OS EFEITOS DA ILUMINAÇÃO SOBRE O DESENVOLVIMENTO INICIAL DE ANFÍBIOS (AMPHIBIA: ANURA E CAUDATA)

THE EFFECTS OF ILLUMINATION ON THE EARLY DEVELOPMENT OF AMPHIBIANS (AMPHIBIA: ANURA AND CAUDATA)

ВЛИЯНИЕ ОСВЕЩЕННОСТИ НА РАННЕЕ РАЗВИТИЕ ХВОСТАТЫХ И БЕСХВОСТЫХ ЗЕМНОВОДНЫХ (AMPHIBIA: ANURA AND CAUDATA)

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RESUMO

Foram estudados os efeitos da iluminação sobre o desenvolvimento precoce de quatro espécies de anfíbios - *Lissotriton vulgaris* (Linnaeus, 1758), *Triturus cristatus* (Laurenti, 1768), *Rana arvalis* (Nilsson, 1842) e *R. temporaria* (Linnaeus, 1758). Em geral, a taxa de desenvolvimento inicial é bastante independente da iluminação. Os ovos de anfíbios sem cauda desenvolvem-se quase igualmente sob quaisquer condições de iluminação, enquanto que os ovos de anfíbios com cauda melhoram a uma iluminação de 700 lx. A iluminação influencia principalmente a taxa de sobrevivência de embriões e prolarvas, que aumenta com baixas intensidades de iluminação e diminui no escuro. Possíveis mecanismos e causas subjacentes aos fatos observados são discutidos.

Palavras-chave: Anfíbios, Desenvolvimento, Mortalidade, Iluminação

ABSTRACT

The effects of illumination on the early development of four amphibian species — *Lissotriton vulgaris* (Linnaeus, 1758), *Triturus cristatus* (Laurenti, 1768), *Rana arvalis* (Nilsson, 1842), and *R. temporaria* (Linnaeus, 1758) — have been studied. In general, the rate of their early development is rather independent of illumination. The eggs of tailless amphibians develop almost similarly under any illumination conditions, whereas the eggs of tailed amphibians better develop at an illumination of 700 lx. Illumination mainly influences the survival rate of embryos and prolarvae, which increases at low light intensities and decreases in the dark. Possible mechanisms and causes underlying the observed facts are discussed.

Keywords: Amphibians, Development, Mortality, Illumination

АННОТАЦИЯ

Изучено влияние освещенности на раннее развитие четырех видов амфибий (*Lissotriton vulgaris* (Linnaeus, 1758), *Triturus cristatus* (Laurenti, 1768), *Rana arvalis* (Nilsson, 1842) и *Rana temporaria* Linnaeus, 1758). Темп их развития мало зависит от освещенности. При этом развитие икры бесхвостых амфибий протекает практически одинаково при всех условиях освещенности, тогда как развитие икры хвостатых амфибий улучшается при освещенности 700 лк. Основное влияние освещенность оказывает на выживаемость эмбрионов и предличинок, которая увеличивается при небольших величинах интенсивности светового воздействия, и уменьшается в темноте. Обсуждаются вероятные механизмы и причины полученных результатов.

Ключевые слова: Амфибии, Развитие, Смертность, Освещенность

INTRODUCTION

Illumination as an abiotic factor plays an important role in the life of juvenile and adult individuals of most animal species except for the species inhabiting caves as well as very turbid or deep water. The intensity of light penetration to aquatic habitats depends on several factors, such as sunlight reflection and refraction by the water surface and their absorption and scattering in the water column. The effects of illumination on the fish egg development have been studied by many researchers (Lyubitskaya, 1951; Chernyaev, 1984; Buchet *et al.*, 1995; Firat *et al.*, 2003; Menu and Girin, 1979; Ronzani and Macedo, 2001; Saka *et al.*, 2001; Karakatsouli *et al.*, 2010), who obtained quite different results. In particular, illumination may have both positive and negative effects on the development and is completely unnecessary for some (indifferent) species. However, the effects of illumination on the amphibian eggs and their early development starting from hatching to the beginning of active feedings are still vague (Hartman and Hailman, 1981; Perry *et al.*, 2008). For example, Terent'ev (1950) reported that the common frog eggs develop in the dark at the same rate as in the light other conditions being equal. Sytina and Nikol'skaya (1984) experimentally demonstrated that the common frog embryos from the upper and thus better illuminated layers of the egg clutch are the first to hatch despite a lower temperature during their development as compared with the centre of the clutch. It is evident that the published data on this issue are poor and contradictory.

