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THE INFLUENCE OF THE EXPOSURE TIME TO THE PREVENTIVE TREATMENTS OF THE PIKE-PERCH (*SANDER LUCIOPERCA* L., 1758) EGGS, AGAINST FUNGAL INFECTION, DURING THE EMBRYONIC DEVELOPMENT PERIOD

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Abstract

Infection with Saprolegnia spp. is reported more and more frequently, becoming endemic in many aquaculture units, having a devastating impact on this sector, especially on embryonated eggs during the incubation period. This paper presents the way to prevent infection with Saprolegnia spp. by applying prophylactic treatments with formaldehyde to pike-perch eggs, during the embryonic development period. The experiments were carried out in triplicate, at SCDP Nucet, Romania, in 2022, at the artificial fish reproduction station. For prevention, formaldehyde solution was used, in a concentration of 1.7ml/l, the exposure time being different: in version V1 (control) of 10 minutes and in version V2 in which the exposure time was based on the respective water temperature 5, 10 and 15 minutes. The results were very good in the V2 variant with losses due to fungal infection of 4.8%, and good in the V1 variant (control) with losses of 14.6%.

Key words*: eggs, formaldehyde, fungal infection, pike- perch, Saprolegnia spp.*

INTRODUCTION

The development of aquaculture depends on the introduction of new species into the culture, as well as on the success of obtaining the fry necessary for stocking (Dobrota et al., 2022).
Compliance of disease and pest control treatment recommendations, especially in juvenile fish, ensures high production (Radu et al. 2022).
The pike-perch period of embryonic development in the hatchery takes place in April, when the water temperature stabilizes in the range of 12-14ºC. In the last decade, due to climate changes, during this period there are sudden changes in environmental parameters such as temperature, pH, organic substance etc.; they can induce outbreaks of saprolegnosis.
In fish culture, saprolegniosis is mainly a problem of eggs during the incubation period (Willoughby, 1970; Czeczuga & Kiziewicz, 1999; Hussein et al., 2001, Gieseker et al., 2006), even if sporadic outbreaks are reported in the situation where the environmental parameters register normal values, without sudden gaps (Thoen et al., 2011). In the past, the disease was adequately treated with malachite green, $Cu_2[(OH)_2|CO_3]$, an organic dye with biocidal effects. However, malachite green was banned for use in aquaculture when its use was found to pose a significant health risk to consumers due to its carcinogenic properties (Fitzpatrick et al., 1995; Kitancharoen et al., 1997).
Consequently, outbreaks of diseases with *Saprolegnia* spp., have increased significantly in the last decade in many areas of the world, with devastating impact on the aquaculture sector.
When incubating the eggs of pike-perch, losses due to microbial diseases can be significant and have significant financial implications. The mortality rate in embryonated eggs can reaches up to 80-100%. Treatments must be effective, safe and cost-effective (Radu et al., 2020).

MATERIALS AND METHODS

The research was carried out in 2022 in the Fish Culture Research and Development Station Nucet (SCDP Nucet), Dâmbovița County, Romania. The artificial breeding station is located in the main bed of the Ilfov brook, downstream of the Ilfoveni reservoir dam. The supply of technological water is carried out from

a settling pondlocated upstream of the reproduction station and its filtration is done through nytal fabric with a mesh of 0.1 mm.
The study material came from the natural-directed reproduction of the pike-perch, which was realized in earthen ponds, with the surface of approximate 1000 m^2. Spawning was done on breeding mats, made of fasciculate willow roots ("whiskers") (*Salix babylonica* L.).
The experimental variants were the following:
- Variant 1 (V1) - where the fertilized eggs were treated for 10 minutes once every 12 hours, was carried out in triplicate, in incubators I1, I2 and I3;
- Variant 2 (V2) - where the fertilized eggs were treated depending on the water temperature respectively for 5, 10 or 15 minutes once every 12 hours, was carried out in triplicate, in incubators I4, I5 and I6.
After harvesting the nests with fertilized eggs from the breeding ponds, they were carefully introduced into the nytal keeping net of the Nucet type incubators (Figures 1 and 2), where a permanent supply of water was ensured, at a flow rate of 8 l/min.

