Features of artemia salina ontogenesis in aquaculture depending on the salt level

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Abstract. The work is devoted to the study of influence of salinity level at the rate of hatching, productivity and morphometric parameters of artemia in aquaculture. The cultivation technology of Artemia salina in aquaculture for obtaining live starter feeds has been used in practice for a long time, but it is still far from perfect, since it is developed for a certain averaged artemia, and in practice we have to deal with specific species and their ecomorphs (ecological morphotypes), the optimal conditions of cultivation of which differ in many ways. One of the most important factors determining the development of artemia is the salinity of the environment. The aim of our study was to assess the effect of the salinity level of culture medium at the rate of hatching, productivity and morphometric parameters of artemia grown in industrial aquaculture. The results of the study showed that with an increase in the concentration of salt in the solution for cultivation of artemia, metamorphosis of the free embryo in nauplia is prolonged and the percentage of their yield decreases. Differences in the average population indicators of length and body weight of nauplia were found, which tended to decrease with an increase in water salt concentration. When studying the absolute fertility of female artemia grown at different concentrations of salt, it was found that an increase in water salinity in reproductive age of females makes it possible to increase their absolute fertility when breeding in artificial conditions.

1 Introduction

Aquaculture in Russia today is in the stage of intensive development. Artemia crustaceans are widely used as a starting live food in early ontogenesis of fish; they are most often used for feeding larvae [1].

Most often, artemia nauplii are used in feeding [1], but decapsulated eggs are also among the valuable feeds, the biomass of juvenile and adult stages of artemia crustaceans is used to feed juveniles and grown-up fish [2, 3]. Artemia (at the stage of nauplia) contain a large amount of vitamins, essential polyunsaturated fatty acids, amino acids, biogenic and microelements.

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In natural ecosystems, artemias live at different levels of salinity, so natural races or ecomorphs are formed. Some species live in water with a salinity of 3%, while others need a salinity of 12-15% [3]. Artemia is able to survive for several days in solutions containing potassium permanganate or silver nitrate [4], but the presence of iodine is poorly beard by them [4, 5]. It is known that artemias living at a low level of salinity have a greenish transparent color, and the inhabitants of ecosystems with a high level of salinity usually have an orange-red color scheme. Abiotic factors, in particular salinity and temperature, in hyperhaline reservoirs have a significant impact on the reproductive indicators of artemia [6].

The most important environmental factors determining the duration of life cycle of artemia include interrelated factors of salinity and water content. The influence of abiotic factors increases at the final stage of reproductive period of the population. The life expectancy of artemia in its natural habitat and the number of its reproductive cycles naturally decreases with an increase in the salinity of the habitat [7].

According to the literature data, water mineralization affects both the morphometric parameters of crustaceans and, in general, the quantity and biomass of artemia, for which the optimal salinity values lie in the range of 100-200 g/dm3 [2, 6]. Also, water salinity has a great influence on the growth and reproduction of artemia crustaceans, both in the natural environment and in aquaculture [7, 8].

In the studies of scientists, the minimum fertility values were noted at critical values of water salinity for the survival of crustaceans, the maximum values were at 150-160 and 250 g/kg [5, 9]. Higher salinity led not only to a decrease in lifespan, but also to a decrease in the rate of linear growth of crustaceans and to later maturation. At the same time, an important indicator of reproductive potential decreased with increasing salt concentration [10-13].

A number of authors believe that a sufficient salt content in the culture medium for artemia is 25-30 g/l [3, 13-16].

The aim of the research is to investigate the nature of influence of salinity level of the environment at the rate of hatching, productivity and morphometric parameters of artemia in aquaculture.

2 Research materials and methods

In the studies, artemia cysts were used, supplied for research by « OOO Dinat-Vneshtorg ».

We studied the effect of water salinity on the development of artemia of the A. var. Principalis race in an artificially created environment. To determine which salinity level is optimal for the cultivation of artemia of the A. var. Principalis race three experiments were carried out.

