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THE EFFECTS OF DENSITY ON MORTALITY AND DEVELOPMENT OF THE *Bufo bufo* EGGS AND TADPOLES

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INTRODUCTION

Some authors who studied the tadpole effect of density in different amphibian species have shown that the excreting of substances produced by tadpoles into the water delays tadpole's growth and development (Brockelman, 1969; Pjastolova and Ivanova, 1981; Rous and Rous, 1964; Schvarts et al., 1976). Other authors supposed that the effect of density is based on the behavioral mechanisms (Wassersug, 1974). The high density inhibits growth of tadpoles (Brockelman, 1969; Brady and Griffiths, 2000, Pjastolova and Ivanova, 1981; Reading and Clarke, 1999; Rous and Rous, 1964; Schvarts et al., 1976; Wassersug, 1974). Tadpoles developing under the high density will be smaller at the metamorphosis stage than the "low density" tadpoles (Brockelman, 1969; Brady and Griffiths, 2000; Rous and Rous, 1964; Schvarts et al., 1976). It was shown also that common toad tadpole mortality may be density-dependent (Brady and Griffiths, 2000; Reading and Clarke, 1999).

It is known that density at the tadpole stages influences not only metamorphosis and post- metamorphosis stages of amphibians' development, but their pubescence as well (Pjastolova and Ivanova, 1981), i.e., "the effect of memory" of developmental conditions was noted. Some authors suppose that we can observe indirect effects of density inhibiting the growth rate and elongating the period of larval development (Rous and Rous, 1964) rather than direct effects of density on the survival of tadpoles.

Unfortunately, information on the influence of amphibian egg density on the developmental and growth rate is not sufficient. Therefore, the purpose of this work was to study the influence of egg density on the mortality and development of embryos and on the growth and development of tadpoles in the common toad (*Bufo bufo*).

Fig. 1. The aquarium for experiments 1 and 2 with 240 eggs.

MATERIAL AND METHODS

The pairs of common toad (*Bufo bufo*) were collected during mass oviposition in May 2002. The parents were placed into separate aquariums for spawning. Right after the termination of spawning two egg clutches were used in the following experiments:

Experiment 1. The parts of the clutches of the first pair were placed into the identical aquariums (diameter of a bottom was 75 mm, and height of a water column was 30 mm, 0.133 liter of water per aquarium) with different egg density. The initial egg densities were 30, 60, 120, 240, 480, and 960 eggs per aquarium (Fig. 1).

Experiment 2. Other parts of the same clutches were placed into the similar aquariums, 480 and 120 eggs per aquarium. All dead eggs had been removed from aquariums with the help of surgical tools and pipettes. These manipulations were carried out daily at the same time.

Experiment 3. Eggs from the clutches of the second pair were placed into the 90 identical aquariums (diameter of a bottom was 30 mm, and height of a water column was 30 mm, about of 0.02 liter of water), 1 egg per aquarium. In addition, 480 eggs from the same clutch were placed into the aquarium with 0.265 liter of water.

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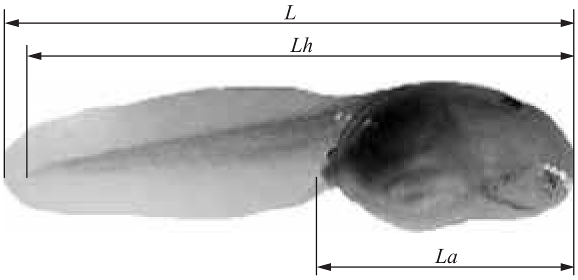

Fig. 2. The measurements of tadpoles.

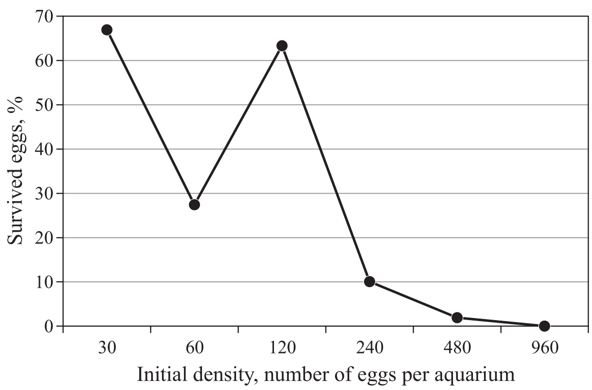

Fig. 3. The dependence of survived eggs percent from initial density.