Figure 1. Detachment of nests with pike-perch fertilized eggs (Original photo)

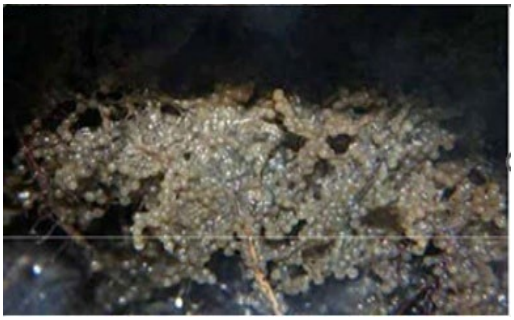

Figure 2. Nest with fertilized eggs before being introduced into the Nucet type incubators (Original photo)

The capacity of the Nucet incubator is 140 litres, with surface water intake and bottom emptying, thus creating a vertical circular current of water, ensuring continuous fresh, well-oxygenated water for the eggs (Figures 3 and 4).

Figure 3. Nests with pike-perch fertilized eggs (Original photo)

Figure 4. The Nucet type incubators with nests of pike-perch fertilized eggs (Original photo)

The duration of the incubation was 7 days (depending on the water temperature), so the

incubation of the eggs started on 04/08/2022 and ended on 04/15/2022.

Considering the fact that the pike-perch is a very sensitive species in the first stages of development, the success of the reproduction expressed in the percentage of viability of the larvae depends to a large extent on the knowledge of the particularities of embryonic development in order to establish the appropriate technological interventions.

The application of prophylactic treatments to prevent infection with fungi was carried out by treating with a 37% formaldehyde solution in a concentration of 1.7 ml/l of water. The first treatment was administered 24 hours after fertilization in both experimental variants.

In the V1 variant, the duration of the treatment time was 10 minutes, without taking into account the water temperature.

In version V2, the duration of the treatment was dependent on the water temperature, as follows: 5 minutes at a water temperature of 8-10°C; 10 min at a water temperature of 11-13°C or 15 min at a water temperature of 14-16°C. The process was repeated every 12 hours, until the embryo surrounded the entire yolk sac, the tail reached the eyes, the pigmentation is accentuated, the movements of the embryo became more intense and the heart pulsations were observed.

For estimation of embryo survival and identification of fungi, during embryonic development, the collected samples were observed under a microscope (10X objective), on which occasion the viability and the degree of infestation were determined. Opaque eggs were determined to be dead, and clear eggs with adequate cell division were considered viable. Fungal infections are easy to spot, appearing as white or brown, cotton-like growths consisting of many small filaments.

The age or stage of embryonic development can be a significant factor in the management of the disease (Radu et al., 2020). In the early stages of embryonic development, stress can be more harmful and influence the survival rate. Understanding the development of *Saprolegnia* spp. is important for improving the hatch rate, which helps in the planning and duration of preventive treatments. During the embryonic development period, spawn samples were collected and used both to assess the hatch rate and to determine the number of hatched larvae.

It was found that, depending on the temperature of the water, after 10 to 14 hours after fertilization, the end of gastrulation takes place. In addition, the stage of development can be determined, during which the antifungal treatment continues. After 150-180 hours of incubation, at a temperature of 9-15ºC, the process of embryonic development ended and hatching began, which lasted 18-24 hours.

Survival from spawn to larvae was determined with the formula (Olaniyi et al., 2013):

$$Sv\ (\%) = \text{Number of larvae} \times 100/\text{Number of eggs for incubation}$$

Losses due to infection with *Saprolegnia* sp. were determined as follows:

$$\text{Losses}\ (\%) = \text{Number of larvae} \times 100/\text{Number of embryonated eggs}$$

Under the conditions of the artificial breeding station at SCDP Nucet, the time required for the incubation of the eggs is directly correlated with the water temperature. Taking into account the exact moment of reproduction correlated with the evolution of temperature, the development of the embryos can be estimated. Temperature is an important environmental factor affecting eggs development, hatch rates and disease susceptibility. Throughout the incubation period, careful monitoring of the physico-chemical parameters of the environment was necessary. These measures can avoid the occurrence of mortality due to the accumulation of large amounts of organic matter, which is a food source for pathogens and can trigger diseases in incubation. Also, high levels of organic matter can reduce the effectiveness of formaldehyde.

The results of the experiment were used in the statistical analyses. The qualitative and quantitative data analysis was performed with MS Excel and represented by tables and graphs obtained from different types of results.

RESULTS AND DISCUSSIONS

Physico-chemical parameters of the technological water were monitored during the whole period of the experiments. The interpretation of the obtained results was carried

out in accordance with the provisions of the "Regulation on the classification of surface water quality", correlated with the data from the specialized literature for waters used for fish farming (Ministry of the Environment and Water Management of Romania, Order no. 161, 2006) (Table 1).

