PROPHYLAXIS METHOD FOR INCUBATING CRAP (*C. carpio* L.) EGGS

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Abstract

The paper presents the results obtained in the experiments carried out to control fungal infestations during the incubation of carp eggs. Experiments were carried out between 15 -24 May 2020 at the artificial fish breeding station at F.C.R.D.S. Nucet. Several concentrations of formalin (1 ml / l, 1.67 ml/l and 2 ml/l) and table salt (NaCl) (1 g / l, 3g /l, 5 g/l) were used to determine the duration and frequency of treatment for carp embryonated eggs. The effectiveness of formalin solutions was evaluated compared to that of table salt solutions by comparing the rate of hatching and larval survival. The results showed that formalin treatments, at a concentration of 1.67 ml / l for 15 minutes, inhibited fungal growth on the surface of embryonated eggs, resulting an incubation survival rate of 79.1% compared to untreated control samples.

Key words: *Cyprinus carpio*, eggs, incubating, prophylaxis method

INTRODUCTION

Aquaculture is a growing industry and an increasingly important supplier of healthy food. The most productive method of obtaining the stocking material is by artificial reproduction. Fish hatchery produce stocking material (larvae and fry) used both in aquaculture and in natural water restocking, to support commercial fishing, recreational fishing and aquatic resource management. One of the most important fish species in the world and in Romania is the carp (*Cyprinus carpio*). The optimal temperature range during the reproduction period is 18 - 23° C and the incubation period is 3 to 5 days. Outside this range, hatching rates are low due to the eggs mortality. During the breeding season, both fish and eggs are particularly susceptible to diseases. Dead or unfertilized eggs are very quickly infected with fungi, if left to proliferate, they affect healthy eggs and carp larvae. Fungi are a group of organisms called heterotrophs that require living or dead matter to grow and reproduce. Fungi are present everywhere: in salt water or fresh water, warm or cold temperatures. In most cases, fungi perform a valuable ecological function by processing dead organic debris. However, fungi can become a problem if fish are stressed by disease, poor environmental conditions, poor nutrition or injury. [4]

Disease control in aquaculture it is more difficult due to the environment in which the fish are reared; the entire production depends on the correlation with the environmental parameters. Poor water quality (low flow rates, low dissolved oxygen, high ammonia water content, high organic load) including the presence of dead eggs, are often associated with *Saprolegnia sp.* infections.[2]

Saprolegniasis is one of the serious fungal infections that causes major economic damage in the aquaculture industry, affecting breeders, fish larvae and eggs during incubation period. When incubating carp eggs, mortality and significant financial losses can occur due to microbial diseases. In embryonated eggs, the mortality rate can reach up to 80-100%. Treatments must be effective, safe and cost-effective.

Malachite green, an organic drug has been used to control saprolegniasis [1]. Regarding to the treatment of fungal diseases and other ectoparasitic diseases such as ichthyophthyrosis, it should be noted that the use of malachite green is no longer allowed on fish farms in the European Union (EU). Alternatively, are recommended therapeutic baths with NaCl, formaldehyde, potassium permanganate, methylene blue, copper sulfate, iodophors etc. Formalin is currently

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used in aquaculture to effectively control saprolegniasis in both fish and eggs. [3]. Table salt (NaCl) is a common harmless substance that has antimicrobial properties.

MATERIAL AND METHODS

The artificial reproduction of *C. carpio*, in 2020 year, at *F.C.R.D.S.* Nucet, was conducted from May 12 to June 9, 2020. To test the effectiveness of prophylactic methods for treating embryonated carp eggs, two experiments were organized that coincided with two batches of breeders selected for artificial reproduction. The experiments were conducted in artificial fish breeding pilot station at Nucet Experimental Base on May 15 and May 20, 2020, respectively. Each batch selected for artificial reproduction consisted of 30 breeders (20 females and 10 males).

Gamete collection and fertilization.

The carp breeders used for artificial reproduction come from the F.C.R.D.S. Nucet live gene bank. Males and females were hormonally stimulated for maturation and reproduction. The collection of gametes (eggs and semen) from breeders was done by "milking" method. A sample of eggs was taken from each female, from which the number of eggs / gram was determined, in order to estimate the number of larvae to be obtained.

