CONDITIONING THE GROWTH OF CARP LARVAE THROUGH APPLICATION OF THERMAL FACTOR

S. Balacci[1*], I. Balan[1], V. Buzan[1], N. Roşca[1]

[1]*Institute of Physiology and Sanocratology, Chișinău, Republic of Moldova*

Abstract

The study aims to highlight the correlation between the temperature of the aquatic environment and the consumption of nutrients of the yolk sac, as well as the determination of the survival of fish larvae. At the same time, the experiments were carried out in order to determine the parameters that could be used as stimulants to increase the adaptive capacities of the animals to the unfavorable action of the environment. The biological material was represented by fish of the species Cyprinus carpio subjected to the application of low temperatures of 9, 12, 15 and 20 °C during different periods of postnatal ontogenesis (1, 3, 5, 7, 10 days). The results obtained showed that the application of the studied temperatures to the carp larvae, whose age at the beginning of the experiments is 1, 2 and 3 days, a variable increase in the studied parameters is recorded and they differ from one experimental series to another and from one temperature to another. It was found that the application of the temperature of 9°C for 10 days leads to the retention of the development of carp larvae with the preservation of the yolk sac for a longer period up to 10-12 days after birth, but the application of temperature of 12°C favors their survival and growth.

Key words: *carp, temperature, yolk sac, survival*

INTRODUCTION

The implementation of modern technologies in aquaculture lead to the emergence of new problems in the process of raising fish associated with a sudden change in maintenance conditions and completely unusual stress factors for them. In order to reduce the negative effects of stress, a new approach to aquaculture is necessary with the involvement of new strategies and tactics for raising animals in order to realize not only the productive potential of the organism, but also the increase of its adaptive capacities, resistance and vitality. Productive fish species, as a rule, have low resistance to the action of environmental factors, which is the result of long-term selection for the purpose of increasing productivity and reducing the level of heterozygosity (Usatîi et al., 2021; Vinogradov, 2021).

In order to correctly assess the huge role that environmental factors play in the life of fish, it is enough to consider the most important of them. Water temperature is the universal and determining environmental factor that has a decisive impact on the life functions of fish determining their growth and development (Gamperl et al., 2021). This factor affects the fish by changing the intensity of enzymatic processes occurring in the body, the activity of food consumption, the nature of metabolism, the course of development of the sexual glands, influencing the improvement or deterioration of the development of the natural food supply (food). Temperature is the external stimulus that determines the beginning of migrations, spawning and wintering of fish (Yarzhombek, 2016; Deutsch et al., 2015; Boltaña et al., 2017).

There are several approaches to solving the problems that arise as a result of the negative impact of stress on the growth and survival of trained fish in aquaculture. We believe that one of the solutions is the application of environmental factors of moderate stressor intensity well determined on organisms in the early postnatal period in order to increase their

[*]Corresponding author: sergiobalacci@gmail.com
The manuscript was received: 26.10.2022
Accepted for publication: 31.10.2022

resistance and adaptive capacities. This would allow, on the one hand, the selection of individuals with increased resistance to stress for the purpose of their further use in commercial cultivation, and on the other hand, the further selection of organisms that would conditionally hereditary adaptive characteristics obtained from the directed action of various environmental factors.

Thus, the aim of these researches was to highlight the effects of low temperatures with a moderate stress intensity on the consumption of nutrients of the yolk sac in carp larvae (Cyprinus carpio), and on their survival. At the same time, the researches were carried out in order to determine the temperature values that could be applied to stimulate the growth of adaptive capacities and the resistance of carp larvae to the unfavorable action of the environment.

MATERIAL AND METHODS

Scientific investigations were carried out on 3 series of carp larvae aged 1, 2 and 3 days. Each series was divided into 4 experimental batches, in which temperatures of 9 ºC, 12 ºC, 15 ºC and 20 ºC were applied. The batch in which the water temperature was 20 ºC (identical to the water temperature during incubation) served as a control. The experiments were carried out in vessels with a capacity of 3 liters of water and a density of 500 larvae per liter. The adaptation period of the larvae to the tested temperatures was 1 hour. The period of application of the studied temperatures was 10 days. In order to maintain the temperature of the water in the aquariums, within the limits of the studied parameters, water of +4+6 ºC was added as necessary. After the end of the thermal factor application period (10 days), the temperature of the water in the aquariums was raised to the temperature of 20 ºC and the experiments were extended until the age of the larvae was 23 days. Throughout the study period, starting from the 3rd day of life, the carp larvae were fed abundantly with zooplankton.

