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Effect of Fluctuations of pH and Illumination on Growth and Development of *Rana ridibunda* Larvae

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Abstract—A study of the influence exerted by fluctuations in pH and illumination on larvae of lake frog (*Rana ridibunda* Pallas) has shown that their growth and development are considerably enhanced under certain variable regimens, with the mortality and variability of tadpole size becoming lower and the duration of the larval stage shorter. First-year individuals raised with the factors studied variying, were larger and showed smaller variation in size and weight, which suggests a positive effect of pH and illumination fluctuations.

The fact that ichthyological studies in the field of ecological physiology are becoming more extensive and in-depth enabled a new approach to the problem of the ecological optimum. A large body of data has been obtained on the positive effect of temperature, pH, illumination, salinity, and oxygen content in water on the growth, energy balance, and physiological condition of young fish (Stroganov, 1962; Konstantinov and Zdanovitch, 1986, 1996; Konstantinov, 1988; Konstantinov and Martynova, 1990; Konstantinov *et al.*, 1998a; Vechkanov *et al.*, 2000). Fluctuations in factors of varied nature lead to about the same effect, which points to their nonspecific action. Thus, it may be assumed that any environmental changes within the limits of ecological valency are favorable for organisms, and just the astatic state makes the ecological optimum (Konstantinov, 1997). Studies of not only fish, but also other hydrobiotic species, confirm this biological pattern. In particular, growth acceleration has been demonstrated for *Daphnia* (Galkovskaya and Sushchenya, 1978), Rotifera (Konstantinov *et al.*, 1995), and green algae (Konstantinov *et al.*, 1998b) under fluctuation of ecological factors.

In this study, the effect of pH and illumination fluctuation on the growth and development of *Rana ridibunda* Pallas tadpoles was analyzed.

MATERIALS AND METHODS

The experiments were carried out at the laboratory of Department of Zoology and Ecology of the Mordovian State University in 1998–1999 years.

Spawn of the lake frog was obtained from a clutch in a natural pond. Before the beginning of the experiment, larvae were equalized along the stage 24 after incubation (Terentiev, 1950). Experiments were carried out in 25-l aquariums with 15-cm liquid column. The filling density of 2–4 individuals per 1-l was equal to that described by Severtsov (1990).

A study of pH fluctuations was conducted in the following varying ranges: 7.0–8.0, 6.5–8.5, and 6.0–9.0. Alternating pH values were changed once a day. Constant pH 7.5 was used as control value known to be the steady-state optimum for this species (Severtsov, 1990). The pH value was adjusted with H_2SO_4 and KOH and monitored using a pH-meter (pH–340) with accuracy within 0.05. To study illumination fluctuations, daily alternating regimens were used: 1900–2900, 1500–3300, and 600–4200 lx. The intensity of illumination was measured with JU-116 luxmeter. All the factors were standardized: temperature was maintained at 24 ± 1ºC, and the oxygen content in water, at 7–7.5 mg/l (compulsory aeration).

Larvae were fed with hard-boiled egg yolk with crushed nettle leaves. The body length, mass, and stage of development (Terentiev, 1950) of experimental individuals were recorded at intervals of 5–15 days. In some cases, the variability of tadpoles was evaluated using the ratio $CV_2 : CV_1$, where CV_2 and CV_1 are, respectively, the coefficients of variation at the end and beginning of experiment. Statistical processing of the numeric material was carried out using the paired comparison method (Lakin, 1990).

RESULTS AND DISCUSSION

The pH value is a very important indicator of the physicochemical properties of water and depends on

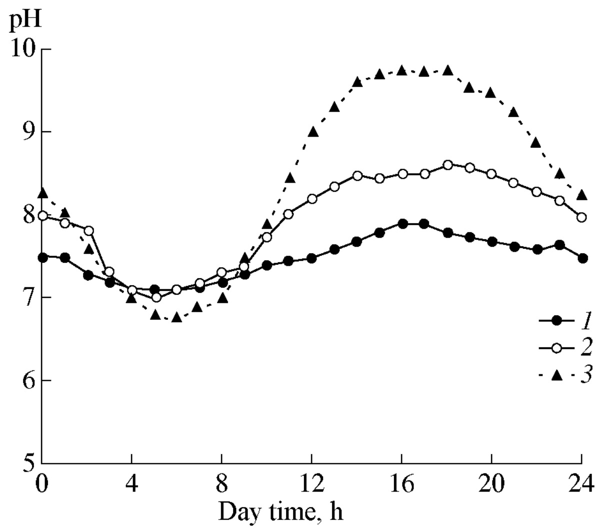

Fig. 1. Daily pH dynamics in June–July 1998 in different reservoir areas, wherefrom the lake frog spawn was taken: *1*, on bottom at a depth of 1 m; *2*, near the surface at the center of pond; and *3*, near the surface in macrophyte bushes.

