Pertanika J. Trop. Agric. Sc. 41 (4): 1889-1897 (2018)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Light Spectrum Impacts on Early Development of Amphibians (Amphibia: Anura and Caudata)

Alexander B. Ruchin

Joint Directorate of Mordovia State Nature Reserve and Smolny National Park, Saransk 430000, Russia

ABSTRACT

The aim of the research was to determine the role of color in the early development of three species of amphibians (*Triturus cristatus*, *Rana arvalis* and *Rana temporaria*). The experiments were carried out in laboratory conditions, there were run 4-7 replicates. Standard filter systems were used. We monitored pace and stage of eggs' development, the mortality of eggs, and length of the hatched larvae. The color of illumination did not affect the rate of embryonic development of species with a short period of early development (*R. arvalis* and *R. temporaria*). Mortality at embryonic stages varied in different species. In all species red light negatively affected the survival of developing eggs. The larvae that started active feeding were larger in all three species with green-blue light than with white and, the more red, light.

Keywords: Development, embryos, light spectrum, mortality, *Rana arvalis*, *Rana temporaria*, *Triturus cristatus*

INTRODUCTION

Light is one of the main factors affecting various aspects of amphibians' life. For example, there were studies of the constant and variable illumination effect on the larval development and growth of some anurans (Konstantinov, Vechkanov, Kuznetsov, & Ruchin, 2000; Kuznetsov & Ruchin, 2001; Ruchin, 2000, 2001, 2004a, 2004b), and effect of photoperiod (Bambozzi, Seixas Filho, Thomaz, & Oshiro, 2004; Delgado, Gutiérrez, & Alonso-Bedate, 1987; Kukita et al., 2015). At the same time, extreme lighting conditions such as 24-hour light or 24-hour darkness adversely affected growth and retarded the metamorphosis of *Xenopus laevis* (Delgado et al., 1987). Increasing photophase significantly retards the development of *Discoglossus pictus* larvae (Gutierrez, Delgado, & Alonso-Bedate, 1984). Increasing photophase significantly retards the development of

ARTICLE INFO

Article history:
Received: 7 June 2018
Accepted: 6 September 2018
Published: 14 November 2018

E-mail address:
sasha_ruchin@rambler.ru

ISSN: 1511-3701
e-ISSN: 2231-8542

Discoglossus pictus larvae (Gutierrez et al., 1984). Larvae reared under longer scotophase developed faster (grew larger and reached further developmental stages) than larvae reared under shorter scotophase. Among other things, light influences behavior, activity time, reproduction of amphibians (Buchanan, 2006; Dastansara, Vaissi, Mosavi, & Sharifi, 2017; Laurila, Pakkasmaa, & Merilä, 2001; Lindgren & Laurila, 2009; Marquis & Miaud, 2008).

The effect of environmental color on animal physiology and behavior is a developing field. As in earlier studies, light spectrum showed both improvement and disruption of animal welfare. These findings are supporting the rising interest to investigate and get a better understanding of the effects of such related rearing conditions on animal performance. As for the color of light impact on the amphibians, there are several studies. For example, we studied the effect of light spectrum on the growth and development of several species of anurans larvae (Ruchin, 2002, 2003, 2004a). Hailman and Jaeger (1974) and Hartman and Hailman (1981) studied some anura species and found some kind of phototaxis for violet, blue, green, yellow, orange and red light, but most species preferred blue and green and were repulsed by violet and red light. The purpose of our research is to determine the role of environmental color in the early development of several species of amphibians.

MATERIAL AND METHODS

In our experiments, we investigated the effect of illumination on the earlier development and mortality of eggs and prolarvae of three species of anurans and caudata, which have different duration of egg development in natural water bodies.

The great crested newt *Triturus cristatus* (Caudata: Salamandridae) is a common species in central Russia. The female lays solitary eggs in a leaf of higher aquatic vegetation and wraps it by the hind legs. The moor frog *Rana arvalis* and the common frog *Rana temporaria* (Anura: Ranidae) belong to the group of brown frogs. These are species with a short embryonic period (5-15 days) and larval development (up to 65 days). Frogspawn of a common frog is always a large cluster, reminding a “mat” that floats on the water’s surface to prevent predators and increase the temperature inside. Moor frog, unlike the common one, has more loose egg mass. The investigated species are grown in zooculture. They are necessary for carrying out experimental works (for example, common and moor frogs) in biology, medicine and pharmacology. Larvae and adult amphibians are used for feeding in aquaculture and rearing reptiles. At the same time, their growing use and withdrawal from nature disturbs the balance in biocenoses, reduces their number in the wild, which increases the demand for animals grown in zooculture.

