

STUDY ON EMBRYO DEVELOPMENT IN CARP (CYPRINUS CARPIO) DEPENDING ON WATER WITH DIFFERENT HARDNESS DEGREES

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Abstract: The cyprinids embryo development biology is well-known in many countries of the world, but in Romanian speciality literature there are relatively few references to this. Also there is little information regarding the embryo development environment and especially the way in which certain environment factors influence the embryogenesis of the cyprinids. Several studies considered the water hardness as a factor that would limit the spread of certain species. The aim of this paper is to emphasize the most important aspects of the ways in which water hardness influences development of carp (*Cyprinus carpio*) embryos. During the experiment 5 variants with different hardness degrees of water were used to monitor the development of carp embryos in 80x15 mm Petri dishes at an optimal density (1 embryo/3 ml) and at a 24°C temperature. We wanted to observe the morphological aspect of the embryos and to establish the embryonic stage in which they are at one point and finally to determine the hatching rate. In the hard water variant (V5=20 °dH) the embryos started to die 10 hours after the beginning of the experiment and 36 hours after the fertilization the mortality was 100%. In the moderate hard water variant (V4=15 °dH), after 76 hours of incubation the hatching was at a 100% rate, but all newly hatched carps died when in direct contact with the environment. In the medium hard water variant (V3=10 °dH) after 144 hours of monitoring the one embryo to be in the hatching period for over 60 hours, died inside the corion resulting in a final mortality of 13.3%. In the first two variants of water of low (V2=5 °dH) and very low hardness (control variant, V1=0 °dH) the hatching was of 100%. Statistically, between V1 and V4, respectively V5, there are very significant differences. Between V1 and V2, respectively V3 there is no difference. The research on the influence of water hardness on *Cyprinus carpio* embryos development emphasized that an incubation environment in which such a factor is present, can induce a different embryo development rate.

Key words: carp, embryo development, water hardness

INTRODUCTION

The cyprinids embryo development biology is well-known in many countries of the world, but in Romanian speciality literature there are relatively few references to it. Also there is little information regarding the embryo development environment and especially the way in which certain environment factors influence the embryogenesis of the cyprinids.

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The aim of this paper is to emphasize the most important aspects of the ways in which water hardness influences the development of carp (*Cyprinus carpio*) embryos.

MATERIAL AND METHODS

During the experiment, environments with various hardness degrees were used: 0°dH - (V₁); 5°dH - (V₂); 10°dH - (V₃); 15°dH - (V₄); and 20°dH- (V₅). In order to obtain them, we used anhydrous calcium carbonate, in various quantities, dissolved in purified water, which was obtained with the help of the water purifying system SIMPLICITY 185-MILLIPORE CO.

The fertilized carp roe introduced in each of the thus prepared environments, were obtained through artificial fertilization (the dry method), at the CEFA fishery (SC ProAcva SA Cefa, Cefa, Bihor county) The roe used for the experiment came from clinically healthy breeders, in the breeding season April-May 2008.

The embryo cultures were prepared in Petri dishes of 80x15mm where we introduced 30 ml environment and 10 fertilized roe, ensuring 1 roe/ 3 ml environment.

For each variant 3 repetitions were undertaken, so that, a total of 30 embryo was studied in each variant.

The culture plates were stationary during the entire experiment period and at a constant temperature of 24°C.