In the 30 aquariums with singly reared embryos (SRE) a half of water was replaced with the same volume of water taken from the aquarium with 480 eggs. Water was replaced every 8 h ("Test").

In the other 30 aquariums with SRE water was mixed thoroughly every 8 h without any replacement ("Control 1"). The rest part of SRE (30 aquariums) developed without any special influence until the hatching ("Control 2").

In all these experiments the numbers of dead eggs were counted, and also the developmental stage of each embryo had been estimated (Caubar and Gipouloux, 1956). "Development rate index" was introduced for the estimation of developmental rate of egg group. For each egg group this index was calculated by the formula:

$$X(t) = \frac{1}{M(t)} \sum_{i=1}^{J} [K(i) n(i,t)],$$

where $X(t)$ is the development rate index of egg group at the time t, $M(t)$ is the number of survive eggs in the group at the time t, J is the number of developmental stage, $n(i, t)$ is the number of embryos at the developmental stage i at the time t; $K(i)$, the correction coefficient for each developmental stage (for example, cleavage, 0, gastrula, 1, and so one to hatching, 6). t-Test for independent samples (Statistica 6.0 for Windows) has been used for the comparison of mortality and development rate indexes in the different egg groups (the 95% confidence intervals).

Experiment 4. This experiment was performed on tadpoles. The Tadpoles hatched in the experiments 1 and 3 were placed into the same aquariums (height of a water column was 60 mm) with 0.265 liters of water (2 and 5 tadpoles per aquarium). Other conditions of tadpoles rearing were identical. The experiment was finished on the 25^{th} day of development, after the formation of "trowel-form" hind-legs (developmental stage, $IV_7(3)$). At the end of experiment the *L*, *Lh*, and *La* were measured for each tadpole (Fig. 2). t-Test for independent samples was used for the comparison of tadpole groups.

The temperature of water in all experiments was 17.6 ± 0.3°C.

RESULTS AND DISCUSSION

The main result of experiment 1: the maximum percentage of surviving embryos was observed at the densities of 30 and 120 eggs per aquarium (Fig. 3). An increase of mortality rate was observed at the densities higher than 120 eggs per aquarium. This effect also appeared at the intermediate density (60 eggs per aquarium). The mass mortality of eggs began at the highest density (480 and 960 eggs) since earlier stages of embryogenesis than at low densities. The development rate was highest at the densities less than the 240 eggs per aquarium (Fig. 4). The inhibition of development (development was stopped at the gastrula stage) was observed at the highest density (960 eggs).

The correlation between the survival of embryos and initial density in the experiments with removing of dead eggs is shown in Fig. 5. The mortality at the density of 480 eggs was higher than at the density of 120 eggs. The rate of development at the density 480 was lower than at the 120 eggs. These results are similar to the results of the experiment 1 (without removing of dead eggs). Therefore, an influence of manipulations associated with the removing of dead eggs levels expected positive effect of the absence of dead embryos and leads to the increase of death rate and slight delay of development.

In the experiment 3 (development of single eggs) the maximum mortality was observed in the Test (16.67%), and the minimum mortality in the Control 2 (3.34%). Thus, the best conditions were in the aquariums with eggs developed without any influence. Water mixing without replacement produced more unfavorable conditions. The worst conditions were observed in the experiments with water replacement. These experimental treatments caused no appreciable effect on the development rates. The development rates of single eggs were higher, than in the clutch (480 eggs per aquarium). The hatching of single eggs came much quicker, than in the clutch. The high synchronization of developmental rate of single eggs was observed.

Tadpoles, reared at a density of 2 tadpoles per aquarium, differed significantly ($p < 0.05$) from the tadpoles, reared at a density of 5 (experiment 4). The means of Lh significantly differed in all cases and the same tendency was observed for *La* and *L*. The tadpoles from density 2 had longer body than tadpoles from density 5 (Table 1). Density had no influence on the developmental rate of tadpoles. Essential distinctions in tadpole mortality were not observed also.

"The effect of memory" of embryonic development was observed at the high density of tadpoles as an effect of the initial density of eggs (Table 1). The tadpoles reared under the different initial egg density significantly ($p < 0.05$) differ by mean values of *La*. This tendency was observed for *Lh* and *L* as well, but distinctions were not always significant because of small number of animals.