Spawn for the research, was selected in spawning reservoirs after fertilization from one couple of breeders (for one experiment), which, among other things, facilitated the identification of species. One set of 10-20 eggs from one frogspawn was taken for every case (per Petri dish). The temperature was maintained at 20 + 0.01°C, the oxygen content in water 7.0-7.5 mg/l. The development stages were determined according to the methods indicated earlier (Ruchin, 2018) and recorded every 2-4 h. The developmental rate was calculated as the time necessary for a certain stage of each individual in the experiment. Daily selection of dead eggs was carried out (mortality was taken into account). Mortality was calculated by the relative number of dead individuals to the total number of individuals in the experiment. Water was changed multiply during development stages observations. The experiment was stopped after the larvae started active feeding. The body length of the hatched larvae was measured with 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 (lamp LB), which do not heat when work, and provide sufficiently strong light flux. During the experiment we scattered light with the help of standard glass (Ruchin, 2004b). Here, one can see that 80-85% of light fall on a narrow zone of spectrum, this zone is a symbol for glass. Light intensity measured on water surface after the passing of light through color filter was 100 lx in all modes. In our experiments light fell at a vertical angle. In this case only 2% of light reflect independently on wavelength. After passing into water depths light was absorbed and diffused, that resulted in the reduction of light intensity depending on spectral structure. If taken into account reflection, absorption and scattering, light intensity on the bottoms of experimental aquariums was: under control lamp – 63.2 lx; by red light – 66.8 lx; by yellow – 64.0 lx; by green – 62.7 lx; by light blue – 62.3 lx; by blue – 58.3 lx. Due to small depth of Petri dishes the decrease of light intensity was insignificant and therefore we can claim that differences in data we got may be explained by spectral structure but not by light intensity.

Data between treatments and sampling times were compared by analysis of variance (ANOVA). The data were statistically processed using a standard method with Student's T Test.

RESULTS

The embryonic development of the crested newt under illumination of the green and blue zones of the spectrum was significantly reduced. In other cases, the rate of development little differed from control group (Table 1). As in other experiments (Ruchin, 2018), mortality of the crested newt was the highest during the period of embryonic development. At the same time, under yellow illumination, it significantly increased by 25.1% ($p < 0.05$), while in the case of blue illumination it decreased by 15.7% ($p < 0.05$) (Table 2). It was also observed that in the yellow light small individuals died and average sizes of surviving larvae became significantly higher (p <0.05) (Table 3). The length of the larvae decreased by 5.5% (p <0.05) in red light, while in the blue illumination it increased, as in the conditions of yellow illumination.

Table 1
Duration of early development in conditions of different colors of illumination (M + SE) (in days from fertilization)

Light spectrum	Beginning of the prolarvae emergence stage			The beginning of the active feeding stage		
	Triturus cristatus	*Rana temporaria*	*Rana arvalis*	*Triturus cristatus*	*Rana temporaria*	*Rana arvalis*
White	15.83±0.10	3.84±0.18	4.55±0.12	15.92±0.18	9.35±0.48	9.86±0.33
Red	15.85±0.11	3.86±0.14	4.51±0.22	16.04±0.14	9.24±0.44	9.80±0.38
Yellow	15.14±0.09	3.80±0.14	4.60±0.18	15.26±0.11	9.34±0.45	9.90±0.34
Green	15.01±0.09*	3.44±0.21	4.07±0.14	15.12±0.13	8.84±0.38	8.90±0.29
Light Blue	14.90±0.11*	3.46±0.11	4.06±0.11	15.08±0.14	8.78±0.36	9.05±0.34
Blue	15.87±0.12	3.55±0.15	4.37±0.17	16.02±0.15	8.94±0.37	9.78±0.25

* - reliable if $p<0.05$

Unlike the crested newt, in common frog, certain monochromatic zones of the spectrum had no significant effect on duration of the early development (Table 1). Some tendency to reduce the development time was traced under green, light-blue and blue light. In contrast to the duration of development, the mortality of embryos and pro-larvae of the common frog significantly increased under red and yellow light. In this case, in the first of these light modes, the length of the larvae decreased by 16.5% (p <0.01). Green light most favorably influenced the early development of the common frog. In this mode mortality decreased and larvae length increased.

