after hatching shows on the front of the head, adhesive papillae, enabling them to remain fixed during resorption of the vitellus bag. During the experiments, according to the analyzed stage, various assays were carried out and interventions. The main physico-chemical water parameters were determined: water temperature, dissolved oxygen, pH. Monitoring was done daily. The water temperature was determined by the classical method with a thermometer. Dissolved oxygen was determined with an oximeter, pH with pH-meter WTW. During the incubation period of the eggs, to prevent the development of *Saprolegnia sp.*, prophylactic baths with 37% formalin solution were made. The dose of formalin used and the duration of treatment have been established consistently with the water temperature and the embryonic development stage. The solution concentration was 1 ml formalin/1 l water, for 10-15 minutes with repetition over 24 hours until hatching. Embryonic and postembryonic development stages were determined by examining embryos and larvae on the OPTIKA B-350 microscope. From the time of hatching, the total length (TL) of the larvae was measured daily and weighed at the Kern analytical scale, with the precision of 0.1 mg. Determination of growth and composition of consumed natural food was made by analyzing the intestinal content of pike larvae. The evaluation of reproductive performance was performed by the comparative analysis of a number of 15 indices characterizing the artificial reproduction process: Experiment variations/year; Females injected/year; Average weight; Mature females; Percentage of maturation; The medium amount of eggs/matured ♀; The amount of eggs/kg matured ♀; Average number of eggs/g; Total number of eggs/mature ♀; Percentage of fertilization; Number of fertilized eggs/matured ♀; Percentage of hatching; No. hatched larvae/mature

♀; Survival from the spawning phase to the larvae for 3-5 days; Number of larvae 3-5 days/matured ♀.

### 3. Results and discussion

This paper presents data and results of artificial reproduction, embryonic and postembryonic development in years 2018 and 2019. Experiments involving artificial reproduction, embryonic and postembryonic development in pike, took place since the second half of March. In 2018, in terms of temperature, considered to be one of the most

important factors in the reproduction process in fish, at the beginning of pike artificial reproduction, was in the optimum range (8-10°C). During the embryonic development period, the water temperature dropped to 4°C and remained at that value for several days. This led to an extension of the embryo period to a number of 15 days. In 2019, pike embryonic development was conducted in a favorable range of water temperature (11.5-13°C). which resulted in massive hatch after a period of 8-9 days. The water temperature situation during the postembryonic development of pike in the years 2018 and 2019 is shown in Figure 1.

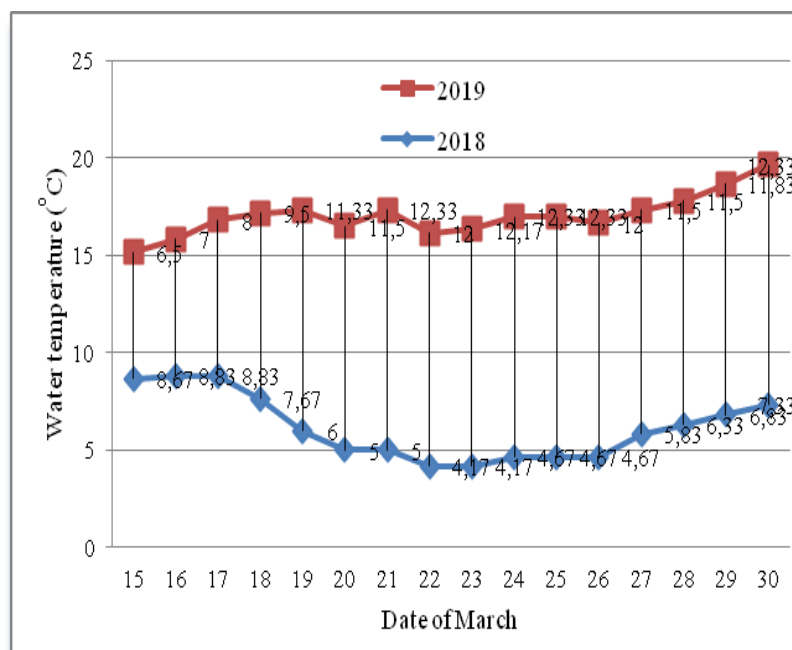


Figure 1. The water temperature situation

The average temperature over the total duration of the experiment was  $11.5 \pm 1.8^\circ\text{C}$ . The mean dissolved oxygen concentration was  $7.8 \pm 0.7$  mg/l or 82% of the ambient air saturation. The mean pH was  $7.8 \pm 0.5$  units.