In order to determine the effect of the action of the thermal factor on the functional state, strength and adaptive capabilities of the organism, the parameters of the yolk sac such as its length and height, as well as the number of larvae survived, were studied. Yolk sac parameters were monitored on the 1st, 3rd, 5th, 7th and 10th day of application of the thermal factor. The studied indices were determined each time on 50 carp larvae. The number of larvae survived was monitored at the end of the application period of low temperatures and additionally at the end of the experiments. The dimensions of the yolk sac were assessed with the aid of the MBS-9 stereoscopic binocular microscope, using the 8x micrometer eyepiece with a scale to evaluate the linear size of the studied object with a precision of ±0.1 mm. The determination of the number of larvae survived was carried out by the direct method of counting the larvae. Thus, after the delicate stirring of the contents, 100 ml of water with carp larvae were collected from the aquarium, after which their counting was carried out. In order to have a truthful result, this procedure was repeated 5 times, after which the mathematical calculations were performed to obtain data on the number of larvae in the entire volume of water.

All numerical data obtained in the process of carrying out investigations on carp larvae were mathematically processed using statistical methods (Ivanter, 2010; Merkuryeva, 1963).

RESULTS AND DISCUSSIONS

As mentioned above, in order to achieve the proposed goal and objectives, the carp larvae used in the study, depending on their age (1 day, 2 days and 3 days) were distributed in 3 experimental series, on which they applied temperatures of 9, 12, 15 and 20 ºC.

Data on yolk sac dimensions in carp larvae subjected to the action of the thermal factor of different intensity are presented in tables 1, 2, and 3. The data of table 1 demonstrate that different values of water temperature act differently on the absorption rate of yolk sac in carp larvae aged 1 day. The lower the temperature, the slower it is absorbed. For example, at the temperature of 9 ºC, the length of the yolk sac in larvae subjected to temperature action for 10 days decreased relative to it in the larvae subjected to action for 1 day by 0.46 mm (15.48%) and constituted 2.51±0.08 mm versus 2.97±0.08 mm ($P<0.05$).

Table 1. Yolk sac dimensions in carp larvae aged 1 day subjected to low temperature actions, (mm)

Studied parameters, (mm)	Duration of temperature application, (days)				
	1	3	5	7	10
Applied temperature 9 ℃					
Length	2.97±0.08	2.95±0.07	2.74±0.07	2.62±0.12	2.51±0.08*
Height	0.78±0.01	0.76±0.02	0.70±0.09	0.64±0.08	0.52±0.07*
Applied temperature 12 ℃					
Length	2.91±0.05	2.87±0.06	2.78±0.06	2.42±0.08*	1.87±0.07*
Height	0.69±0.02	0.64±0.03	0.61±0.02*	0.59±0.06	0.33±0.04*
Applied temperature 15 ℃					
Length	2.87±0.04	2.68±0.09	2.46±0.05*	1.62±0.11*	0
Height	0.62±0.02	0.52±0.04	0.46±0.03*	0.34±0.02*	0
Applied temperature 20 ℃					
Length	2.82±0.06	1.52±0.14	0.27±0.07*	0	0
Height	0.37±0.03	0.30±0.04	0.07±0.03*	0	0

Note: ** - the differences are statistically truthful between the experimental and control group (P<0.05).*

At the temperature of 12 ℃ the yolk sac length also decreased significantly by 1.04 mm (35.73%) and was 1.87±0.07 mm compared to 2.91±0.05 mm (P<0.05). When the larvae are exposed to temperatures of 15℃ and 20 ℃, the yolk sac on the 10th day is completely absorbed. More than that, in the control group (20 ℃) the yolk sac is not registered even after 7 days of the experiment, i.e. at the age of 8 days of the larvae, which corresponds to the results obtained in specialized research *(Komlatsky et al., 2020; Kozlov, 1998; Park et al., 2017)*. At the same time, when the larvae were exposed at the temperatures of 15℃ and 20℃, both the length and height of the yolk sac changed to veridical values starting from day 5 (P<0.05).