The eggs were fertilized by the dry method, after which they were spread on the nytal surface of the frames in a thin layer and then they were incubated in the Nucet type incubator. The frame used to incubate carp eggs consists of a thicker wire frame that is covered with a nytal screen (mesh size: 200-250 µ). The size of a frame is 44.5 x 30 cm = 1335 cm^2. On this type of frame can be incubated a quantity of 45-50 g of eggs, respectively, 30,000-35,000 pieces, corresponding to a density of 25-30 eggs / cm^2. The volume of a Nucet type incubator is 150 l of water and the incubation capacity is 1 kg of eggs (20 frames). The eggs are spread on the frame in a thin layer and as evenly as possible to avoid their agglomeration which could thus facilitate the transmission of diseases. The risks that arise during the incubation of carp eggs are represented mainly by bacterial and fungal infections. The main causes of fungal and bacterial attack during the spawning period are determined by the quality of the environment (low dissolved oxygen values, low water flow, large amount of organic matter, agglomeration and / or eggs overlaying).

Fungal infections are easy to notice, they appear as white or brown growths, similar to cotton, consisting of many small filaments. If left untreated, the filaments can invade and kill healthy adjacent eggs, spreading rapidly and covering the entire mass. For antifungal treatments used during the embryonic period in carp eggs, we tested the effectiveness of two chemicals, respectively, formalin and NaCl, substances approved for such treatments. In order to determine the most effective method of prophylaxis that could reduce / decrease the degree of infestation with *Saprolegnia sp*., the experiments were carried out according to the working protocol which established three treatments in three different concentrations and in three repetitions. (R1, R2, R3). The working protocol that includes the concentration of the substance, the form of administration and the treatment time is presented in table no. 1.

Table 1 Working protocol - (concentration of substance, form of administration and treatment time)

Therapeutic agent / mode of administration	Concentration	Number of repetitions
Formalin (baths of 15 min., 3-4 days depending on water temperature)	1.0 ml /l	R1
		R2
		R3
	1.67 ml/l	R1
		R2
		R3
	2.0 ml/l	R1
		R2
		R3
NaCl (baths of 15 min., 3-4 days depending on water temperature)	1.0 g /l	R1
		R2
		R3
	3.0 g/l	R1
		R2
		R3
	5.0 g /l	R1
		R2
		R3
Control sample	-	R1
		R2
		R3

The baths were performed in specially prepared treatment containers with previously established concentrations solutions. After the

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end of the treatment time, the frames with embryonated eggs are taken out of the bath and introduced back into the incubator where the water flow is adjusted according to the stage of embryonic development.

Estimation of embryos survival and fungal identification

To estimate the eggs survival during embryonic development, eggs samples were collected and observed under a microscope (10X objective), on which occasion the viability and fungal infestation were determined. It has been determined that opaque eggs are already dead; transparent eggs with adequate cell division were considered viable.

Hatching rate determination

The age or stage of embryonic development may be a significant factor in the management of the disease. In the early stages of embryonic development, stress can be more harmful and can influence survival rate. Understanding the development progress is important for improving the hatching rate and estimating the age of embryonated eggs, which helps to plan chemical treatments. During the embryonic development period, eggs samples were collected and used both to assess the hatching rate and to determine the number of hatched larvae.

It was found that, depending on the water temperature, after 10 to 14 hours after fertilization the end of gastrulation occurs (stage 13). In addition, depending on the development stage, the period in which antifungal treatment continues, can be determined. After 55-72 hours of incubation, at a 22ºC temperature, the embryonic development process has ended and hatching began, which lasted 7 - 8 hours.

Hatching rate determination

Hatching rate = Number of larvae × 100 / Total number of fertilized eggs

After hatching, the survival rate of larvae up to four days old was determined.

Larval survival rate estimation

The total survival rate was determined by the total number of larvae that survived after treatment.

Survival = Number of live eggs up to the larval stage × 100 / Total number of hatched larvae

In the conditions of the artificial reproduction fish station from S.C.D.P. Nucet, the time required for incubation for carp eggs is in direct correlation with the water temperature. Taking into account the exact moment of reproduction correlated with the evolution of temperature, the development of embryos can be estimated. Temperature is an important environmental factor that affects the eggs development, hatching rates and the disease susceptibility. Throughout the incubation period it was necessary to carefully monitor the physico-chemical parameters of the incubation environment. These measures can avoid the occurrence of mortality due to large quantities of organic matter accumulation, which, is a source of food for pathogens and can trigger diseases in incubation. Equally, high levels of organic matter can reduce the effectiveness of formalin and NaCl.