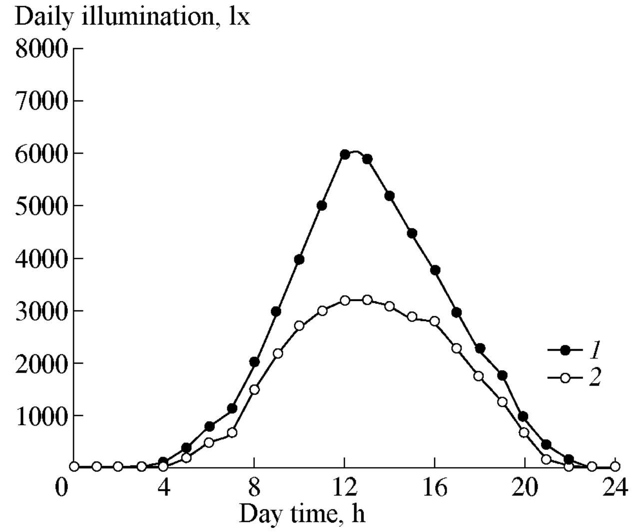

Fig. 2. Daily illumination dynamics in June–July of 1998 at a depth of 1 m, wherefrom the lake frog spawn was taken: *1*, on clear sunny day; *2*, on cloudy day.

the nature and concentration of substances dissolved in water (Alekin, 1953). The seasonal and daily fluctuations of this factor are observed in fresh water reservoirs (Lukanov *et al*., 1971). We observed that, in June–July 1998, water of a natural standing lake, which was the source of experimental frog spawn, had alkalescent pH (Fig. 1). At the same time, the average daily pH values varied between different pond areas: 7.35 near the bottom at a depth of 1 m, 7.85 near the surface at the pond center, and 8.26 near the surface inside macrophyte bushes. It is well known (Alekin, 1953) that, during a day, plants absorb carbon dioxide in the course of photosynthesis, which leads to water alkalization, and the inverse process takes place at night. The strongest pH oscillation was also recorded by us inside a macrophyte bush near bank (6.76 at night, 9.76 in daytime), and the narrowest, near the bottom (7.10 and 7.61, respectively).

During a day, the atmospheric illumination may change by a factor of tens of millions from thousandths up to tens of millions of lux similarly to pH fluctuations. The greatest overfalls occur during the crepuscular period (Girsa, 1970). The light incident onto the water surface is partly reflected, and that penetrating inside is absorbed and diffused by water molecules and suspended particles, and this determines the clarity of water. No more than 5–10% of solar radiation usually reaches a depth of 1 m in lakes and reservoirs having 1–2-m transparency (Konstantinov, 1979). Our measurements made in June–July 1998 showed that the strongest illumination in experimental reservoirs at a depth of 1 m is 6000 lx on sunny days and no more than 3200 lx on cloudy days (Fig. 2). Significant (by a factor of 1000) illumination changes were observed in the morning (5–7 h) and evening (20–22 h) twilight.

Thus, both pH and illumination undergo significant oscillations during 24 hours. In predominant habitats of frog tadpoles, the range of pH variation did not exceed 1–1.5 units in day and night (average value 7.48), and noon illumination did not exceed 5000 lx.