The horizontal analysis of the obtained data shows a varied decrease in the dimensions of the yolk sac at all parameters of the thermal factor of the experiment. At the temperatures of 9 and 12 ℃, the length and height of the yolk sac decreased veridical from day 10 (P<0.05). The influence of temperatures of 15 and 20℃ produced significant changes in the studied parameters starting from the 5th day, with a more essential pronouncement at the action of the temperature of 20 ℃ (P<0.05).

Next, research was carried out on the influence of low temperature with a moderate stress intensity on the organism of 2-day-old carp larvae. The results obtained are presented in table 2.

Table 2. Yolk sac dimensions in carp larvae aged 2 days subjected to low temperature actions, (mm)

Studied parameters, (mm)	Duration of temperature application, (days)				
	1	3	5	7	10
Applied temperature 9 ℃					
Length	2.71±0.06	2.67±0.07	2.49±0.09	2.23±0.08*	1.82±0.13*
Height	0.68±0.02	0.61±0.03	0.57±0.03	0.48±0.02*	0.32±0.04*
Applied temperature 12 ℃					
Length	2.59±0.11	2.53±0.09	1.95±0.13*	1.34±0.11*	0.82±0.12*
Height	0.53±0.03	0.47±0.04	0.44±0.06	0.33±0.03*	0.14±0.02*
Applied temperature 15 ℃					
Length	2.32±0.07	2.17±0.08	0.74±0.13*	0	0
Height	0.38±0.06	0.33±0.04	0.16±0.05*	0	0
Applied temperature 20 ℃					
Length	1.97±0.08	0.89±0.12*	0	0	0
Height	0.31±0.02	0.21±0.05	0	0	0

Note: ** - the differences are statistically truthful between the experimental and control group (P<0.05)*

From table 2 it is observed that the yolk sac in 2-day-old larvae subjected to the action of the thermal factor of 9°C is present in important dimensions also at the age of 10 days, which at the same time decreased to veridical values (P<0.05). The same evolution supports the yolk sac of the larvae subjected to the action of 12°C temperature, where a decrease in its dimensions is recorded (P<0.05). At the influence of temperature of 15°C, the yolk sac, even if it is reduced to true dimensions, is still present at the age of 5 days and constitutes 0.74±0.13 mm and 0.16±0.05 mm, corresponding to its length and height. The yolk sac dimensions of carp larvae in the control group (20°C) rapidly decreased on the 3rd day (P<0.05) and by the 5th day it was absorbed completely. The linear analysis of the data in the table shows that at the action of the temperature of 9°C applied over 5 days to the larvae, the yolk sac is practically integral (2.49±0.09 mm), and at the action of the temperature of 20°C, the yolk sac is not registered.

From the comparative analysis of the data of tables 1 and 2 it is observed that at all temperatures studied and throughout their application the size of the yolk sac in 2-day-old larvae subjected to the action of the thermal factor is smaller compared to the size of the yolk sac in 1-day-old larvae. The yolk sac in 2-day-old larvae subjected to the action of 9 °C temperature for 10 days shrank compared from this in 1-day-old larvae by 0.69 mm (27.49%), at 12 °C temperature by 1.05 mm (56.14%). At the same time, at higher temperatures (15°C and 20°C) the yolk sac is absorbed completely. More than that, it should be noted that at 15°C it is absent in 2-day-old larvae compared to the yolk sac in 1-day-old larvae, the length and height of which at the application of temperatures for 7 days constituted respectively 1.62±0.11 and 0.34±0.02 mm. When applying temperatures of 20°C for 5 days (which corresponds to the age of the larvae of 7 days), the yolk sac is practically not noted, which corresponds to the data of the specialized literature (Komlatsky et al., 2020; Kozlov, 1998; Park et al., 2017). It is also worth mentioning that the absorption rate of the yolk sac in the series of experiments with 2-day-old larvae is higher compared to the absorption time in the experiment with 1-day-old carp larvae. The intensity of this process is predetermined by the fact that the larvae of the first series of experiments were maintained at the temperature of 20°C (the temperature that is considered physiologically optimal for the development of carp larvae) for only one day, and the larvae of the second series of experiments were maintained at this temperature 2 days until the start of the experiment, a fact that allowed their organism to develop physiologically normally for twice compared to those of one day.