In the first experiment, artemia cysts were cultured in 3% NaCl solution (30 g per 1 liter of water), in the second experiment - at 6% NaCl (60 g per 1 liter of water), in the third - at 9% NaCl (90 g per 1 liter of water). Cultivation was carried out at fixed other abiotic factors: temperature - 28 ° C, acidity of the medium - pH 8.2, luminous intensity – 2000 lux.

The laying of cysts for incubation was carried out at the rate of 2.5 g per 1 liter of solution. The proportion of hatched nauplii was determined by direct counting of individuals under a biological microscope Micromed 2 (var. 3-20).

To make the calculation, with running aeration, 10 ml of culture medium with artemia was taken three times, samples were combined, mixed, a third part was taken, which was diluted tenfold. 1 ml of diluted sample was placed on a slide for counting and 1 drop of formalin was added to paralyze artemia. The calculation was carried out under a small magnification. Calculations were made in three repetitions to obtain averaged results. The average number was multiplied by 10 (to record dilution).

At the end of the mass hatching, aeration was temporarily turned off to remove the empty shell of the cysts that floated to the surface, undeveloped cysts settled, and the nauplia remained in thickness of solution.

Dried spirulina preparation was used to feed nauplii artemii. Before feeding, it was previously pound in a pestle into a fine powder, which was dispersed over water surface during feeding. The cultivation process was monitored every eight hours under a binocular magnifier. At the same time, the state of artemia was evaluated at all stages of ontogenesis, characteristic features of metamorphosis were noted, measurements of the body and its parts were made using an eyepiece micrometer.

During morphometry of nauplii, not only body length was measured using an eyepiece micrometer, but also the mass of 1 individual was determined. To do this, a small volume of nauplia was taken from the culture medium and left to drain in a sieve, then drained with filter paper, the resulting biomass was weighed and divided by the number of individuals in sample.

3 Research results

When doing the work, we researched:

- influence of salinity level on hatching rate and dynamics of artemia metamorphosis in aquaculture conditions;
- parameters of artemia productivity at different levels of salinity;
- morphometric parameters of artemia at different levels of salinity of aquatic environment.

3.1 Influence of salinity level on hatching rate and metamorphosis dynamics

When cultivating artemisia in vitro, water salinity is important for realizing the potential of productivity. In order to select the optimal salinity, three experiments were laid, as noted above. As further studies have shown, the artemia cysts we have belonged to an ecomorph living in conditions of low salinity.

In the first experiment, water salinity was 3%, in the second experiment the salt concentration was 6%, in the third experiment artemia cysts were cultured at a salt concentration of 9%.

At the first stage of the experiment, we studied such an indicator as percentage of hatching of artemia embryos at different levels of salinity of culture medium.

In the first and second experiments, the appearance of free embryos was observed after 15 hours of incubation. The percentage of free embryos that were released as of 15 hours of incubation was 10% and 2%, respectively, in the 1st and 2nd experiments, and then continued for 7 hours.

In the third group, the release of free artemia embryos was slightly delayed and occurred after 16 hours of incubation. The recorded hatching by this time was 10%.

The results of all three experiments are shown in Figure 1.

The result analysis presented in Figure 1 shows that the highest rates of embryo yield were characteristic of the first experiment, in which culture medium was used in a 3% NaCl solution. The output of embryos in the first experiment was 84%.

In the second experiment, in which artemia cysts were cultured in a 6% NaCl solution, the proportion of hatched embryos was 5 percent lower compared to the first experiment.

The hatching of artemia embryos in the third group, in which a 9% NaCl solution was used for cultivation, with an increasing total of 72%, which is 12% lower than in the first experiment (Figure 1).