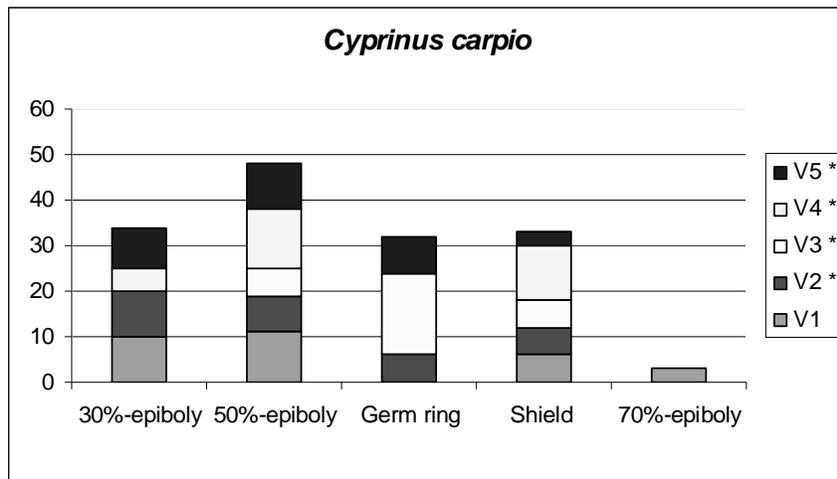
The observations were carried out under the OLYMPUS laboratory optic microscope.

At each reading, the mortality rate and the development stage of the embryos were established. Then, at the end of the experiment, the hatching rate was calculated as the percentage of young larvae from the total incubated roe.

For the statistic processing of the experimental data, the non-parameter concordance χ^2 test was used.

RESULTS AND DISCUSSIONS

Graphs were used for a more suggestive presentation of the data (graphics 1-4).



Graph 1. Water hardness influence upon embryo development in *Cyprinus carpio*, 10 hours from fertilization (* p>0,05; ** p= 0,01 - 0,05; ***p=0,001 - 0,01; ****p<0,001)

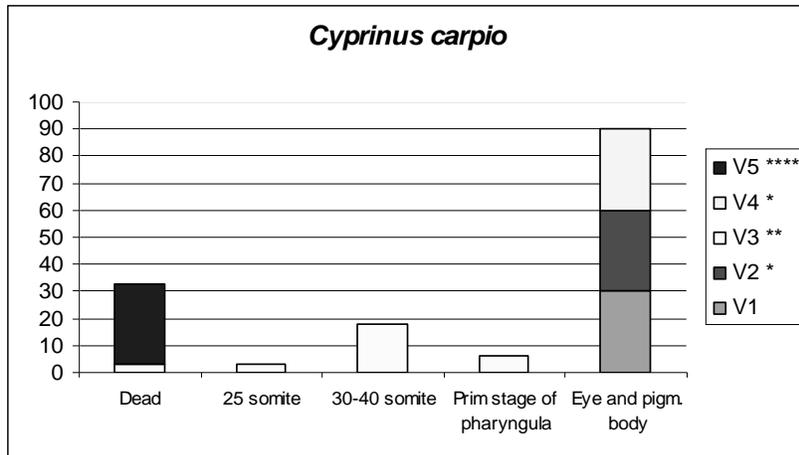
10 hours from fertilization, most carp embryos are in various stages of the gastrula period. The most developed ones represent 10% from the witness variant in a 70%-epiboly stage. No embryo has died and, from the point of view of the statistical analysis, there are no differences among the variants.

24 hours from incubation, the mortality rate is of 0% in all variants, all embryos are in the segmentation period, but we have to mention that in V3 and V5, 50% are slightly behind, respectively 90% are in the incipient stage of somite appearances.

28 hours from fertilization, in the last variant, with hard water (V5), the 90% embryos, which were at the beginning of the segmentation 4 hours before, stopped their development

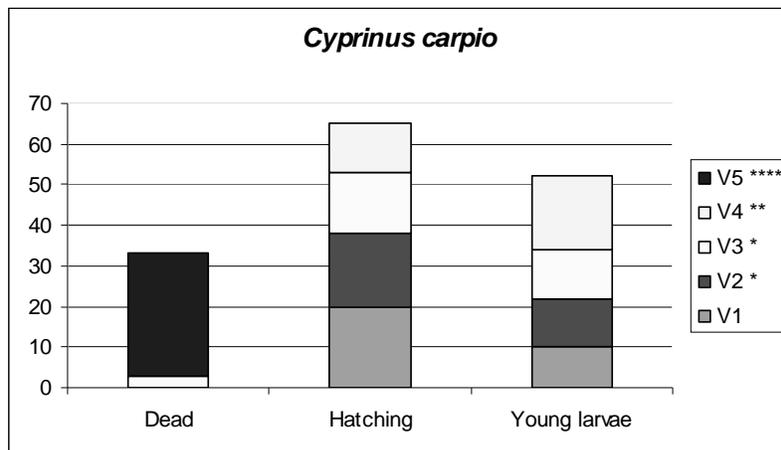
and died. The other 10% have got 4 somites. In V2, V3, V4 the embryos are in the middle of the segmentation period. The most developed embryos from V1 86.7% have got 30-40 somites, and 13.3% 25 somites.