Table 1. Average values of the physico-chemical indicators of water in the experimental period

Curt. No.	The chemical parameter		Unit of measure	Parameter values		
				Source	Incubators	Optimum according to quality standards
				The average of the year 2022		
1	pH		pH units	7.1	7.5	7-7.8
2	Alkalinity		mg/l	162	201	200-400
3	Calcium (Ca^{2+})		mg/l	46.8	44.6	90-120
4	Magnesium (Mg^{2+})		mg/l	19.8	21.4	10-40
5	Ca^{2+} / Mg^{2+}		mg/l	2.36	2.25	5
6	Organic substance		mg $KMnO_4$ /l	15	24.5	20-60
7	Oxygen		mg/l	10.6	8.4	5-12
8	Ammonia (NH_3^+)		mg/l	lack	lack	lack
9	Nitrates (NO_3^-)		mg/l	lack	0.24	2.5-4
10	Nitrites (NO_2^-)		mg/l	0.001	0.002	0.03
11	Phosphates (PO_4^{3-})		mg/l	lack	0.05	0.05-1.5
12	Chloride	Cl^-	mg/l	8.65	8.36	30
		NaCl	mg/l	14.21	14.00	20
13	Ammonium (NH_4^+)		mg/l	lack	0.018	0.5-1
14	Total hardness		(°D)	12.8	13.1	12

During the study period, the dissolved oxygen recorded variations that were between 8.4-10.6 mg/l. The pH of the water was between 7.1 and 7.5, the optimal range for the hatching of the eggs. The organic matter content of the water recorded values between 15-24.5 mg/l. The water temperature measured during the entire experiment recorded values in the range of 9-13°C (Figure 5).

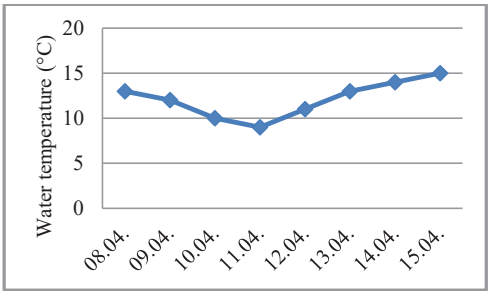

Figure 5. Evolution of the average water temperature

After harvesting the nests with fertilized eggs from the breeding ponds, they were very carefully introduced into the nytal keeping net of the Nucet type incubators (Figures 3 and 4), where a permanent supply of water was ensured, at a flow rate of 8 l/min.

From the specialized data and from the observations made, it was possible to find out the fact that the duration of the embryonic development process in the pike-perch eggs directly depends on the temperature of the technological water at which the embryonic development takes place. In Table 2 and Figure 5 it shows the duration of this process at the pike-perch depending on the water temperature. From the macro/microscopic observations it was found that, shortly after fertilization (1-2 hours), the diameter of the eggs varies between 1.2 and 1.4 mm. After 48-50 hours, under the conditions of embryonic development at water temperatures of 12-14°C, the embryo is in the gastrula stage. After 70-72 hours, the formation of the body of the embryo (in which 21

myomeres can be distinguished) and which half surrounds the yolk sac was observed. The eyes are slightly pigmented, the heart and blood circulation can be seen. After 4-5 days, a visible growth of the embryo became visible with the naked eye, whose body surrounds the yolk sac almost one and a half times, microscopically about 34 myomeres are visible. The eyes of the embryo are pigmented and have a brown colour. The primordia of the nasal orifice, the heart and the blood circulation can be distinguished, and inside the egg it is easy to observe the wriggling movements of the embryo, a sign that the moment of hatching is close.

Table 2. Average water temperature during the incubation period

Specification	Date								
	08.04	09.04	10.04	11.04	12.04	13.04	14.04	15.04	Total
Water temperature (°C)	13	12	10	9	11	13	14	15	-
No. baths/day	0	2	2	2	2	2	2	1	13
Exposure time (min.) V1	0	20	20	20	20	20	20	10	130
Exposure time (min.) V2	0	20	10	10	20	20	30	30	140
Amount of formaldehyde (liters) V 1	0	0.476	0.476	0.476	0.476	0.476	0.476	0.238	3.094
Concentration of formaldehyde (ml / water liter / treatment)	0	1.7	1.7	1.7	1.7	1.7	1.7	1.7	-

After the 7th to the 8th day, at the time of hatching, it was observed that the embryo surrounds the yolk sac twice, the eyes are strongly pigmented. Under the binocular microscope, small brown chromatophores were visible in the head area, on the yolk sac and to a lesser extent on the rest, while the digestive tube, the heart and pink blood and the fin fold were also visible. Figure 6 shows the larva of the pike-perch before the moment of hatching.