Table 2
Mortality rates of amphibian eggs in conditions of different colors of illumination (M±SE)

Light spectrum	Mortality in embryonic stages, %			Mortality in the prolarval stages, %		
	Triturus cristatus	*Rana temporaria*	*Rana arvalis*	*Triturus cristatus*	*Rana temporaria*	*Rana arvalis*
White	36.2±2.1	27.2±2.4	20.4±2.3	6.8±1.0	0	5.3±1.0
Red	38.6±3.8	47.1±6.7*	30.3±5.8	6.4±0.6	12.4±1.2***	4.7±2.3
Yellow	45.3±2.8*	40.5±2.4**	10.7±4.2	4.5±1.2	11.7±1.6***	0
Green	33.6±2.6	40.1±2.8*	9.1±3.6*	4.3±1.1	0	0
Light Blue	30.5±1.8*	33.0±4.6	20.6±2.5	5.1±0.9	0	1.2±0.6*
Blue	34.3±6.5	20.6±3.4	9.8±1.3*	6.3±2.2	0	0

* - reliable if $p<0.05$, ** - reliable if $p<0.01$, *** - reliable if $p<0.001$

As in the experiments on common frog, the duration of early development of the moor frog, differed little under various conditions (Table 1). Mortality at the embryonic stages in experiments with the moor frog has significantly decreased with green and blue light. After hatching, a significant mortality of individuals was observed only in control group and in red light. A considerable increase ($p < 0.05$) in the length of the moor frog larvae was recorded under green and blue light, while in red light this index decreased significantly ($p < 0.01$).

Table 3
Larvae body length in conditions of different colors of illumination (M±SE)

	Larvae body length, mm		
Light spectrum	Triturus cristatus	*Rana temporaria*	Rana arvalis
White	10.23±0.10	8.34±0.09	8.10±0.09
Red	9.67±0.07*	6.96±0.12***	6.90±0.13**
Yellow	10.75±0.09*	8.27±0.08	8.08±0.11
Green	10.45±0.05	7.39±0.08**	7.69±0.09*
Light Blue	10.84±0.11*	8.30±0.07	8.51±0.08*
Blue	10.30±0.04	8.56±0.06*	8.41±0.10

* - reliable if p<0.05, ** - reliable if p<0.01, *** - reliable if p<0.001

DISCUSSION

The species of amphibians we have studied lay their eggs in the upper layers of the water to a depth of no more than 15-20 cm. Considering that the water in the spawning ponds is transparent at this time, the rays of different wavelengths penetrate well into the water and must affect the embryonic development of the species. Amphibian eggs range in color from cream to black. Melanin pigmentation, which imparts the dark color, is typically found on the dorsal hemisphere. Eggs that are hidden from the sun and laid under debris, among leaves, or in foam nests tend to be lighter in color than those that are fully exposed to sunlight. Eggs of aquatic-breeding salamanders tend to be brown, but the eggs and embryos of most species of frogs and toads that breed in open water are nearly black (Wright & Wright, 1949).

Most research focus on the effect of ultraviolet radiation on the eggs of amphibians (Anzalone, Kats, & Gordon, 1998; Blaustein & Belden 2003; Cummins, Greenslade, & McLeod, 1999; Langhelle, Lindell, & Nyström, 1999), and there is practically no evidence on how does visible spectrum part effect the frogspawn. For example, Terentiev (1950) noted that eggs of common frog ceteris paribus developed in the dark and in the light with the same speed. Sytina and Nikol'skaya (1984) had experimentally proven that, despite the temperature increase in in the center of the spawn, eggs of this species from the upper, most illuminated layers, developed and hatched faster, even though the temperature was lower. In the experiments of Ankley et al. (2000) intense sunlight caused high mortality of developing embryos.

Our experiments showed that environmental color affects the development of amphibians' eggs. However, its influence differs depending on parameters of development, on species, and on embryonic development duration. This is also confirmed by experiments on four similar in biology species of anurans (Ding, Lin, Zhao, Fan, & Wei, 2014). For example, light spectrum doesn't have any significant influence on separate stages of embryogenesis in anurans with short embryonic development (*Rana temporaria* and *Rana arvalis*). On the other hand, eggs of newts, which have four times longer development cycle than the eggs of anurans, developed faster than control group when exposed to green and blue light. Apparently, a certain time is necessary for the manifestation of a stimulating or negative effect of the light spectrum. We obtained reliable information in the analysis of egg survival in different lighting conditions. It turned out that the mortality of embryos and prolarvae increases within the long-wavelength part of the spectrum and decreases with green-blue illumination. Unfortunately, there is very little information about the light spectrum effect on the eggs of amphibians. However, our data are consistent with those obtained in some fish species: eggs development of *Acipenser baerii*, *Dicentrarchus labrax*, *Gadus morhua* and *Scophthalmus maximus* improved when exposed to blue-green spectrum rays (Ruchin, 2016; Sierra-Flores et al., 2016; Villamizar et al., 2011). We assume that the short-wave part of the spectrum (green and blue light) induces the development of amphibian embryos, actively affecting certain stages of development. At the same time, this effect does not become evident immediately, but manifests itself after a certain period of time. It becomes clear from experiments with eggs of anurans, in which the development of eggs is faster than that of caudata.