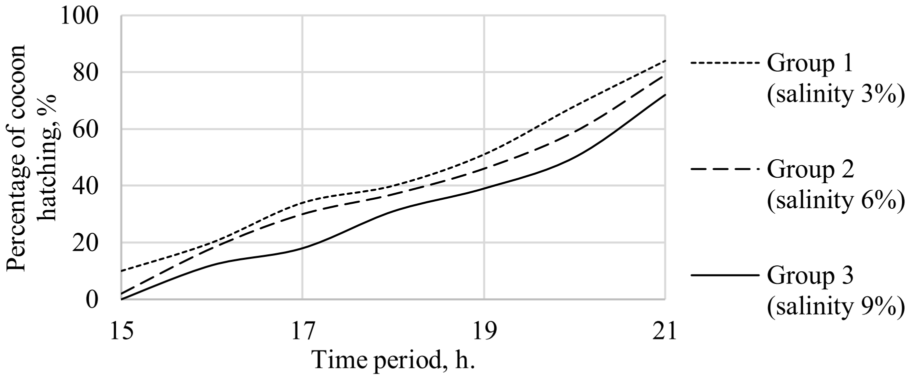

Fig. 1. Dynamics of hatching of artemia embryos depending on the salinity of the culture medium.

At this stage of research, we can conclude that an increase in the concentration of NaCl in the culture medium to 9% during the cultivation of artemia cysts has an inhibitory effect on the cracking process and the hatching of free embryos. For the ecomorphs of artemia at our disposal, the most optimal medium for cultivation was a 3% NaCl solution.

At the next stage of work, the dynamics of hatching of artemia nauplii and the influence of the salinity of culture medium on this process were studied.

After 30 hours of cultivation, the hatching of nauplia in the first and second experiments averaged 35±3.8% and 31±2.4%, respectively; in the third experiment, the proportion of developed nauplia was 28± 2.6% (Figure 2).

Fig. 2. Stages of nauplia development in the conditions of aquaculture.

In our experiments on the cultivation of artemia at different concentrations of NaCl, the asynchrony of nauplia development was noted. The period of nauplia development was stretched in time and from the laying of cysts to the hatching of nauplia was 48-72 hours. The research results are presented in Figure 3 as a cumulative result.

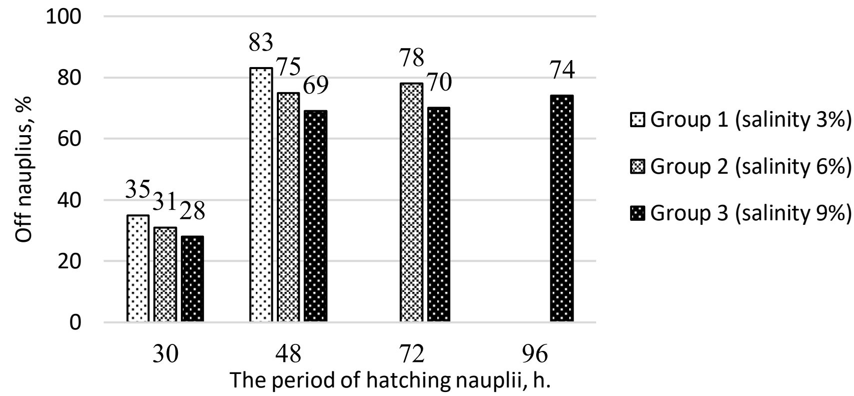

Fig. 3. Dynamics of metamorphosis (cumulative) at different levels of water salinity.

Analysis of the dynamics of metamorphosis into nauplia at different water salinity showed that the fastest development took place in a culture medium with 3% NaCl content, the entire period of development from the nauplia egg was 48 hours. After 30 hours of cultivation in the first experiment, the proportion of nauplia in the culture medium was 35 ± 3.8%, on the second day, if we consider the cumulative total, the proportion of nauplia reached 83 ± 4,1%.

In the second experiment, where cultivation was carried out in an environment with a 6% NaCl content, the period of hatching of nauplias was more extended, compared with the first experiment. In this case, on the first day hatching was 31 ± 3.2%, and the cumulative total on the second day was 75 ± 2,7%. The completion of embryo transformation in nauplia occurred on the third day. During this time, in an environment containing 6% NaCl, the yield of nauplium was 78 ± 2.7%, which is lower than in the first experiment.