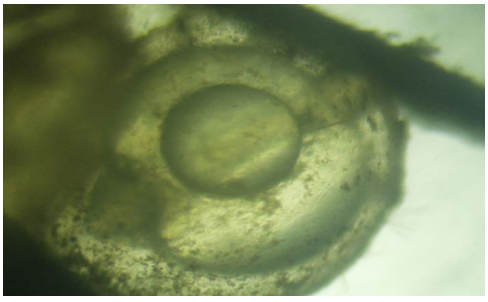

Figure 6. Pike-perch larva before the moment of hatching (Original photo)

During the incubation period, the average number of eggs deposited in the nests and introduced for incubation was calculated. The percentage of fertilization was determined by sampling the eggs deposited on the willow whiskers from each incubator and counting those with embryos and those not fertilized (which are opaque and whitish). Also, during this period, the percentage of hatching was determined, which was achieved in the phase when the heart pulsations and the movement of the embryo could be observed, and depending on the value of this percentage, the number of pike-perch larvae that were to hatch was determined.

The incubation period was 6-8 days at the average daily water temperature of 12-15°C.

After hatching, the remains of the willow whiskers were removed from the Nucet incubators, the larvae were kept in the incubators until they were 7-8 days old, during which time the water intake rate of the incubators was reduced to 4-5 l/min. Taking into account the fact that the larvae are sensitive and swim slowly, 2-3 frames of nytal were placed in the incubators for them to support and rest.

The comparative analysis took into account the values of the technological indicators from the experimental variant in which the treatments were carried out for 10 minutes/treatment, compared to the values of the technological indicators from the variant in which the treatments were carried out differently depending on the water temperature, respectively 5 minutes at the temperature of 8-10°C, 10 minutes at a temperature of 11-13°C and 15 minutes at a temperature of 14-16°C (Figure 7).

During the incubation period, the water temperature was recorded daily, the average number of eggs deposited in the nests and

introduced to the incubation, as well as the fertilization percentage, were evaluated.
During the incubation, the water temperature was recorded daily, the average number of eggs deposited in the nests and introduced to the incubation, as well as the percentage of fertilization was evaluated (Table 3 and Figure 8). The incubation period was 7-8 days at an average daily water temperature of 13.5°C.

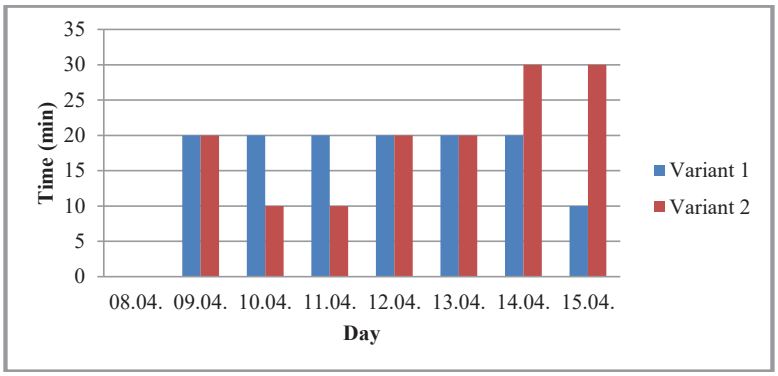

Figure 7. Evolution of treatment duration

Table 3. Biotechnological indicators obtained after treatmentof the embryonated eggs in the two experimental variants

Curt. No.	Biotechnological indicators	Unit of measure	V1	V2
1	Eggs for incubation	1000 pcs.	820.20	825
2	No. incubators	pcs.	3	3
3	No. eggs/incubator	1000 pcs.	273.40	275
4	Fertilization percentage	%	94.30 ± 3.77	94.20 ± 4.02
5	Number of fertilized eggs	1000 pcs.	773.4	777.2
6	Losses due to infection with *Saprolegnia spp.*	%	14.60	4.80
7	Hatching larvae	1000 pcs.	645.50	729.30
8	Incubation survival	%	78.70 ± 4.32	88.40 ± 4.27

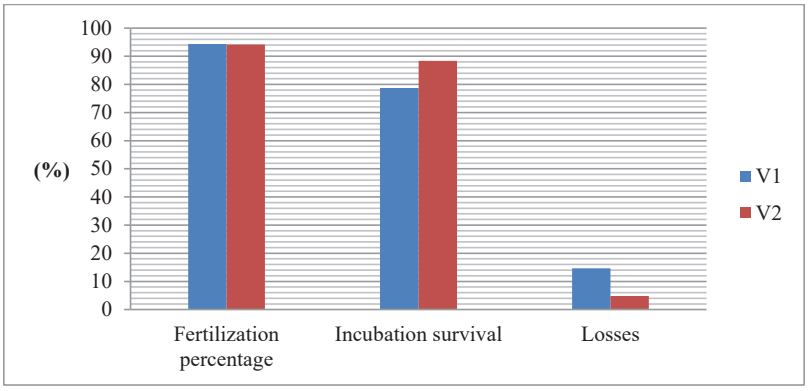

Figure 8. Fertilization percentage, incubation survival and losses recorded during the incubation period