The data obtained correlate with the results of our previous experiments (Ruchin, 2002, 2003, 2004a), which showed better growth and development of larvae in certain amphibian species under blue-green illumination. Thus, the early development parameters of these species, as well as the prolonged larval development, depended on the environmental color. Obtained results partially agree with Jung's conclusion (Berkovich, 1953) on the negative effect of red light on the growth of tadpoles.

CONCLUSION

Thus, the influence of monochromatic illumination in the visible part of the spectrum for different species is specific. The authors showed that light intensity effect on the growth and development of larvae depends on the species. We showed that the light spectrum does not affect the rate of embryonic development of species with a short period of early development, but affects species with a long embryonic period. At the same time, different zones of the spectrum differently affect such significant development parameters as the survival rate and size of the larvae that started active feeding. Favorable effect of green-blue light is common for all the three species of amphibians. Unfortunately, our and literary data are still not enough to make assumptions about the effect of illumination color on amphibian eggs. Therefore, for the construction of hypotheses in the future, it is necessary to conduct similar studies on species with other ecological needs.

REFERENCES

Ankley, G. T., Tietge, J. E., Holcombe, G. W., DeFoe, D. L., Diamond, S. A., Jensen, K. M., & Degitz, S. J. (2000). Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. *Canadian Journal of Zoology, 78*(6), 1092-1100. doi:10.1139/z00-040

Anzalone, C. R., Kats, L. B., & Gordon, M. S. (1998). Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina*, *Hyla regilla*, and *Taricha torosa*. *Conservation Biology*, *12*(3), 646–653.

Bambozzi, A. C., Seixas Filho, J. T. D., Thomaz, L. A., & Oshiro, L. M. Y. (2004). Effect of the photoperiod on bullfrog (*Rana catesbeiana* Shaw, 1802) tadpoles development. *Revista Brasileira de Zootecnia*, *33*(1), 1-7. doi:10.1590/S1516-35982004000100001

Berkovich, E. M. (1953). Influence of white and monochromatic light on animal organisms. *Biology Bulletin Reviews*, *36*(1), 43–63.

Blaustein, A. R., & Belden, L. K. (2003). Amphibian defenses against ultraviolet-B radiation. *Evolution and Development*, *5*(1), 89–97.

Buchanan, B. W. (2006). Observed and potential effects of artificial night lighting on anuran amphibians. In C. Rich, & T. Longcore (Eds.), *Ecological consequences of artificial night lighting* (pp. 192-220). Washington, USA: Island Press.

Cummins, C. P., Greenslade, P. D., & McLeod, A. R. (1999). A test of the effect of supplemental UV-B radiation on the common frog, *Rana temporaria* L., during embryonic development. *Global Change Biology, 5*(4), 471–479.

Dastansara, N., Vaissi, S., Mosavi, J., & Sharifi, M. (2017) Impacts of temperature on growth, development and survival of larval *Bufo* (Pseudepidalea) *viridis* (Amphibia: Anura): Implications of climate change. *Zoology and Ecology*, *27*(3-4), 228-234. doi: 10.1080/21658005.2017.1360037

Delgado, M. J., Gutiérrez, P., & Alonso-Bedate, M. (1987). Melatonin and photoperiod alter growth and larval development in *Xenopus laevis* tadpoles. *Comparative Biochemistry and Physiology Part A: Physiology*, *86*(3), 417-421.

Ding, G. H., Lin, Z. H., Zhao, L .H., Fan, X. L., & Wei, L. (2014). Effects of light intensity on activity in four sympatric anuran tadpoles. *Zoological Research*, *35*(4), 332−337. doi:10.13918/j.issn.2095-8137.2014.4.332

Gutierrez, P., Delgado, M. J., & Alonso-Bedate, M. (1984). Influence of photoperiod and melatonin administration on growth and metamorphosis in *Discoglossus pictus* larvae. *Comparative Biochemistry and Physiology Part A: Physiology*, *79*(2), 255–260.

Hailman, J. P., & Jaeger, R. G. (1974). Phototactic responses to spectrally dominant stimuli and use of colour vision by adult anuran amphibians: A comparative survey. *Animal Behavior*, *22*, 757-795.

Hartman, J. G., & Hailman, J. P. (1981). Interactions of light intensity, spectral dominance and adaptational state in controlling anuran phototaxis. *Zeitschrift fur Tierpsychologie*, *56*(4), 289-296.

