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THE EFFECTS OF ILLUMINATION ON THE EARLY DEVELOPMENT OF TAILED AND TAILLESS AMPHIBIANS

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Abstract. The objective of this work consists in valuing the carob pods (very rich in sugars and in nourishing elements) as being a medium of culture for growth of *Lactobacillus bulgaricus* and comparison this natural syrup with MRS medium for lactic acid production curve. By its biochemical composition, the carob syrup is very rich in carbohydrates 16 g/L, protein 0.25 %, ash 0.8 % including potassium (110 mg/100 mL of MF), sodium (80) and calcium (150). Kinetics study of growth of *Lactobacillus bulgaricus* in MRS and carob pods syrup showed that high biomass and lactic acid were obtained in the end of carob fermentations respectively (5.51 and 13.8) compared to MRS fermentation (4.57 and 10.9). This study clearly showed that carob pods have potential to be used as feedstock for lactic acid production by *Lactobacillus bulgaricus* using batch culture.
Keyword: Abiotic Factor; Common Frog; Common Newt; Crested Newt; Development; Larvae; Moor Frog; Mortality.

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, Terentev [TERENTEV, 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 Nikolskaya 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 [SYTINA and NIKOLSKAYA, 1984].

It is evident that the published data on this issue are poor and contradictory.

Material and methods

In our experiments, we 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, FERENCZ *et al.*, 2012, BUTU *et al.*, 2014] (Caudata: Salamandridae) are the 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 the 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 the species identification.

Each variant (one petri dish) of one experimental series contained 10–20 eggs from the same clutch.

The cultivation temperature was 20±1°C, and oxygen concentration in the water was 7.0–7.5 g/L.

The developmental stages were identified at 2–4 h intervals according to Liozner [Liozner, 1975] for the common and crested newts and according to Dabagyan and Sleptsova [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 dead individuals relative to their total number in the 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 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 with 0.01 mm accuracy.

All experiments were conducted with four to seven replications. The tabled data were averaged over all experimental series. For illumination, we used luminescent lamps, which do not heat during their work, and provide sufficiently strong light flux. The "dark" conditions (0 lx) were provided by a light–proof hood.

The illumination intensity was determined on the water surface with a Yu–116 light meter accurate up to +5%. The data were statistically processed using a standard method with Student's *t*–test [LAKIN, 1990].

Results and discussion

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).

In these variants, we recorded earlier hatching by respectively 3.0 and 4.3%, as compared to 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. In our experiments, we observed a similar situation; however, a short period between hatching and active feeding was still recorded.

Table 1.

Duration (days after fertilization) of the early development of tailed amphibians depending on illumination (*M*±*m*).

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

* Statistically significant at $p<0.05$.

This is associated with the fact that the yolk plug needs some time to gradually leave the gut. The mortality rate of the common newt embryos and prolarvae demonstrated a statistically significant decrease at low illumination (1

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and 10 lx). The actively feeding larvae grown under the illumination of 1 lx were slightly smaller in size (Table 2). At 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).

Table 2.

Mortality rate and body length of 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	
	Tr. cristatus	*L. vulgaris*	*Tr. cristatus*	*L. vulgaris*	*Tr. cristatus*	*L. vulgaris*
0,control	45.6 ± 2.8	26.5 ± 1.6	8.9 ± 1.4	17.5 ± 0.8	8.95 ± 0.09	9.01 ± 0.07
1	42.3 ± 2.4	20.0 ± 2.3*	7.8 ± 0.8	12.8 ± 1.2*	9.75 ± 0.07*	8.95 ± 0.12
10	34.0 ± 2.6*	19.3±2.0*	8.0 ± 1.2	10.3 ± 0.9†	10.56 ± 0.09†	10.34 ± 0.08†
700	42.5 ± 3.7	18.9 ± 3.4	8.2 ± 0.6	14.9 ± 2.0	9.83 ± 0.07*	10.83 ± 0.08†
1500	43.1 ± 4.5	20.6 ± 3.6	7.7 ± 0.9	15.3 ± 1.7	9.12 ± 0.07	9.42 ± 0.04*

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

The crested newt embryos developed faster only at the illumination of 700 lx (exceeding the control by 3.8%, p<0.05).

At 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 the 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 showed a statistically significant increase at low and medium illumination intensities (Table 2).

Thus, larger–sized larvae of the common newt developed at medium and high light intensities, and those of the crested newt at low and medium light intensities.

Illumination had no statistically significant effect on the egg development rate of the both tailless amphibian species (Table 3).

The time of the prolarvae emergence in all variants was similar.

We only noted a certain trend of the embryonic development time shortening observable at illuminations of 550, 600, and 800 lx.

However, the prolarval stage demonstrated that this trend was not retained, while the switch to active feeding accelerated at low light intensity.

Table 3.

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

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

On the other hand, the embryos displayed different survival rates.

In the tailless amphibians, in all cases death occurred 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 was registered at light intensities of 150, 550, and 1400 lx.

The differences in the remaining illumination variants were statistically insignificant.

Table 4.

Mortality rate and body length of hatched larvae of tailless amphibians depending on illumination (*M±m*).

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

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

As Table 4 shows, the survival rate of common frog embryos displayed no distinct dependence on illumination intensity. The mortality rate after hatching was lower at low illumination values (150 and 550 lx), i.e., a high survival percentage was observed at these light modes. Yet, the mortality rate of prolarvae decreased in other variants but the differences from the control were statistically insignificant.

Similarly, to the developmental rate, the body sizes of the actively feeding larvae differed from the control variant but this divergence was statistically insignificant. The experiments with the moor frog gave almost identical results.

Statistically significant mortality rate differences from the variant with zero illumination were recorded in the variants with light intensities of 150, 600, and 800 lx ($p < 0.01$ and $p < 0.001$). It must be noted that the moor frog displayed a very high (up to 70 %) mortality rate 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, it was partially due to the fact that the measurements were taken of the survived larvae. As we mentioned above, the 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 zero illumination (in the control) can be explained by the elimination of small individuals and survival of larger ones, the measurements of which contributed to the final result.

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 and RUCHIN, 2001; RUCHIN, 2002; 2003].

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 differed in a statistically significant manner depending on illumination.

The larvae grown in the dark and at the 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

My results suggest that the effect of illumination on the early development of amphibians is species–specific. In general, the rate of their development 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 the illumination of 700 lx.

Presumably, this is associated with the extended embryonic development of the tailed amphibians and an increase in the light radiation accumulated by the embryo. It is possible that certain illumination levels elevate the 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 grows 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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