MATERIALS AND METHODS

In our experiments, we have studied the effects of illumination on the early development and mortality rate of the eggs and prolarvae of four tailed and tailless amphibian species that differ in the duration of egg development in natural water bodies.

The common newt *Lissotriton vulgaris* (Linnaeus, 1758) and crested newt *Triturus cristatus* (Laurenti, 1768) (Caudata: Salamandridae) are typical species in central Russia. Both species have similar developmental biology. The clutches contain solitary eggs wrapped into a leaf of a higher aquatic plant by the hind limbs of the female. The moor frog *Rana arvalis* (Nilsson, 1842) and common frog

R. temporaria (Linnaeus, 1758) (Anura: Ranidae) belong to the group of true frogs. Characteristic of these species are short embryonic (5–15 days) and larval (to 65 days) development (Pyastolova and Ivanova, 1978). The egg clutches are always aggregated, forming a sort of mats, which protect them from predators and enhance temperature increase within the clutch. The clutches of the moor frog are looser as compared with the common frog.

The eggs were sampled from an individual pair (for one experiment) in the spawning water bodies directly after fertilization; among other matters, this simplified species identification. Each variant (one petri dish) of one experimental series contained 10–20 eggs from the same clutch. The cultivation temperature was $20 \pm 1^\circ\text{C}$ and oxygen concentration in water, 7.0–7.5 g/L. The developmental stages were identified 2–4 h later according to Liozner (1975) for the common and crested newts and according to Dabagyan and Sleptsova (1975) for the common and moor frogs. The developmental stages were identified on a regular basis. The developmental rate was calculated as the time necessary for a certain stage of each individual in experiment. The mortality rate was calculated as the number of died individuals relative to their total number in experiment.

Dead eggs were counted daily to record the mortality rate. In the “dark” variant (zero illumination), all manipulations were performed on a daily basis at a very low (0.001 lx) scattered illumination. The experiment was stopped after the larvae started active feeding. The body length of the hatched larvae was measured with the help of an eyepiece micrometer accurate to 0.01 mm (Fig. 1).

All experiments were conducted in four to seven replicates. The data listed in tables were averaged over all experimental series. Luminescent lamps, not heating during their work and providing sufficiently strong light flux, were used for illumination. “Dark” conditions (0 lx) were provided by a light-proof hood. Illumination intensity was determined on the water surface with a Yu-116 lux meter accurate to +5%. The white light fluorescent lamps (thereafter – fluorescent lamps) fluorescent lamps are intended for illumination of the closed premises, and also for external usage. They operate in AC networks with a voltage of 127–220 V, a frequency of 50 Hz. These lamps connect to the

AC network together with the corresponding start-control equipment, in the starter-ignition circuits. A fluorescent lamp has G13 socle type. These fluorescent lamps are characterized by high light output, low energy consumption and high life duration. The colour temperature of these lamps is 4200 K. The main reason that we have used this lamp type was that they have a low operating temperature (5–25 °C) (Afanasyeva & Skobelev, 1986). Thus, the lamps used in our study could not cause the water heating due to their low operating temperature, and also due to the location of these lamps at 15 cm from the water surface.

The data were statistically processed using a standard method with Student's *t*-test (Lakin, 1990).