The data obtained undoubtedly confirm the assumption of the stimulating effect of fluctuations in ecological factors within tolerance limits on the growth and development of lake frog larvae. It is clear from Table 1 that oscillation of hydrogen ions within 1 unit around the constant optimum markedly and statistically significantly ($P < 0.01$) accelerates the linear growth by a factor of 1.12, and mass growth by a factor of 1.25 in comparison with the control during the whole experiment. This variable regimen exerted the most favorable influence on the growth and development of the lake frog. The increase in fluctuation range up to 6.5–8.5 units also has growth stimulating effect, which is, however, somewhat weaker than that within the 7.0–8.0 range. A more complex dependence was observed with pH fluctuating outside the tolerance limits (6.0–9.0 units). Table 1 demonstrates that such profound pH fluctuations tended to suppress the growth

Table 1. Growth and developmental stages of *Rana ridibunda* tadpoles under fluctuating pH

pH regimen	Days after hatching	Number of individuals	Length, mm	Mass, mg	Stage of development
7.5	15	48	7.1 ± 0.1	61.2 ± 2.0	25–26
	30	46	10.1 ± 0.2	206.6 ± 11.0	26–27
	40	42	11.9 ± 0.3	308.7 ± 14.2	26–28
	45	39	13.8 ± 0.3	489.8 ± 32.2	26–28
7.0–8.0	15	49	7.5 ± 0.1*	73.2 ± 1.7***	25–26
	30	49	11.3 ± 0.3**	261.5 ± 11.7**	26–28
	40	46	13.4 ± 0.3***	384.8 ± 25.4*	26–28
	45	45	15.8 ± 0.4***	607.0 ± 28.5**	27–30
6.5–7.5	15	47	7.2 ± 0.2	68.8 ± 1.7**	25–26
	30	46	11.5 ± 0.2***	250.0 ± 9.6**	26–27
	40	45	12.9 ± 0.2*	366.5 ± 24.2*	26–28
	45	43	15.1 ± 0.4*	579.6 ± 33.7	27–29
6.0–9.0	15	45	6.6. ± 0.5	53.8 ± 2.7	25–26
	30	40	9.9 ±0.3	192.5 ± 10.7	26
	40	33	11.9 ± 0.3	305.7 ± 20.3	26–28
	45	31	14.4 ± 0.4	530.9 ± 37.7	26–28

*—Significant difference ($P < 0.05$),
**—significant difference ($P < 0.01$),
***—significant difference ($P < 0.001$).

Table 2. Growth and developmental stages of *Rana ridibunda* tadpoles under illumination fluctuations

Illumination regimen, lx	Number of individuals	Days after hatching	Length, mm	Mass, mg	Stages of development
2400	17	92	7.2 ± 0.2	63.3 ± 2.4	25–26
	32	92	9.1 ± 0.1	109.8 ± 3.0	26
	41	88	11.1 ± 0.2	235.3 ± 6.5	26–28
	47	83	15.1 ± 0.3	693.1 ± 31.2	26–28
1900–2900	17	90	7.7. ± 0.2	72.3 ± 2.2**	25–26
	32	86	10.4 ± 0.1***	153.6 ± 4.9***	26–27
	41	86	12.1 ± 0.1***	260.1 ± 7.1**	26–28
	47	86	15.8 ± 0.3	699.9 ± 32.7	26–28
1500–3300	17	92	7.8 ± 0.3*	74.1 ± 2.6**	25–26
	32	90	11.1 ± 0.1***	180.2 ± 5.7***	26–27
	41	88	12.6 ± 0.2***	320.2 ± 10.3***	26–28
	47	87	16.7 ± 0.4**	813.1 ± 39.8*	26–29
600–4200	17	91	7.8 ± 0.3*	76.9 ± 2.8***	26
	32	91	11.4 ± 0.1***	189.5 ± 4.4***	26–28
	41	89	14.5 ± 0.2***	405.1 ± 6.3***	26–28
	47	86	17.1 ± 0.4***	902.0 ± 28.2***	26–29

*—Significant difference ($P < 0.05$),
**—significant difference ($P < 0.01$),
***—significant difference ($P < 0.001$).