In a culture medium with 9% NaCl, metamorphosis was slower than in the first and second experiments and ended on the fourth day. The total percentage of nauplia hatching by the cumulative total in the third experiment was 74 ± 2.1%, which is lower than in culture media with lower salinity (Figure 3).

Thus, we came to the conclusion that with an increase in the concentration of salt in the solution for artemia cultivation, the period of embryo metamorphosis in nauplia is prolonged and the percentage of their yield decreases.

For the production of nauplia as live starter feeds, the 3% concentration of NaCl, for the ecomorphs of artemia at our disposal, turned out to be optimal.

The nauplias hatched from the egg shells are shown in Figure 4. The color of hatched nauplias was greenish-beige, this is confirmation that the crustaceans of this ecomorph live in hyperhaline reservoirs with a low level of salinity [1, 7].

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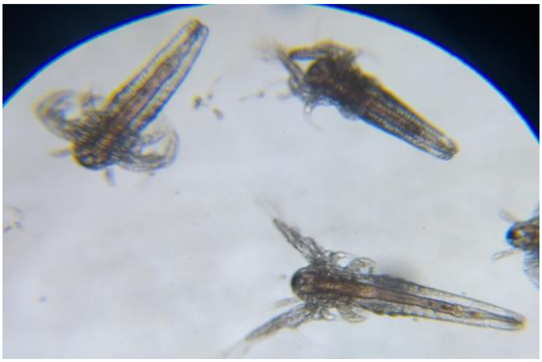

Fig. 4. Hatched nauplii.

At the end of hatching, nauplia morphometry was performed. Body length and mass of 1 individual were measured. To do this, a sample of nauplii was weighed and divided by the number of its constituent individuals. The average individual mass of hatched nauplia in experiments with different levels of salinity of the medium had no significant differences.

There were significant differences in body length between the nauplias cultivated in an environment with a content of 3% and 9% NaCl, the results are shown in Figures 5 and 6.

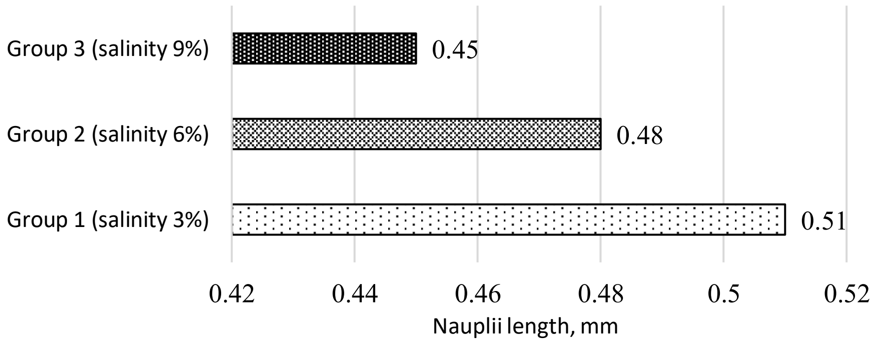

Fig. 5. The length of nauplium body in medium with different NaCl content.

The study of length parameters of hatched nauplii allowed us to establish that with an increase in concentration of salt in water, the average population parameters of length and body weight of nauplii tended to decrease (Figure 5).

The highest indices of body length and mass of nauplia were noted in the first experiment with a 3% NaCl solution, where the linear length of their body was 0.51 ± 0.02 mm, body weight 0.022 ± 0.01 mg. With salt concentration increasing to 6% solution, the indicators of the length and body weight of nauplii decreased, average body length was 0.48 ± 0.02 mm, mass was 0.02 ± 0.01 mg. Even lower values compared to the 1st and 2nd experimental groups were recorded in the 3rd experiment, where body length was 0.45± 0.02 mm, and mass was 0.017 ± 0.01 mg.

As a result, it can be noted that at the stage of nauplia cultivation, with increased salt concentrations up to 9%, there is a tendency to decrease length and body weight of nauplia.

Based on the data obtained, it can be concluded that the concentration of NaCl in the culture medium affects the development of nauplia. The use of increased concentrations at the early stages of cultivation extends hatching process in time and reduces the growth characteristics of the nauplii.