#### Embryonic development

This period encompasses a period of time after fertilization that goes through a series of special qualitative processes: blastula segmentation and formation, gastrulation and embryonic foils formation, embryo shaping and development, differentiation of the main systems and organs, appearance of system functions, etc. The period ends with the embryo hatching.

At the release of the embryo from egg, an important role plays the hatching gland, the secretion of which contributes to weakening the resistance of the outer shells of the egg and diminishing the effort of the embryo.

Although the process of embryo development takes place inside the egg, it requires the existence of certain external environment conditions. Among these are important: temperature, oxygenation regime, salinity, light, etc. The thermal regime is one of the decisive factors in embryogenesis, as this process can only take place within certain limits. If the development speed is considered normal under optimal temperature conditions, a decrease, causes the development process to slow down, and under a minimal limit,

development stops and death occurs. Also, increasing the water temperature in the first stage leads to the development intensification, but then gradually makes the process more difficult, and above the maximum temperature the embryo dies. Changing the temperature conditions can cause a deregulation of the process of differentiation and development of the new organism. Thus, under conditions of high temperature, embryos can hatch before time and can be shorter in length and in weight compared to the normal. They may also present a series of abnormalities in the structure of the heart and nasal capsules, have no formed blood elements, and the oral cavity is closed. These embryos are not viable and sometime after hatching they die.

In the hatchery conditions of S.C.D.P. Nucet and depending on the mechanism of breathing, in the course of embryonic development in pike, we have established several characteristic stages, which emphasize the role of the movement in the embryo respiration:

- *the first stage*, shortly after fertilization, in the morula stage, the respiration mechanism takes place at the intracellular level. The process of oxygen penetration is achieved through the membrane of the eggs and the perivitelline liquid inside the eggs has a passive diffusion character.

- *second stage*, before the start of eggs pigmentation, breath is no longer intracellular, gas exchange being provided by specialized centres.

- *third stage*, is characterized by shaping the embryo, with the formation of nerve centres involved in the respiration and takes place an intensification of the gas exchange.

The evolution of the embryonic development process in pike, in 2019, is presented below:

- 5 hours (**56 degrees-hours**) from fertilization the eggs are semi-transparent (Figure. 2).

- 24 hours (**264 degrees-hours**) from fertilization, the nucleus is located at the animal pole and the eggs are semi-transparent. It is also now possible to see the perivitelline space. In the morula stage the respiration mechanism is carried out at the intracellular level.

- 72 hours after fertilization (**792 degrees-hours**) the embryo is expanded. The body of the embryo surrounds up to 1/3 of the circumference of the vitellus sac.

- 96 hours after fertilization (**1056 degrees-hours**), the embryo is well-accounted for and covers almost 50% of the circumference of the vitellus sac. The body of the embryo in the posterior part is conical and in the anterior part the primary eye vesicles can be observed.

- 144 hours from fertilization (**1584 degrees-hours**) the embryo covers up to 75% of the circumference of the vitellus sac and the eyes are slightly pigmented (Figure 3). Embryonic development is almost complete; the caudal sector begins to separate from the vitellus sac. The first movements of the embryo can be observed and heart pulses are visible.

- 168 hours after fertilization (**1848 degrees-hours**) surrounds the entire embryo yolk sac, caudal reaches the eyes, eye pigmentation is stronger and embryo movements become more intense.

- At 192 hours of fertilization (**2110 degrees-hours**) the embryo is fully developed, the caudal goes over the head, the embryo's movements are intense and the hatching begins. Massive hatching (fig.4) began after accumulating a number of **93 degree-days** (8-9 days).

#### Larval development

After hatching (Figure 5), the frames on which the eggs were incubated are removed from the pool to clean the dead eggs. The frames are cleaned and disinfected with a solution of formalin and are put back into the pool of freshly hatched larvae, to be used as a support. Pike larvae require little time until they can detach and swim easily.

The length of the pike larvae immediately after hatching is 7-7.5 mm. Yolk sac is almost spherical, presents numerous fat droplets and the body is surrounded by the fins fold, starting from the forward third of the dorsal side, to the back of the yolk sac. Pectoral fins are rudimentary at the beginning of their forming. The mouth is unworkable. Gills are not formed, and breathing is ensured by a network of blood vessels of the ventral vein on the vitelline sac. In this step, the oxygen consumption corresponds to 0.7-0.8 mg O<sub>2</sub>/g larvae. The larvae are sensitive; they are hung on the walls of the tank or objects due to a sticky substance secreted at this stage. When they are disturbed, they swim difficult through wave-like movements of the body. They are indifferent