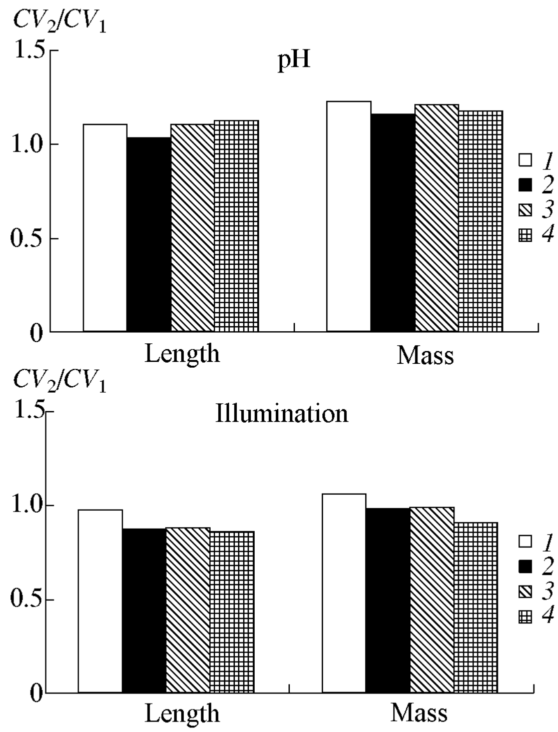

Fig. 3. Variability of length and mass in lake frog larvae under various pH and illumination regimens, calculated from the ratio of variation coefficients at the beginning (CV_1) and the end (CV_2) of experiment. pH ranges: *1*, 7.5; *2*, 7.0–8.0; *3*, 6.5–8.5; *4*, 6.0–9.0. Illumination regimens: *1*, 2400 lx; *2*, 1900–2900 lx; *3*, 1500–3300 lx; *4*, 600–4200 lx.

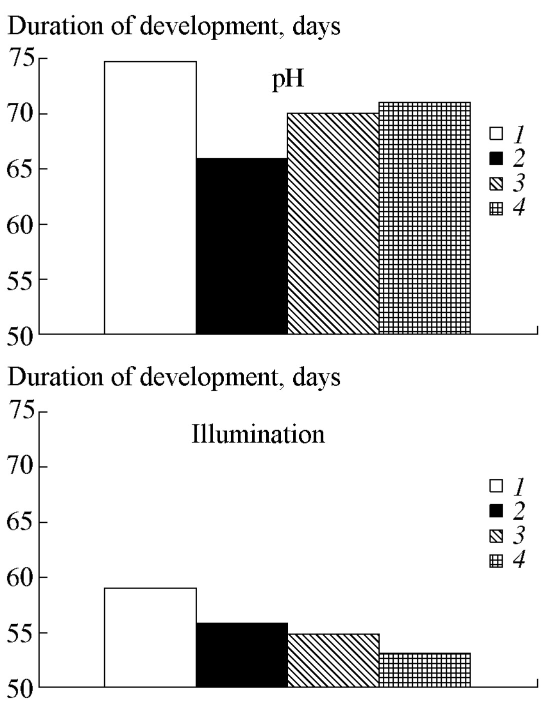

Fig. 4. Duration of larval development of lake frog under pH and illumination oscillations. For designations, see Fig. 3.

of experimental individuals, and, virtually till the end of experiment, their length and mass were smaller than those of the control. However, by the end of experiment, the larval growth rate sharply increased, and linear dimension-weight characteristics exceeded those in the control (with the difference being statistically non-significant). Such a result can be probably accounted for by the eurybionticity and improvement of the adaptation mechanisms in older individuals (Zhukova and Doroshenko, 1989).

Similar results were obtained under the influence of varying illumination (Table 2). The most favorable conditions for the growth and development of tadpoles were fluctuations within the range 600–4200 lx. At the same time the linear and weight growth was accelerated by factor of 1.19 and 1.49, respectively. The significance of the means difference exceeded a significance level of 99%. Reduction of oscillations range to 2800 lx around the stationary control also statistically significantly stimulated the growth. Such an effect was virtually manifested at all during the first 47 days of experiment under minor illumination oscillations within the range 1900–2900 lx.

Fluctuations in pH and illumination within the optimal ranges also led to acceleration of larval development of the lake frog. It can be seen from Tables 1 and 2 that a tendency to pass developmental stages faster was also noted. Under the optimal conditions, experimental larvae exceeded in length and mass the control by, on the average, 10–15% ($P < 0.05$). In addition, these pH fluctuations within a unity range reduced the mortality of tadpoles by 10% as compared with the control ($P < 0.05$). At a wider range of pH fluctuations the mortality increased (Table 1). A high larval mortality (38%) was recorded in the case of fluctuations within the range 6.0–9.0. This is in agreement with published data (Severtsov and Surova, 1979; Zhukova and Doroshenko, 1989), which indicate a decrease in survival of tadpoles at pH exceeding 8.4 and smaller than 6.0. It is necessary to mention that the larval survival of the lake frog is virtually the same under constant and varying illumination (83–86%) and shows no significant difference (Table 2).