The tadpoles reared under the egg density 30 were significantly larger compared with tadpoles under egg density 120 (Table 1). Tadpoles developed from the SRE Test and the SRE Control 2 differed significantly ($p < 0.05$) (Table 1). The best way to show this result is the comparison of *La* means. The tadpoles under the Test had a larger *La*, than the tadpoles under the Control 2 (Table 1). This tendency was observed for *Lh* and *L* as well, but the distinctions were not always significant because of small sampling size. These differences occurs at the low (2 tadpoles) and at the high (5 tadpoles) density. Thus, "the effect of memory" of embryonic development was observed, the tadpoles passed embryogenesis without any special influences were larger than the tadpoles that developed from the eggs reared under the water replacement.

Thus, dependence of mortality and developmental rate from the egg density has a non-monotone segment. At the density of 120 eggs the survival and the developmental rate appeared to be much higher than one could expect,

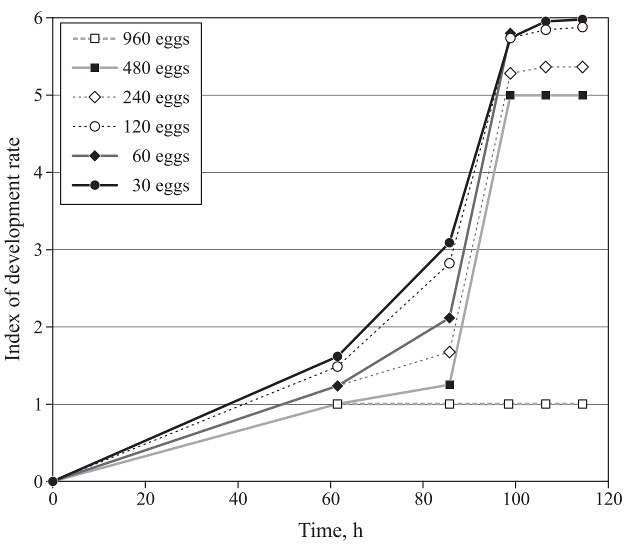

Fig. 4. The time-dependence of the development rate index at different density.

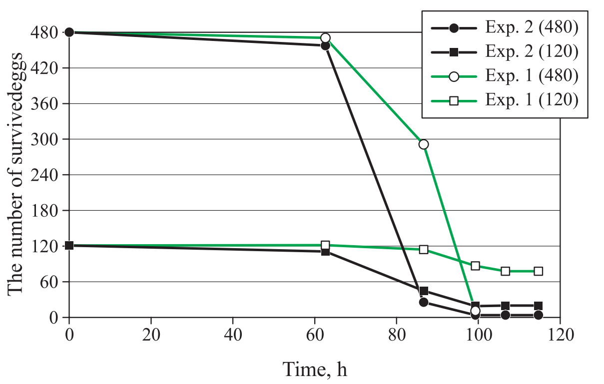

Fig. 5. The dependence of survival rate of eggs on density at removal of dead eggs.

TABLE 1. Measured Characteristics of Tadpoles at Different Density

Initial density of eggs	Density of tadpoles					
	L		*Lh*		*La*	
	2	5	2	5	2	5
120	13.37 ± 0.55*	11.66 ± 0.30*	12.94 ± 0.54*	11.24 ± 0.29*	5.89 ± 0.23*	5.37 ± 0.13*
30	13.91 ± 0.10*	12.13 ± 0.16*	13.46 ± 0.10*	11.72 ± 0.16*	6.23 ± 0.06*	5.69 ± 0.09*
SRE Test	13.91 ± 0.18	13.33 ± 0.22	13.46 ± 0.17*	12.84 ± 0.21*	6.31 ± 0.09	6.17 ± 0.11
SRE Control 2	13.55 ± 0.31	12.75 ± 0.12	13.14 ± 0.30*	12.32 ± 0.12*	5.86 ± 0.12	5.71 ± 0.05

SRE, the singly reared embryos from experiment 3.
*, indicates that the distinction is significant at $p < 0.05$.

and were similar to the survival and developmental rate of animals at a low density (30 eggs). By contrast, at a density of 60 eggs the survival appeared to be lower than expected. This effect was described for the first time. In the experiments with eggs, the inhibition of development was observed and mass mortality began since the earliest stages of embryogenesis at the highest egg density. The tadpoles under the high initial egg density were smaller than the tadpoles under the low initial egg density. However, the density had no effect on the developmental rate of tadpoles, which is consistent with the data of other authors (Brady and Griffiths, 20001).

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