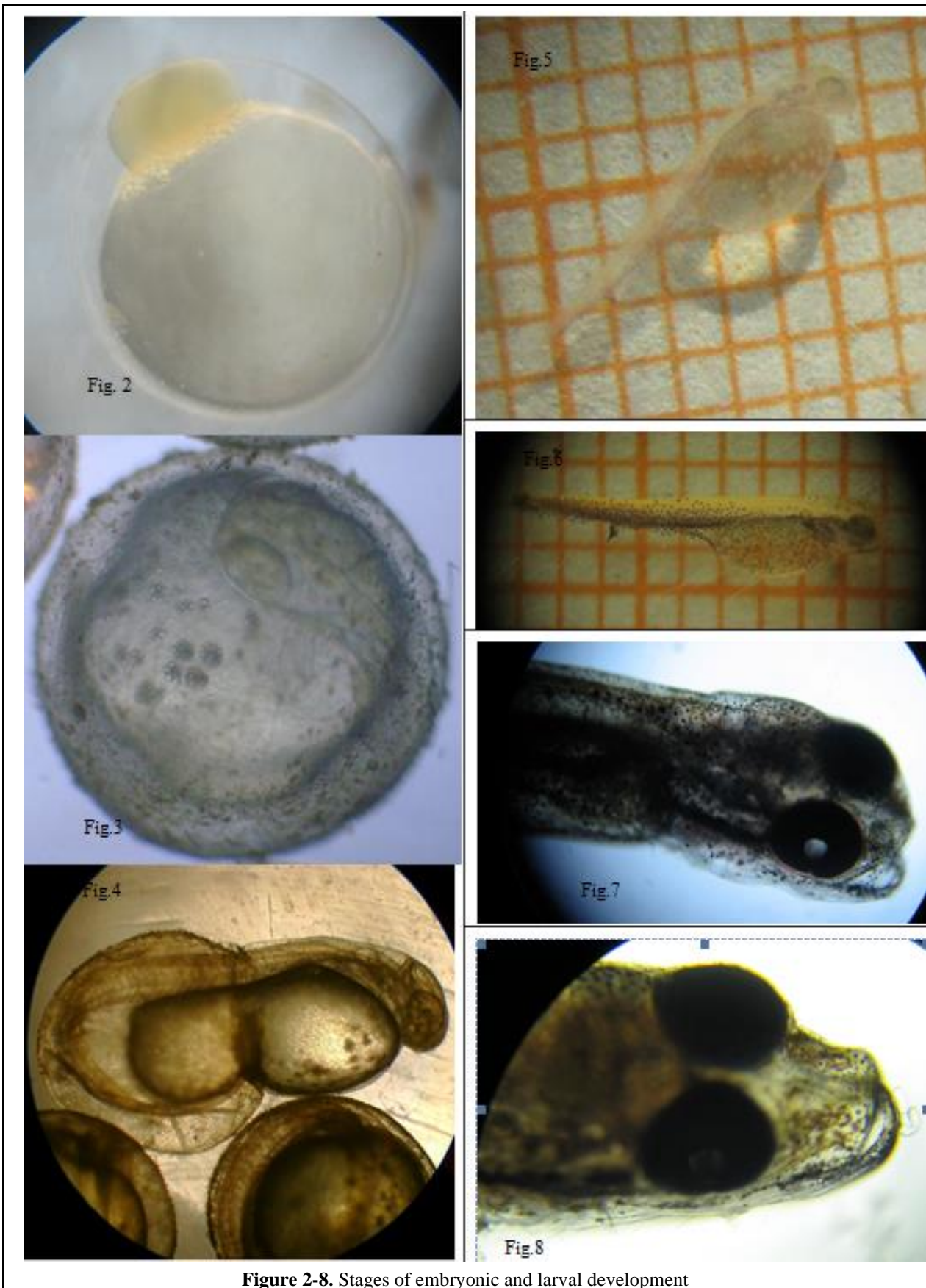


Figure 2-8. Stages of embryonic and larval development

to the light; nutrition is endogenous. The rhythm of growth is quite fast.

At the age of **2-3 days** (water temperature of 10-12°C), the larvae are 9-10 mm long, the head is separated from the vitellus sac, which, decreasing, becomes oval.

At the age of 3 days pigmentation of the larva becomes more pronounced and the mouth is easily observed. Haemoglobin appears.

At the age of **4 days**, the length of a larva is 10-11 mm (Figure 6). The pigmentation of the body is accentuated. Transparency shows heart beats (88-90 beats/min). The number of myotomas is 58-60. The finning membrane is continuous. Pigmentation becomes more pronounced, especially on the ventral side of the body.