RESULTS

The experiments with the common newt showed that the rate of its embryonic development accelerated at light intensities of 10 and 700 lx (Table 1). The hatching in these variants was recorded by 3.0 and 4.3% earlier, respectively, as compared with the control ($p < 0.05$). Unlike the tailless amphibians, characteristic of which is a considerable time span between hatching and the beginning of active feeding, the larvae of tailed amphibians start active feeding almost immediately. The situation observed in our experiments was analogous; however, a short period between hatching and active feeding was still recorded. This is associated with that the yolk plug needs some time to gradually leave the gut. The mortality rate of the common newt embryos and prolarvae decreased at a low illumination (1 and 10 lx) in a statistically significant manner. Note that the actively feeding larvae grown under an illumination of 1 lx were slightly smaller (Table 2). At a high illumination values, the mortality rate decreased insignificantly but the linear size of the larvae exceeded the control in a statistically significant manner ($p < 0.05$ and $p < 0.01$).

The crested newt embryos developed faster only at an illumination of 700 lx (exceeding the control by 3.8%, $p < 0.05$). At the other light intensities, the developmental rate was close to the control. Unlike the common newt, the mortality rate of the crested newt embryos significantly decreased only at an illumination of 10 lx. The differences for the remaining illumination modes were statistically insignificant.

The mortality rate at prolarval stages was almost equal in all variants. The body length of the actively feeding crested newt larvae increased in a statistically significant manner at low and medium illumination intensities (Table 2). Thus, larger-sized larvae of the common newt developed at medium and high light intensities and of the crested newt, at low and medium light intensities.

Illumination had no statistically significant effect on the egg development rate of both tailless amphibian species (Table 3). The time moments for prolarvae emergence in all variants were similar. Note only a certain trend of the shortening in embryonic development observable at illuminations of 550, 600, and 800 lx. However, the prolarval stage demonstrated that this trend was not retained, while the switching to active feeding accelerated at a low light intensity.

On the other hand, the embryos displayed different survival rates. Note that the tailless amphibians in all cases died at early developmental stages (stages 25–27). As is evident from Table 4, the maximum mortality rate was observed in the variant without any illumination and the minimum mortality rate, at light intensities of 150, 550, and 1400 lx. The differences in the remaining illumination variants were statistically insignificant.

As is evident from Table 4, the survival rate of common frog embryos displayed no distinct dependence on the illumination intensity. The mortality rate after hatching was lower at low illumination values (150 and 550 lx), i.e., a high percentage of survival was observed in the same modes. On the other hand, the mortality rate of prolarvae decreased in the other variants but the differences from the control were statistically insignificant. Similar to the developmental rate, the body sizes of the actively feeding larvae differed from the control variant but in a statistically insignificant manner. The experiments with the moor frog gave almost identical results. Statistically significant differences in the mortality rate from the control variant with a zero illumination were recorded in the variants with light intensities of 150, 600, and 800 lx ($p < 0.01$ and $p < 0.001$). Note that the moor frog displayed a very high mortality rate (at the level of 70%) during the embryonic development in the cases of high illumination, medium illumination (550 lx), and in the dark (Table 4).

Illumination also had no statistically significant effect on the body length of the hatched larvae. Presumably, the body length (correspondingly, measurements) of the survived larvae had a certain effect in this case. As is mentioned above, embryos mainly died at early developmental stages. Thus, it is rather likely that the observed increase in the size of the moor frog larvae at a zero illumination (in the control) is explainable by elimination of small individuals and survival of larger ones, the measurements of which contributed to the final result.

DISCUSSION

The overwhelming majority of amphibians have well developed organs of sight, represented by the lateral eyes and pineal complex. Interestingly, the latter develops earlier than the true eyes and can control the primitive looming escape response (Roberts, 1978; Moriya *et al.*, 1991). In addition, the pineal organ is an endocrine gland secreting melatonin. This hormone is present in the amphibian pineal gland and inhibits the growth of their larvae (Delgado *et al.*, 1987; Edwards and Pivorun, 1991). Its concentration is always higher in the dark and lower in the light (Gutierrez *et al.*, 1984; Green *et al.*, 1999; Kuznetsov, Ruchin, 2001; Ruchin, 2002, 2003, 2004). Thus, the pineal gland secretes a considerable amount of melatonin in the constant absence of light (in the dark), which can slow down the development of embryos and larvae. Melatonin appears as early as embryonic stages, and its effect becomes significant already in the spawn and further increases in the hatched larvae (Green *et al.*, 1999).