Data on yolk sac dimensions in carp larvae subjected to thermal factor actions aged 3 days are presented in table 3.

Table 3. Yolk sac dimensions in carp larvae aged 3 days subjected to low temperature actions, (mm)

Studied parameters, (mm)	Duration of temperature application, (days)				
	1	3	5	7	10
Applied temperature 9 ℃					
Length	1.84±0.07	1.49±0.09*	1.17±0.05*	0.91±0.03*	0.55±0.06*
Height	0.34±0.05	0.28±0.12	0.15±0.04*	0.13±0.06*	0.09±0.01*
Applied temperature 12 ℃					
Length	1.62±0.08	1.24±0.04*	0	0	0
Height	0.21±0.01	0.16±0.02	0	0	0
Applied temperature 15 ℃					
Length	1.47±0.03	0.28±0.07*	0	0	0
Height	0.18±0.07	0.05±0.01	0	0	0
Applied temperature 20 ℃					
Length	1.38±0.04	0.18±0.03*	0	0	0
Height	0.23±0.02	0.04±0.02*	0	0	0

Note: ** - the differences are statistically truthful between the experimental and control group (P<0.05)*

The data of table 3 show that the yolk sac in carp larvae is preserved during all periods of application of the temperature of 9 ℃. At the temperature of 12, 15 and 20 ℃ the yolk sac is recorded only up to the duration of the application of the stresoric factor for 3 days. At the duration of application of low temperatures for 5 days, the yolk sac is no longer determined. It is worth noting that this period corresponds to the physiological age of the larvae of 8 days and is consistent with the data from the specialized literature regarding the duration of the existence of the yolk sac (Komlatsky et al., 2020; Kozlov, 1998; Park et al., 2017).

The duration of application of low temperature of a moderate stress intensity during the first 3 days produces changes in the yolk sac throughout the range of experimental application temperatures (9-20 ℃). At the temperature of 9 ℃, the absorption intensity of the yolk sac occurs with a slow uniform speed having truthful values throughout the application of the thermal factor. Therefore, carp larvae at experimental ages, including and at the 10th day, benefit from the yolk sac resources. At the temperature of 12 ℃ yolk sac absorption occurred at a rapid rate between days 3-5, from 1.24±0.04 mm to 0 mm. The sudden reduction in yolk sac sizes at the influence of temperatures of 15 and 20 ℃ occurred earlier between 1 and 3 days.

The size of the yolk sac in carp larvae aged 3 days, which were subjected to the action of the stressor factor according to the comparative analysis of the results of the tables mentioned above is smaller compared to its size in larvae of 1 and 2 days at all varieties of temperatures and throughout experimental duration. The action of the temperature of 9 ℃ for 10 days caused the reduction of the yolk sac at the 3-day-old carp larvae in obvious relation to those aged 2 and 1 day, which constitutes 0.55±0.06 mm, 1.82± 0.13 mm and 2.51±0.08 mm respectively, with a difference of 1.27 mm between 3 and 2 days and 1.96 mm between 3 and 1 day. At the influence of temperature of 12 ℃ yolk sac dimensions in 3-day-old larvae were 2.04 times smaller compared to the yolk sac values of 2-day-old larvae and 2.31 times in 1-day-old larvae. At this temperature in the following days the yolk sac at the 3-day larvae was totally absorbed, and at the 2- and 1-day-old larvae the yolk sac was absorbed more slowly and served as an energy source until the end of the experimental series (10 days).

In this series of experiments, the rapid absorption of the yolk sac in the carp larvae was conditioned by the fact that until the initiation of the experiments, they were maintained in optimal conditions (+20 ℃) of physiological development of the organism for 3 days, which favored the intensity of energy consumption and the metabolism of nutrients, causing the reduction of the yolk sac. Therefore, at the beginning of the experiments in this series, the yolk sac of the carp larvae was already much more consumed compared to its dimensions in the other experimental series.