The mouth can be observed easily due to the movement of the lower jaw. Breathing is through the gills, but also directly, sanguineous.

Oxygen consumption is 1.9 mg O<sub>2</sub>/hour/g larva. The organs of the lateral line are formed and the pectoral fins are well contoured. At this stage, the larvae are more active, but they are still swimming with difficulty.

They attach easily to objects in water and generally maintain a vertical position.

At the age of **10 days** corresponding to the third stage of development, the body length is 12-13 mm and a weight of 12-13 mg.

The sticky glands are very small, the swim bladder was filled with air, and the swim membrane sharpened in the tail area, where the mesenchymal tissue began to form. The blood vessel network on the surface of the vitellus sac has been reduced and breathing is exclusively through the gills. Oxygen consumption increases to 4-5 mg/hour/g larva.

Pike larvae swim with more ease and they can keep themselves in the thickness of the water layer. Feeding is still endogenous. Until this stage, the intestine has the shape of a straight tube.

At the age of **11-12 days**, the body reaches lengths of 13-14 mm (Figure 7). Feeding is mixed (endogenous - exogenous), and the vitellus

reserves are almost completely consumed. As the growth and development increases, the swimming membrane is reduced.

At the age of **15 days**, the length of the body of the larvae exceeds 15 mm. The formation of mesenchymal tissue is advanced.

The caudal fin has become heterocercal, the body is almost entirely covered with brown pigments. Separation of digestive tract segments is achieved (previous, middle and posterior). Food is exclusively exogenous. Larvae swim easily, and they can keep themselves in the thickness of the water layer, swimming vertically or horizontally.

At the age of **25 days** (TL=20-29 mm), fins are formed, pigmentation of the body is enhanced, and the shape of the head acquires the specific features of adults due to the extension of the snout (Figure 8). Towards the end of this stage, when the pike features are outlined, the larvae begin to become ichthyophagous (feeding on the larvae of other fish), however food is predominantly zooplanktonic.

Their movement is very fast, swims easily, but continues to remain at the shore area, at small depths, with a rich trophic base.

The larval period ends at **30-35 days** of age (TL=13-37 mm), the scales begin to form, and body coloration is darker on the dorsal side and has small spots in lighter colour. The fry of pike are fast swimming in the water depth and in shore areas, becoming mostly ichthyophagous. If the larvae of other fish are not present, pike fry feed on planktonic and benthic organisms.

In the first stage, the larvae food was varied and strictly zooplanctonophagous, consisting essentially of small cladoceran species (*Chydorus sphaericus*, *Bosmina longirostris*, *Daphnia sp.*). Better ingestion and subsequent survival with this type of prey have been observed. From day 4, zooplanktonic fractions of 500 µm were observed (*Copepode*, *Cyclopidae* and *Daphnia sp.*).

The results obtained are highlighted on the basis of 15 indices characterizing the artificial reproduction process, and are presented in Table 1.

**Table 1.** Technological indices of artificial reproduction process

No.	Technological indices	UM	2018	2019
			CPE	CPE
0	1	2	3	4
1	Experiment variations/year	2	2	2
2	Females injected/year	ex.	50	50
3	Average weight	kg/ex	1.5	1.7
4	Mature females	ex	42	45
5	Percentage of maturation	%	84	90
6	The median amount of eggs/matured♀	g/♀	160	185
7	The amount of eggs/kg mature♀	thous	13.3	13.0
8	Average number of eggs/g	pic./g	126	120
9	Total number of eggs/mature♀	thous	20.2	22.2
10	Percentage of fertilization	%	85	88
11	Number of fertilized eggs/matured♀	thous	17.2	19.5
12	Percentage of hatching	%	52	55
13	No. hatched larvae/mature♀	thous	8.9	10.7
14	Survival from the spawning phase to the larvae for 3-5 days	%	84.2	88.8
15	Number of larvae 3 to 5 days/matured ♀	thous	7.5	9.5

#### 4. Conclusions

Water quality parameters such as temperature, dissolved oxygen and other gases are of particular importance in the pike incubation period. Water temperature is an important factor during embryogenesis. The optimum for the species is between 8-10°C and it can be between 4-22°C. If the water temperature stays for several days at 5°C, there is a fairly high mortality rate. At an average water temperature of 12.5°C, 93 degrees-days were required to incubate the eggs. The amount of dissolved oxygen should be between 7 and 9 mg/l. Larvae began to feed actively day 3 from hatching.

The results obtained in the research on artificial reproduction, embryonic and postembryonic development at the pike can be useful in the practice of fish breeding technologies.

In order to better understand the processes of artificial breeding technologies, it is necessary to continue the research in the field.

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