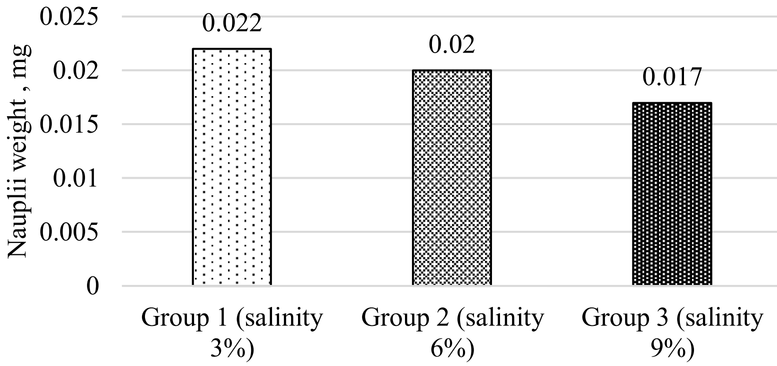

Fig. 6. Body weight of nauplia cultivated in medium with different NaCl content.

If the purpose of cultivation is to quickly obtain nauplia for their use as feed, then the salt content in water at the level of 3% for the ecomorph available to us will be optimal.

3.2 Study of productivity indicators at different levels of salinity in aquaculture

One of the tasks solved within the framework of this study is focused on increasing reproductive potential and production of artemia cysts in conditions of artificial breeding.

The leading factor that has a fundamental impact on the launch of artemia cysts is the salinity of aquatic environment. All types of artemia live in hypergaline reservoirs.

In Kazakhstan, in such lakes as Maraldy, Tuz, Kazy, Kalatuz, salinity reaches 250-300 g/l, and the survival threshold is 360 g/l concentrations [2, 6]. Externally, artemia, living with a high salt content, differ from those living in reservoirs with a low level of salinity by the color of skin [9].

Artemia are inhabitants of reservoirs with a low salt level – greenish or transparent, and artemia are inhabitants of reservoirs with a high salt content approaching the upper threshold of allowable salt concentration is orange [8, 10].

In nature, the spread and development of artemia depends on the salinity of the habitat. The effect of salinity level on the vital activity of artemia is shown in Table 1 (given by Browne, Wanigasekera, 2000).

Table 1. Natural populations of artemia at salinity (g/l).

Salinity, g/l	The influence result on artemia
30-400	The limit of occurrence of crustaceans
70-230	The artemia population is developing normally
70-150	Optimal for increasing the biomass of crustaceans
110-200	Optimal for the production of cysts
30-50 and 250-400	Crustaceans are rare

The race (ectomorph) of artemia that we have in breeding is greenish-transparent. According to the results of our studies, the highest nauplium hatch is observed at 3-6% NaCl content in the culture medium. At this level of salinity, eggs in females were formed on the 21st day. The population was parthenogenetic.

Under favorable conditions, the development of eggs proceeds completely in the broodsac and ends with ovoviviparity: the female casts out either free-floating nauplii, or eggs in which embryogenesis is completed in a few hours.

If living conditions deteriorate, ovoviviparaty stops and females cast out cysts (over 300 within one to two weeks), surrounded by a thick opaque multi-layered shell. A dent forms on dried cysts, which disappears when wet.

To stimulate the production of cysts, we gradually increased the salinity level in the culture medium. For this purpose, 3 additional experiments were laid, in which the first group of mature artemias was grown in a culture medium with 9% NaCl, the second group of mature artemias – with a salt content of 12%, the third group of mature artemias - in a 15% NaCl solution.

The specifics of these 3 experiments were a gradual, even increase in the salinity level: in the first experiment up to 9%, in the second up to 12%, in the third – up to 15%.

Further, daily observations of females were carried out in order to determine the proportion of females who had eggs and their average number was calculated. This was done to determine productivity. The results of the studies are shown in Figures 7 and 8.