The following research focused on the study of the survival index of carp larvae after the application of low temperatures for 10 days. The obtained data of these researches are presented in table 4.

Table 4. Survival of carp larvae after application of low temperatures for 10 days, (heads)

Series of experiments	Applied temperature			
	9 ℃	12 ℃	15 ℃	20 ℃
I	1359.7±15.23*	1377.2±14.73*	1362.4±16.77*	1298.6±17.08
II	1278.4±13.98	1383.9±16.11*	1365.2±16.28*	1271.4±16.49
III	1173.6±15.43	1326.7±16.07*	1204.9±14.32	1168.1±17.23

Note: ** - the differences are statistically truthful between the experimental and control group (P<0.05).*

The data of table 4 demonstrate that after the application of the stressor factor the highest number of surviving larvae was recorded at 1-day-old larvae at all studied temperatures compared to 2- and 3-day-old larvae (with exception of the experimental

series II at 12 ℃ and 15 ℃). For example, at the temperature of 9 ℃, the number of 1-day-old larvae was 1359.7±15.23 heads, and the number of 3-day-old larvae was 1173.6±15.43 heads, i.e. by 186.1 heads less. At the temperature of 12 ℃ the number of 1-day-old larvae was 1383.9±16.11 heads, and of 3-day-old larvae - 1326.7±16.07 heads, with 57.2 heads less. The most pronounced difference at the temperature of 15 ℃ is recorded in the experimental series II and III, and this constituted 160.3 heads. Therefore, the highest variability in the number of surviving larvae was recorded at the application of the temperature of 9℃, decreasing after 10 days of thermal action on the 3-day-old larvae compared to the 1-day-old larvae by 13.68%.

The obtained results show that the highest survival of carp larvae was recorded at the temperature of 12 ℃ in all experimental groups I, II, III and constituted 1377.2±14.73; 1383.9±16.11 and 1326.7±16.07 heads compared to the number of surviving larvae in all three experimental series from the control group (P<0.05). At the temperature of 15 ℃, the number of surviving larvae maintained high at statistically authentic values in experimental series I and II (P<0.05). In this context, it should be mentioned that, although the number of larvae that survived at the action of the temperature of 15 ℃ has essential values compared to those of the control group, they are still lower than the number of larvae subjected to the action of the temperature of 12 ℃. In the control group, the lowest survival values of fish larvae were recorded, which decreased by 13.42%, 15.24% and 22.12%, for the series of experiments I, II and III, respectively. The values of this index are not consistent with some statements in the specialized literature, which demonstrate that the optimal maintenance temperature of carp larvae in the first days of life is 20 ℃.

Thus, the most favorable temperature for the survival of carp larvae at the age of 1, 2 and 3 days is 12 ℃, applied within 10 days, at which the most efficient metabolism of nutrients in the yolk sac takes place, which contributed to the increase of resistance and well-being of the organism.

Next researches was applied on the survival of carp larvae after applying low temperatures for 10 days and after their subsequent maintenance under industrial biotechnology conditions (20 ℃) until the age of 23 days. The results obtained are presented in table 5.

Table 5. Survival of carp larvae at the end of the experiment, aged 23 days, (heads)

Series of experiments	Applied temperature			
	9 ℃	12 ℃	15 ℃	20 ℃
I	1284.8±12.04*	1305.7±13.89*	1287.4±14.34*	1244.3±10.07
II	1263.2±13.52	1323.9±10.78*	1245.7±13.96	1223.8±14.43
III	1120.5±11.17	1266.3±14.06*	1074.1±14.08*	1114.5±13.86

Note: ** - the differences are statistically truthful between the experimental and control group (P<0.05).*

The data of table 5 show that the survival of carp larvae at the end of the experiment, aged 23 days, undergoes variable changes, both in increasing the values of the survival indicator and in decreasing them. Moreover, significant changes were found in the researched parameters within the same series of experiments, as well as in all research series. In the first experimental series, the major survival of carp larvae was determined at all temperatures of 9 ℃, 12 ℃ and 15 ℃, being higher by 1.5, 4.9 and 3.5% compared to the control group (P<0, 05). Similarly the processes also evolved at the larvae of experimental series II. Thus, the values of the survival of the larvae at the temperatures of 9℃, 12 ℃ and 15 ℃ were 1263.2±13.52, 1323.9±10.78 and 1245.7±13.96 heads and were higher by 3.2%, 8.2% and 1.8%. In the experimental series III, the increase in the value of larval survival was recorded only at the application of the temperature of 9 ℃, 12℃, and at the temperature of 15 ℃ its value decreased (P<0.05).