RESULTS AND DISCUSSIONS

The first experiment began on May 15, 2020. Fertilized carp eggs were placed in incubation at 00^{10} a.m. The first antifungal treatment was done 24 hours after incubation started. The following treatments were then repeated every 12 hours until the first larvae appeared.

The second experiment started on May 20, 2020. Fertilized carp eggs were incubated at 7^{15} a.m. The treatment protocol was identical to the one used on May 15, 2020.

Many treatment guidelines suggest that the treatment applications should be made until the eyes pigmentation is highlighted (black spots).

Because not all hatching eggs are in the same stage of development, some of them begin to hatch shortly after the eyes appear on most embryos. As a good practice, the literature recommends stopping treatment

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when the embryo's eyes are formed, as there is a risk of killing the hatched larvae.

When the first larvae appeared, the last bath with formalin (concentration 1 ml/ l) was taken for 5-7 minutes depending on the water temperature. Water temperature and quality affect not only the development and survival of carp embryos, but also the potential efficacy and toxicity of chemical treatments.

The physico-chemical parameters of the water monitored at the incubation station were:

Dissolved oxygen registered variations that were between 5.1-7.8 mg / l.

The pH of the water was between 7.5 - 7.8, the optimal range for carp growth.

The organic matter content of the water recorded values between 17.43 - 21.15 mg /l.

The water temperature measured during the whole experiment has recorded values in the range of 19.5 - 23 ° C. The recorded data are represented in figure 1.

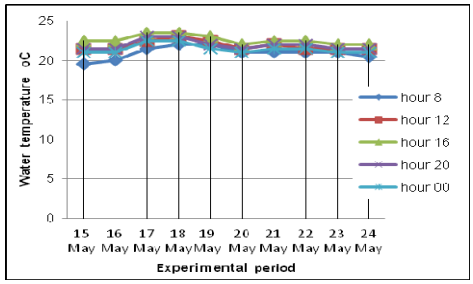

Figure 1 The water temperature during the experimental period

Eggs incubation results and antifungal treatment.

For *C. carpio* species, the number of determined eggs/g was 650 ± 100 pieces /g.

Each group received an unique treatment concentration (formalin, NaCl) that were administered daily in 15-minute baths.

Evaluation of hatching rate results

Hatching began 55-72 hours after incubation. Embryonated eggs were treated with two chemicals in three different concentrations.

The results obtained in the experiment no. 1 when applying formalin and NaCl treatments are shown in Figure 2. The results when applying formalin treatment were as follows: 54.8 ± 4.32%, 79.1 ± 5.29% and 61.5 ± 3.74% at 1 ml / l, 1.67 ml / l and 2.0ml/l, respectively.

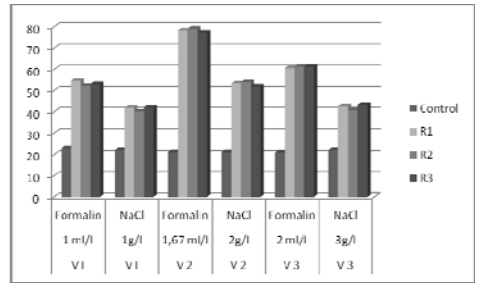

Figure 2 Experiment I hatching rate results

In the case of NaCl treatment the results were as follows: 42.2 ± 5.32%, 54.2 ± 4.34% and 43.67 ± 5.62% at 1 g/l, 3 g /l and 5 g/l.

The results regarding the hatching rate within the experiment no. 2 for formalin and NaCl treatments are shown in Figure 3. For formalin treatment the situation was as follows: 51.4 ± 4.52%, 68.3 ± 4.32% and 56.3 ± 4.78% at 1 ml / l, 1.67 ml / l and 2.0 ml / l, respectively.

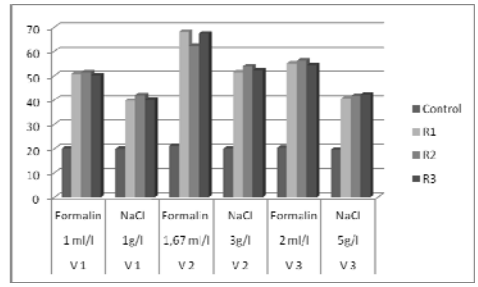

Figure 3 Experiment II hatching rate results

The results for NaCl treatment were as follows: 42.2 ± 5.67%, 53.9 ± 4.89% and 43.3 ± 5.49% at 1 g / l, 3 g / l and 5 g / l, respectively.