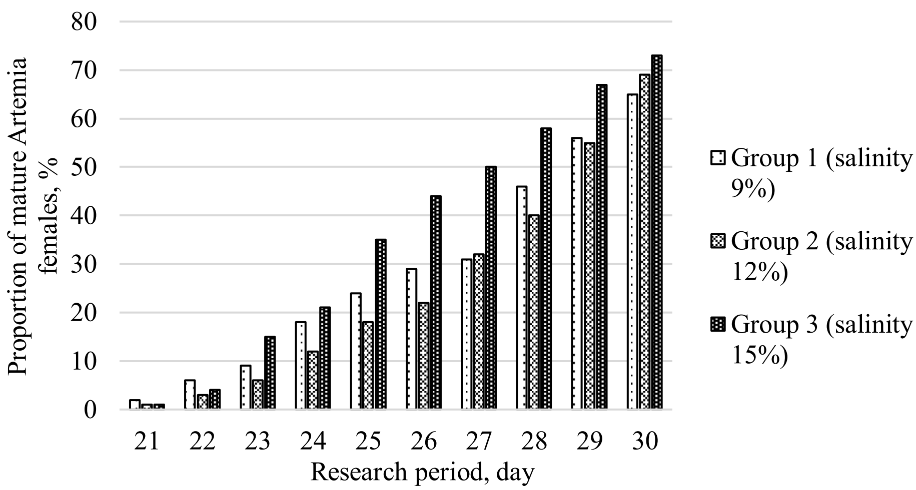

Fig. 7. The proportion of female egg carriers depending on the salinity level.

Analysis of the diagram reflecting synchronous fruiting of females at different levels of salinity showed that this process was most pronounced in the third experiment. The proportion of mature females in the third experiment was the most numerous and amounted to 73± 1.6% of their total number in the experiment.

The number of synchronously fruiting females in the second experiment was 69±2.8%, which is lower than in the experiment with a 15% salt level. The difference is statistically unreliable.

The proportion of female egg carriers in the first experiment at 9% NaCl concentration was 65% ± 2.2%. In the first experiment, there was a tendency to decrease the proportion of fertile females, compared with experiments with a higher level of salinity.

Summing up, it should be noted that for our ecomorph artemia, with an increase in concentration of NaCl in the culture medium up to 15%, the proportion of synchronously fruiting females increased. Growing artemia at different levels of water salinity affects its reproductive process and its indicators.

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According to the literature data, absolute fertility of artemia varies quite widely and ranges from 16 to 184 eggs [3]. Artemia in the fruiting stage is shown in the Figure 8.

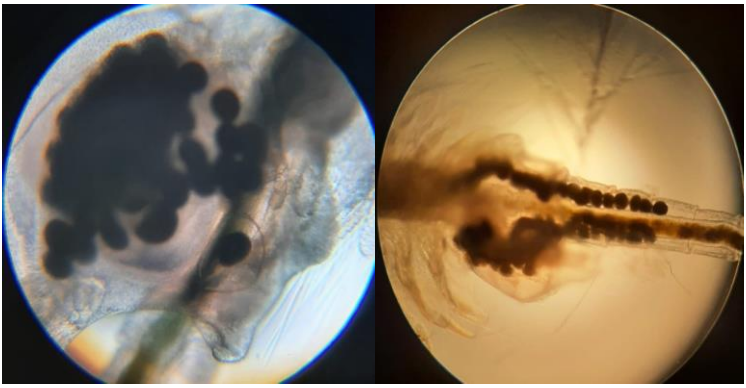

Fig. 8. Artemia in the fruiting stage. Egg sac with unswept eggs.

The conducted studies of absolute fertility of females of available ecomorphs of artemia, depending on the salt content in the culture medium, showed that with an increase in the salinity of water up to 15%, the reproductive activity of females increased. The results obtained are shown in the Figure 9.