The hatching rate was 88.4 ± 4.27% in the V2 variant and 78.7 ± 4.32% in the V1 variant.
The fertilization percentage had similar values in both experimental variants, with the following values: 94.3 ± 3.77% in variant V1 and 94.2 ± 4.02% in variant V2. Losses due to infection with *Saprolegnia* spp. recorded different

values, good results in the V2 variant (4.8%) and satisfactory in the V1 variant (14.6%).

CONCLUSIONS

The economic losses caused by fungal infestation can be severe and consequently disinfection measures must be taken. Each treatment has an economic value which includes the cost of treatment and the expected economic benefits.

Proper use of regulated products, some of which are quite expensive, can be important in preventing significant economic losses.

After the treatment carried out in the two experimental variants, the results demonstrated that the most effective treatment for preventing and combating *Saprolegnia* spp. infection is in variant 2 where the treatments were carried out depending on the water temperature.

Formaldehyde has the ability to inhibit the growth and spread of the fungus to live or dead eggs with superior results when taking into account the two essential factors in this process, namely: temperature and exposure time.

For the prevention and control of the disease, the treatments must be carried out rigorously, and the technological water must fit within the characteristics of the regulations in force from the point of view of environmental factors.

The correct use of regulated products, some of which are quite expensive, can be important in preventing significant economic losses.

REFERENCES

Czeczuga, B., & Kiziewicz, B. (1999). Zoosporic fungi growing on the eggs of *Carassius carassius* L. in oligo - and eutrophic water. *Polish Journal of Environmental Studies*, *8*(2), 63–66.

Dobrota, G., Cristea, V., Dobrota, N. G., Simionov, I. A., & Anghelescu, A. C. (2022). The influence of the population density on the development of the species *Sander lucioperca* (Linnaeus, 1758) in the postembryonic period. *Scientific Papers. Series D. Animal Science*, *LXV*(1), 581-587.

Fitzpatrick, M. S., Shreck, C. B., Chitwood, R. L. & Marking, L. L. (1995). Evaluation of three candidate fungicides for treatment of adult spring chinook salmon. *Progressive Fish-culturist*, *57*, 153-155.

Gieseker, C. M., Serfling, S. G., & Reimschuessel, R. (2006). Formalin treatment to reduce mortality associated with *Saprolegnia parasitica* in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *253*(1-4), 120–129.

Hussein, M. M. A., Hatai, K. & Nomura, T. (2001). Saprolegniosis in salmonids and their eggs in Japan. *Journal of Wildlife Diseases*, *37*(1), 204–207.

Kitancharoen, N., Yamamoto, A. & Hatai, K. (1997). Fungicidal effects of hydrogen peroxide on fungal infection of rainbow trout eggs. *Mycoscience*, *38*(4), 375–378.

Ministry of the Environment and Water Management of Romania (2006). Order no. 161, February 16, 2006. *Official Gazette no. 511, June 13, 2006*.

Olaniyi, A. O., Solomon, O. A., & Olatunde, O. F. (2013). Growth performance and survival rate of *Clarias gariepinus* fed *Lactobacillus acidophilus* supplemented diets. *IOSR Journal of Agriculture and Veterinary Science, 3*(6), 45–50.

Radu, D., Costache, M., Costache, M., & Marica, N. (2020). Prophylaxis method for incubating crap (*C. carpio* L.) eggs. *Scientific Papers-Animal Science Series: Lucrări ştiinţifice – Seria Zootehnie*, *74,* 128–133.

Radu, D., Costache, M., Marica, N., Barbu, A., Nicolae, C. G. (2022). Innovative treatment to combat philopod crustacean (*Cyzicus* sp.) in fish nurseries. Scientific Papers. Series D. Animal Science, *LXV*(2), 426–431.

Thoen, E., Evensen, Ø ., & Skaar, I. (2011). Pathogenicity of *Saprolegnia* spp. to Atlantic salmon, *Salmo salar* L., eggs. *Journal of Fish Diseases*, *34*(8), 601–608.

Willoughby, L.G. (1970). Mycological aspects of a disease of young perch in Windermere. *Journal of Fish Biology*, 2(2), 113–116.