Survival rate assessment

After hatching, the survival rate was determined up to four days old larvae.

The results regarding the survival rate in the experiment no. 1 are shown in Figure 4. When using formalin treatment, the larval

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survival rate was 53.2 ± 4.51% 79.8 ± 9.64% and 61.8 ± 6.24% at 1 ml / l, 1.67 ml / l and 2 ml / l, respectively.

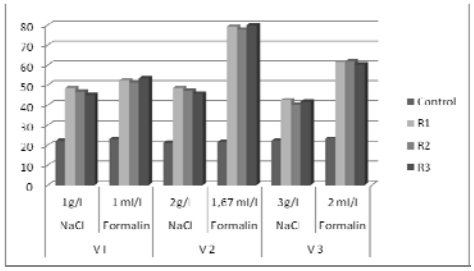

Figure 4 Experiment 1 survival rate

The results obtained in larval survival in the experiment no. 1 with NaCl treatment were as follows: 48.3 ± 4.62%, 48.4 ± 4.53% and 42.4 ± 5.67% at 1 g / l, 3 g / l and 5 g / l, respectively.

The results on the survival rate in the experiment no. 2 are shown in Figure 5. In formalin treatments, the results in larval survival were 54.5 ± 4.62%, 78.4 ± 7.46% and 60.2 ± 6.67% at 1 ml / l, 1.67 ml / l and 2.0 ml / l, respectively.

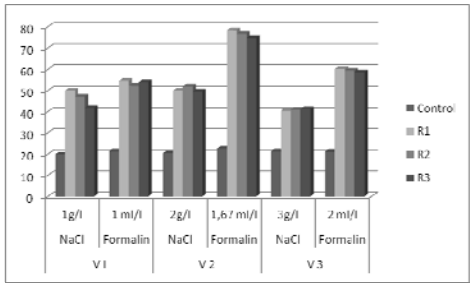

Figure 5 Experiment II Survival rate

The survival rate resulting from NaCl treatment was as follows: 50.0 ± 5.45%, 52.0 ± 4.48% and 41.3 ± 5.74% at 1 g / l, 3 g / l and 5 g / l.

Data analysis

The results obtained from the experiment were used in statistical analyzes. Qualitative and quantitative analyzes of the data were performed. MS Excel has also been used to represent tables and graphs obtained from different types of data.

DISCUSSIONS

After the treatment performed in the experiments with embryonated eggs from experiment no. 1 and 2, the results have shown that the most effective treatment for saprolegniasis is the formalin concentration of 1.67 ml /l. Results of the hatching rate was 79, 1 ± 5.29% and 68.3 ± 4.32%. The survival rate, results were 79.8 ± 9.64% and 78.4 ± 7.46%, respectively.

In formalin treatments with of 1 ml / l and 2.0 ml /l concentration, hatching and survival rates were much lower, but significantly higher comparatively to the NaCl treatment.

Treatment with formalin in a higher concentration resulted in a lower hatching and survival rate. The use of 1 ml / l formalin effectively reduced fungal infection in embryonated eggs of *Cyprinus carpio* larvae.

Saprolegnia sp. has been developed on all the carp eggs from the untreated control incubators, until the eye stage appeared and persisted by the end of the process. The survival rate observed in the control samples was 21%, which was significantly lower than any of the antifungal treatment groups. The larvae malformations were similar between all treatment groups and controls.

Formalin has the ability to inhibit fungal growth and the spread of the fungus from dead to live eggs. In addition, the concentration of 1.67 ml / l formalin would also inhibit any bacterial growth and may explain the increased survival rate observed at this specific treatment concentration.

The low concentrations of both antifungal agents used in the treatment of saprolegniasis allowed the development of fungi in some cases. It should be noted that formalin treatments at a 1 ml / l concentration did not prevent fungal growth in incubators.

Daily formalin treatments at concentrations of 1.67-2.0 ml / l for 15 minutes have been shown to be equally effective in completely inhibiting fungal growth even under suboptimal culture conditions and the use of any treatment is recommended to maximize eggs survival during incubation.

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CONCLUSIONS

For disease prophylaxis and control, treatments should be done as soon as the first signs of fungal infestation are detected.

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.

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