The survival of carp larvae at all thermal parameters applied in the research groups shows that it is decreasing, with minimum values recorded in the experimental series III. The decrease in the number of larvae according to the applied temperatures was 164, 39 and 213 heads for the temperatures of 9 °C, 12 °C and 15 °C, respectively.

So, the results presented in the table established that the highest number of live carp larvae was recorded under the action of the temperature of 12 °C in all three experimental series. At the same time, the maximum survival of the larvae in the conducted study was notified in the experimental series II, the same after the action of the temperature of 12 °C, series in which the larvae at the onset of the experiments were 2 days old.

CONCLUSIONS

From the data obtained as a result of the experiments it was established that at the application of the temperature of 9, 12 and 15 °C on the carp larvae, whose age at the beginning of the experiments is 1, 2 and 3 days, there is a variable increase in the studied parameters and they differ from one experimental series to another and from one temperature to another.

The application of low temperatures to carp larvae leads to the retention of their growth with the preservation of the yolk sac for a period of up to 10-12 days from birth. These results are more evident when applying temperatures of 9 °C in the series, in which the larvae at the onset of the experiment were 1 day old.

The most favorable temperature for the survival of carp larvae in the three series of experiments is 12 °C. We believe that it is at this temperature that the most efficient metabolism of nutrients in the yolk sac takes place, which contributes to increasing the body's resistance and well-being.

So, by applying the thermal factor of 9 °C, it is possible to slow down in a directed manner the duration of the absorption period of the yolk sac of the carp larvae in all series of experiments, and by applying the temperature of 12 °C, it is possible to increase in a directed manner the survival of the larvae in the experimental series II and III in relation to the control.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from the Project 20.80009.7007.25 „Methods and procedures for maintenance and conservation of biodiversity depending on the integrity of gametogenesis and food variability".

REFERENCES

1. Boltaña, S., Sanhueza, N., Aguilar, A., Gallardo-Escarate, C., Arriagada, G., Valdes, J.A., Soto, D., Quiñones, R.A. (2017). Influences of thermal environment on fish growth. Ecol Evol., nr. 17: p. 6814-6825.
2. Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H-O., Huey, R.B. (2015). Climate change tightens a metabolic constraint on marine habitats. Science, nr. 348: p. 1132-1135.
3. Gamperl, A.K., Syme, D.A. (2021). Temperature effects on the contractile performance and efficiency of oxidative muscle from a eurythermal versus a stenothermal salmonid. J. Exp. Biol., nr. 15: p. 1-14.
4. Ivanter, E.V. (2010). Elementary biometrics. Petrozavodsk, RU: PetrGU.
5. Komlatsky, V.I., Komlatsky, G.V., Velichko, V.A. (2020). Fish farming: a textbook for open source software. St. Petersburg, RU: Lan.
6. Kozlov, V.I. (1998). Handbook of a fish farmer. Moscow, RU: Publishing house VNIRO.
7. Merkuryeva, E.K. (1963). Fundamentals of biometrics. Moscow, RU: Publishing house of Moscow State University.
8. Park, J.M., Mun, S.J., Yim, H.S., Han, K.H. (2017). Egg Development and Larvae and Juveniles Morphology of Carp, Cyprinus carpio in Korean. Dev. Reprod., nr. 3: p. 287-295.
9. Usatîi, A., Șaptefrați, N., Bulat, D. (2021). Good practices in fish farming in the context of climate change. Practical guide for agricultural producers. Chișinău, MD.
10. Vinogradov, E.V. (2021). Stress resistance of carp (Cyprinus carpio, L.) in early ontogenesis and its influence on fish breeding and biological characteristics. Moscow, RU: Dissertation for the degree of candidate of biological sciences.
11. Yarzhombek, A.A. (2016). Lifestyle and behavior of commercial fish. Moscow, RU: Publishing House of VNIRO.