When the duration of experiments was extended (as in the case with newts), we observed that the larval body length differ in a statistically significant manner depending on illumination. The larvae grown in the dark and at an illumination of 1500 lx were the smallest. These results suggest the following scheme of how light influences the newt larvae. Different illumination intensities, acting via the visual analyzer (pineal organ and eyes), stimulate the anterior pituitary gland, which in turn releases the growth-stimulating hormones enhancing an increase in body size (Crim, 1975; Sakoe *et al.*, 1980).

CONCLUSIONS

Our results suggest that the effect of

illumination on the early development of amphibians is species-specific. In general, the rate of their development taking place under strictly controlled conditions is weakly dependent on illumination. In this process, the eggs of tailless amphibians develop almost identically under any illumination conditions, while the eggs of tailed amphibians better develop at an illumination of 700 lx. Presumably, this is associated with an extended embryonic development of the tailed amphibians and an increase in the light radiation accumulated by the embryo. It is possible that certain illumination level elevates secretion of growth-stimulating hormones, for example, prolactin. On the other hand, illumination has a considerable effect on the mortality rate of amphibian embryos and prolarvae. Thus, illumination mainly influences the survival of embryos and prolarvae, which elevates at a low level of light intensity and decreases in the dark. In any case, the mechanisms underlying the effect of light on the early development of amphibians require serious studies and clarification.

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Table 1 Duration (days after fertilization) of the early development of tailed amphibians depending on illumination ($M \pm m$)

Illumination, lx	Beginning of prolarval stage		Beginning of active feeding	
	<i>Tr. cristatus</i>	<i>L. vulgaris</i>	<i>Tr. cristatus</i>	<i>L. vulgaris</i>
0, control	16.14 $\pm$ 0.10	16.20 $\pm$ 0.11	16.22 $\pm$ 0.12	16.33 $\pm$ 0.32
1	16.33 $\pm$ 0.12	16.15 $\pm$ 0.13	16.37 $\pm$ 0.10	16.34 $\pm$ 0.31
10	16.17 $\pm$ 0.11	15.72 $\pm$ 0.11*	16.28 $\pm$ 0.10	15.81 $\pm$ 0.29
700	15.52 $\pm$ 0.09*	15.50 $\pm$ 0.12*	15.60 $\pm$ 0.12	15.62 $\pm$ 0.26
1500	16.37 $\pm$ 0.12	16.34 $\pm$ 0.31	16.45 $\pm$ 0.14	16.57 $\pm$ 0.42

* Statistically significant at $p < 0.05$.

Table 2 Mortality rate and body length of the hatched larvae of tailed amphibians depending on illumination ($M \pm m$)

Illumination, lx	Mortality rate at embryonic stages, %		Mortality rate at prolarval stages, %		Body length, mm	
	<i>Tr. cristatus</i>	<i>L. vulgaris</i>	<i>Tr. cristatus</i>	<i>L. vulgaris</i>	<i>Tr. cristatus</i>	<i>L. vulgaris</i>
0, control	45.6 $\pm$ 2.8	26.5 $\pm$ 1.6	8.9 $\pm$ 1.4	17.5 $\pm$ 0.8	8.95 $\pm$ 0.09	9.01 $\pm$ 0.07
1	42.3 $\pm$ 2.4	20.0 $\pm$ 2.3*	7.8 $\pm$ 0.8	12.8 $\pm$ 1.2*	9.75 $\pm$ 0.07*	8.95 $\pm$ 0.12
10	34.0 $\pm$ 2.6*	19.3 $\pm$ 2.0*	8.0 $\pm$ 1.2	10.3 $\pm$ 0.9†	10.56 $\pm$ 0.09†	10.34 $\pm$ 0.08†
700	42.5 $\pm$ 3.7	18.9 $\pm$ 3.4	8.2 $\pm$ 0.6	14.9 $\pm$ 2.0	9.83 $\pm$ 0.07*	10.83 $\pm$ 0.08†
1500	43.1 $\pm$ 4.5	20.6 $\pm$ 3.6	7.7 $\pm$ 0.9	15.3 $\pm$ 1.7	9.12 $\pm$ 0.07	9.42 $\pm$ 0.04*

Statistically significant at * $p < 0.05$ and † $p < 0.01$.