Konstantinov, A. S., Vechkanov, V. S., Kuznetsov, V. A., & Ruchin, A. B. (2000). Oscillation of abiotic environment are required optimal growth and development of a frog *Rana temporaria* L. *Doklady Akademii Nauk*, *371*(4), 559–562.

Kukita, S., Gouda, M., Ikeda, S., Ishibashi, S., Furuya, T., & Nakamura, K. (2015). Effects of photoperiod and temperature on growth and development in clouded salamander (*Hynobius nebulosus*) larvae. *Zoological Science*, *32*(3), 266-271. https://doi.org/10.2108/zs140220

Kuznetsov, V. A., & Ruchin, A. B. (2001). Effect of pH and illumination oscillations on growth rate and development of *Rana ridibunda* larvae. *Zoologicheskii Zhurnal*, *80*(10), 1246-1251.

Langhelle, A., Lindell, M. J., & Nyström, P. (1999). Effects of ultraviolet radiation on amphibian embryonic and larval development. *Journal of Herpetology*, *33*(3), 449-456.

Laurila, A., Pakkasmaa, S., & Merilä, J. (2001). Influence of seasonal time constraints on growth and development of common frog tadpoles: A photoperiod experiment. *Oikos*, *95*(3), 451-460. doi:10.1034/j.1600-0706.2001.950310.x

Lindgren, B., & Laurila, A. (2010). Are high-latitude individuals superior competitors? A test with *Rana temporaria* tadpoles. *Evolutionary Ecology*, *24*(1), 115–131. doi: 10.1007/s10682-009-9294-4

Marquis, O., & Miaud, C. (2008). Variation in UV sensitivity among common frog *Rana temporaria* populations along an altitudinal gradient. *Zoology*, *111*(4), 309-317. doi: 10.1016/j.zool.2007.09.003

Ruchin, A. B. (2000). The effect of light oscillations on the growth of fish juveniles and the brown frog (*Rana temporaria*). *Zoologicheskii Zhurnal, 79*(11), 1331-1336.

Ruchin, A. B. (2001). Some peculiarities of the fish young growth under light-gradient conditions // *Journal Evolutionary Biochemistry and Physiology*, *37*(3), 312-313. DOI: 10.1023/A:1012635827380

Ruchin, A. B. (2002). Effects of monochromatic light on growth and development of the clawed frog *Xenopus laevis* larvae. *Zoologicheskii Zhurnal*, *81*(6), 752-756.

Ruchin, A. B. (2003). Effect of monochromatic light on the growth and development of brown frog (*Rana temporaria* L.) larvae. *Biology Bulletin*, *30*(5), 482-484.

Ruchin, A. B. (2004a). Effects of temperature and illumination on growth and development of brown frog larvae (*Rana temporaria*). *Zoologicheskii Zhurnal*, *83*(12), 1463-1467.

Ruchin, A. B. (2004b). Influence of colored light on growth rate of juveniles of fish. *Fish Physiology and Biochemistry*, *30*(2), 175-178. doi: 10.1007/s10695-005-1263-4

Ruchin, A. B. (2016). Effect of light on the development of the hard roe of *Acipenser baerii* Brandt, 1869. *Indian Journal of Science and Technology*, *9*(29). doi:10.17485/ijst/2016/v9i29/89110

Ruchin, A. B. (2018). The effects of illumination on the early development of amphibians (Amphibia: Anura and Caudata). *Periodico Tche Quimica*, *15*(30), 152-159.

Sierra-Flores, R., Davie, A., Grant, B., Carboni, S., Atack, T., & Migaud, H. (2016). Effects of light spectrum and tank background colour on Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) larvae performances. *Aquaculture*, *450*, 6-13. doi:10.1016/j.aquaculture.2015.06.041

Sytina, L. A., & Nikol'skaya, N. G. (1984). Individual variability of development rate in the grass frog embryos (*Rana temporaria* L.) in natural and experimental conditions. *Ecological-morphological investigations of the early ontogenesis in vertebrates* (pp. 70–96). Moscow, Russia: Nauka.

Terentiev, P. V. (1950). *Frog*. Moscow, Russia: Modern Science.

Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S., & Sánchez-Vázquez, F. J. (2011). Effects of light during early larval development of some aquacultured teleosts: A review. *Aquaculture*, *315*(1-2), 86–94. doi:10.1016/j.aquaculture.2010.10.036

Wright, A. H., & Wright, A. A. (1949). *Handbook of frogs and toads of the United States and Canada.* New York, NY: Comstock Publishing.