The level of variability of dimension-mass characteristics can serve as an optimality criterion for the living conditions of organisms (Polyakov, 1975). Our results indicate a decrease in the variability of larval length and mass under the optimal conditions for growth and development (Fig. 3). At the same time, all varying-illumination regimens resulted in a decrease in

Table 3. Dimensional characteristics of *Rana ridibunda* yearlings after completion of metamorphosis under fluctuating pH

Regimen of pH fluctuations	Number of individuals	Length, mm	Mass, mg	Coefficient of variation	
				length	mass
7.5	33	16.2 ± 0.2	421.3 ± 13.1	8.00	17.82
7.0–8.0	43	17.7 ± 0.2***	488.4 ± 8.8***	6.20	11.74
6.5–8.5	40	17.0 ± 0.2**	437.0 ± 12.0	6.54	17.40
6.0–9.0	26	16.4 ± 0.2	424.4 ± 16.4	6.25	16.69

*—Significant difference ($P < 0.05$),
**—significant difference ($P < 0.01$),
***—significant difference ($P < 0.001$).

Table 4. Dimensional characteristics of *Rana ridibunda* yearlings after completion of metamorphosis under fluctuating illumination

Regimen of illumination fluctuations	Number of individuals	Length, mm	Mass, mg	Coefficient of variation	
				length	mass
2400	63	17.6 ± 0.1	556.5 ± 11.7	6.02	16.80
1900–2900	72	17.9 ± 0.1*	584.1 ± 9.5	4.81	13.02
1500–3300	71	18.2 ± 0.1***	615.5 ± 7.1**	4.55	9.19
600–4200	74	19.4 ± 0.1***	723.3 ± 6.4***	3.74	7.11

*—Significant difference ($P < 0.05$),
**—significant difference ($P < 0.01$),
***—significant difference ($P < 0.001$).

these characteristics by 7–11% as compared with the control. However, only pH fluctuations within the optimal range 7.0–8.0 significantly reduced the $CV_2 : CV_1$ ratio; under other regimens no such distinct pattern was observed (Fig. 3).

As pointed out above, the sequence of developmental stages of tadpoles was accelerated under fluctuations of the factors described, which resulted in a decrease in the duration of the *Rana ridibunda* larval stage (Fig. 4). For example, in the optimal variable pH regimen, this characteristic decreases significantly ($P < 0.05$), by 10% as compared with the control, with the difference being non-significant in other variants. A clear pattern was observed under alternating illumination: with increasing fluctuation amplitude, the duration of larval development decreases gradually (on the average by 10% in relation to control at $P < 0.05$).

After metamorphosis, yearlings grown up under alternating hydrogen ion concentrations had the best size characteristics (Table 3). At pH 7.0–8.0, their length was 1.1 and mass 1.2 times higher than those for the controls ($P < 0.001$). No statistically significant difference was revealed between dimension-mass characteristics of yearlings grown at constant pH and with pH fluctuating within the range of 3 units. The variability of these characteristics decreased in alternating pH regimens. The dimension-mass characteristics increased to even greater extent under alternating illumination (Table 4). A stepwise widening of the range of illumination fluctuations resulted in their increase. A reverse dependence was obtained for the variability of length and mass. Simultaneously, the mortality of metamorphosing tadpoles decreased under the optimal alternating pH and illumination conditions.

Thus, the obtained data well conform to the general concept concerning the effect of environment astaticism, and confirm the hypothesis of the ecological optimum (Konstantinov, 1997). It is probable that optimization of the living conditions of organisms at varying ecological factors is a general biological property, and that any kind of static environment is disadvantageous for them. The fluctuations of environmental parameters cause the "general adaptation syndrome," which in this case improves, rather than impairs, the physiological state of organisms. It is well known that Selye (1974) distinguished 2 forms of "general adaptation syndrome"—eustress (positive) and distress (negative). Moderate deviations of ecological factors, causing positive, or physiological (Arshavsky, 1982) stress, demand an additional work from the organism which results in reconstruction of the metabolism, and shifts it toward anabolism owing to hypercompensation of energy expenditure. In its turn, this leads to stronger mass accumulation, acceleration of development, and raises the resistance of organism (for example, frog larvae) to unfavorable factors. The results obtained can be interpreted in just this aspect.

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