Table 3 Duration (days after fertilization) of the early development of the tailless amphibians depending on illumination ($M \pm m$)

Illumination, lx	Beginning of prolarval stage		Beginning of active feeding stage	
	<i>R. temporaria</i>	<i>R. arvalis</i>	<i>R. temporaria</i>	<i>R. arvalis</i>
0, control	3.54 $\pm$ 0.17	4.78 $\pm$ 0.09	9.36 $\pm$ 0.58	10.05 $\pm$ 0.33
5	3.50 $\pm$ 0.14	4.69 $\pm$ 0.12	9.04 $\pm$ 0.45	10.12 $\pm$ 0.24
150	3.65 $\pm$ 0.13	4.79 $\pm$ 0.15	9.65 $\pm$ 0.35	10.15 $\pm$ 0.25
550	3.33 $\pm$ 0.09	4.98 $\pm$ 0.11	9.41 $\pm$ 0.36	9.94 $\pm$ 0.45
600	3.29 $\pm$ 0.12	4.75 $\pm$ 0.09	9.34 $\pm$ 0.42	9.96 $\pm$ 0.23
800	3.36 $\pm$ 0.10	4.89 $\pm$ 0.11	9.26 $\pm$ 0.56	10.22 $\pm$ 0.40
1400	3.68 $\pm$ 0.12	4.95 $\pm$ 0.14	9.33 $\pm$ 0.78	10.08 $\pm$ 0.61

Table 4 Mortality rate and body length of the hatched larvae of tailless amphibians depending on illumination ($M \pm m$)

Illumination, lx	Mortality rate at embryonic stages, %		Mortality rate at prolarval stages, %		Body length, mm	
	<i>R. temporaria</i>	<i>R. arvalis</i>	<i>R. temporaria</i>	<i>R. arvalis</i>	<i>R. temporaria</i>	<i>R. arvalis</i>
0, control	30.3 $\pm$ 5.0	50.3 $\pm$ 5.6	20.0 $\pm$ 2.9	20.5 $\pm$ 2.6	10.42 $\pm$ 2.31	10.17 $\pm$ 5.51
5	16.6 $\pm$ 3.6	40.6 $\pm$ 2.3	14.4 $\pm$ 2.5	20.7 $\pm$ 5.3	11.76 $\pm$ 1.62	8.65 $\pm$ 1.54
150	12.2 $\pm$ 2.4†	17.4 $\pm$ 5.6†	8.5 $\pm$ 1.8†	11.4 $\pm$ 1.8*	11.55 $\pm$ 2.35	8.58 $\pm$ 1.42
550	8.6 $\pm$ 2.3†	50.8 $\pm$ 2.3	2.5 $\pm$ 0.3†	19.8 $\pm$ 2.4	10.37 $\pm$ 1.83	7.37 $\pm$ 2.58
600	18.5 $\pm$ 4.3	12.0 $\pm$ 2.5†	15.4 $\pm$ 5.7	4.4 $\pm$ 1.1†	10.53 $\pm$ 1.44	9.41 $\pm$ 2.39
800	22.8 $\pm$ 5.2	7.4 $\pm$ 3.6†	13.4 $\pm$ 6.4	5.6 $\pm$ 2.0†	10.35 $\pm$ 1.38	8.88 $\pm$ 1.82
1400	16.3 $\pm$ 3.0†	45.4 $\pm$ 4.5	14.7 $\pm$ 3.5	25.3 $\pm$ 6.4	9.56 $\pm$ 2.90	7.20 $\pm$ 3.38

Statistically significant at $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$.

Figure 1. Scheme of the experiment.