After another 8 hours of monitoring, in V5, the mortality rate is of 100%. In V1 on the other hand, we find the most developed embryos, 100% are in the first stage of the pharyngula period. In the other 3 variants, the living embryos are in various stages of the segmentation period.



Graph 2. Water hardness influence upon embryo development in *Cyprinus carpio*, 48 hours from fertilization (* $p > 0,05$; ** $p = 0,01 - 0,05$; *** $p = 0,001 - 0,01$; **** $p < 0,001$)

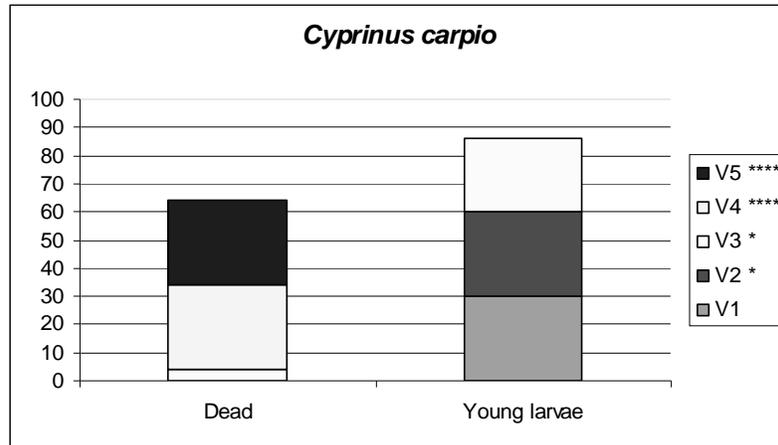
According to graph 2, 48 hours from fertilization, in V1, V2 and V4, all embryos are in the pharyngula stage with pigmented eyes and body. In V3 10% have died, the other 70% are still in the segmentation period, and 20% are at the beginning of the pharyngula period. Between V1 and V2, respectively V4, there are no differences, between V1 and V3, there are differences, and between V1 and V5 there are quite significant differences.



Graph 3. Water hardness influence upon embryo development in *Cyprinus carpio*, 72 hours from fertilization (* $p > 0,05$; ** $p = 0,01 - 0,05$; *** $p = 0,001 - 0,01$; **** $p < 0,001$)

72 hours from incubation, according to graph 3, the young larvae have appeared, 33.3% in V1, 40% in V2 and V3, 60% in V4. Another 4 hours later, 76 hours from incubation in V1 and V4, we have got a 100% hatching rate. In V1 - 90%, and in V3 - 50% young larvae. Another 2 hours later, 78 hours from fertilization, the larvae in V4 have all died (100%).

Since they were not protected by the corion, the larvae died when in direct contact with that environment.



Graph 4. Water hardness influence upon embryo development in *Cyprinus carpio*, at the end of the experiment (* $p > 0,05$; ** $p = 0,01 - 0,05$; *** $p = 0,001 - 0,01$; **** $p < 0,001$)

At the end of the experiment, after 144 hours of monitoring, the embryo in V3, after experiencing an over 60 hour long hatching period, died inside the corion.

There are very obvious, significant differences between V1 and V4, respectively V5. There are no differences between V1 and V2, respectively V3.

CONCLUSIONS

The research regarding the water hardness influence upon the carp embryo have highlighted the fact that this element can induce various embryo growth and development rhythms during the incubation period.

The first embryo development stage is very sensitive to higher hardness degrees, a fact made obvious by the stagnation, and implicitly, by the death of the embryos.

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