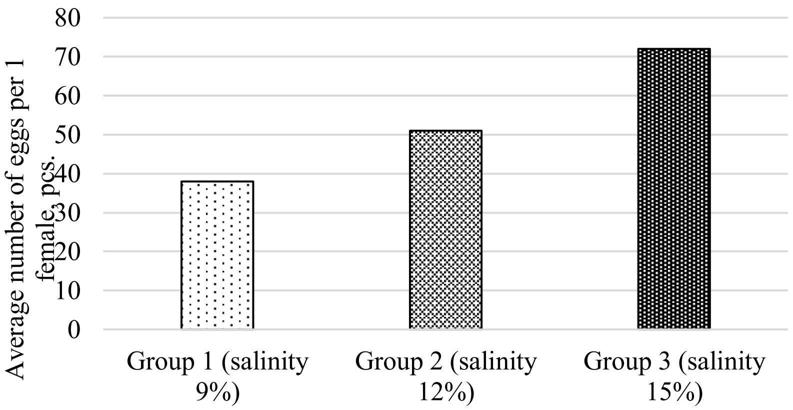

Fig. 9. The effect of salinity level on the number of eggs in one oviposition.

The research results on the fertility of artemia showed that it was mainly realized in the form of eggs and cysts, the percentage of live births was low. The absolute fertility of females increased with increasing salt concentration, the highest rates were noted in the 3 experimental group with 15% saline solution, the average number of eggs per 1 female was 72 ± 10.5 specimens. In the second population group with 12% salt content in the culture medium, the number of eggs per 1 female was 51 ± 8.1 pieces. Lower indicators of absolute fertility were found in females of the first population group at 9% salinity level. The average number of eggs per 1 female was 26 ±6.3 pieces.

The study of absolute fertility of female artemia grown at different concentrations of salt allows us to conclude that an increase in water salinity in the reproductive age of females makes it possible to increase their absolute fertility when breeding in artificial conditions.

The results obtained match the literature data on the reproductive indicators of artemia in natural populations. [4].

4 Discussion of results

The conducted studies allow us to conclude that an increase in concentration of NaCl in culture medium to 9% during the cultivation of artemia cysts has an inhibitory effect on the cracking process and the hatching of free embryos. For the artemia ecomorphs at our disposal, the most optimal medium for cultivating artemia nauplia, widely used in fish farming, was a 3% NaCl solution.

Studies of dynamics of artemia nauplia hatching and the influence of salinity of the culture medium on this process have shown the asynchrony of nauplia development. The period of nauplia development was stretched over time and from the laying of cysts to the hatching of nauplia was 48-72 hours.

Thus, we came to the conclusion that with an increase in the concentration of salt in the solution for cultivation of artemia, embryonic period of development and hatching of nauplia is prolonged and the percentage of their yield decreases.

The study of length parameters of hatched nauplias allowed us to establish that with an increase in the concentration of salt in water, the average population parameters of nauplia length and body weight tended to decrease.

If the purpose of cultivation is to obtain nauplia quickly for their use as feed, then the salt content in water at the level of 3% for the ecomorph at our disposal will be optimal.

For our ecomorph artemia, with an increase in the concentration of NaCl in culture medium up to 15%, the proportion of synchronously fruiting females increased. Growing artemia at different levels of water salinity affects its reproductive process and its indicators.

The research results on the fertility of artemia showed that it was mainly realized in the form of eggs and cysts, the percentage of live births was low.

The high sensitivity of artemia to such an abiotic environmental factor as salinity is consistent with the data of other literary sources.

5 Conclusion

Based on the data obtained, it can be concluded that the concentration of NaCl in culture medium affects the development cycle and cultural characteristics of artemia. The use of increased concentrations of NaCl at the initial stages of cultivation slows down the hatching process over time and reduces the growth characteristics of artemisia.

The degree of sensitivity to high salt content in water increases with the process of puberty of artemia.

The results of the conducted studies have shown that with an increase in concentration of salt in the culture medium during cultivation of artemia, the period of embryo metamorphosis in nauplia is prolonged and the percentage of their yield decreases.

Studies of the absolute fertility of female artemisia grown at different concentrations of salt allow us to conclude that an increase in water salinity in reproductive age of females makes it possible to increase their absolute fertility when breeding in artificial conditions.

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