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Impact of Electromagnetic Field on Peculiarities of Rana Temporaria Linnaeus, 1758 (Anura, Ranidae) Ontogeny

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This work is dedicated to the study of electromagnetic irradiation influence on European grass frog tadpoles. Peculiarities of its impact on their linear features and mortality were investigated. Growth and mortality intensification were detected according to the increasing of number of irradiation sessions. The presence of morphological abnormalities in post-metamorphic individuals was also assessed. The experiment showed that electromagnetic irradiation did not cause the mass occurrence of morphological anomalies. The preliminary results of the work show that this field of experiment is promising and further investigations should be conducted. The results of the experiment can be used in herpetoculture in creating the stable breeding stock of amphibians, development of nature conservation management-plans for objects of the nature reserve fund as well as in studying the impact of an electromagnetic field on living organisms in general.

Keywords: amphibians, European grass frog, ontogeny, electromagnetic irradiation, ecology.

Introduction

With no doubt humanity's use of modern devices that operate at high frequencies such as 3 MHz – 3,000 GHz (mobile phones, microwaves, physiotherapy apparatus, radios, etc.) has increased in recent few decades and the progressive growth in the number of cancer and congenital disorders among human beings are a matter of fact. As a consequence, experiments with irradiation are becoming increasingly relevant and the number of works on this topic has grown significantly in order to study the impact of different irradiation types on living organisms. The study of the effects of environmental factors on the progress of embryonic and post-embryonic ontogenesis is an important trend in modern investigations. Amphibians are often used for various scientific purposes as model objects. Various factors can affect amphibians' ontogeny (BeeBee, Griffiths, 2005), e.g., temperature, nutrition, population's density, various chemical and physical pollutions, etc. Experiments with high frequency irradiation (900 MHz) have yielded interesting effects of electromagnetic radiation on bird and fish ontogeny (Romaniuk et al., 2011; Jyoti, 2014; Orizaola et al., 2014; Tsybulin, 2016).

Other similar experiments of different objects have shown results that are rather contradictive. For example, in one of the earliest variants of such experiments, a group of British scientists have studied the effect of low frequency irradiation (from 100 to 1000 Hz) on embryogenesis of chickens. As a result, the percentage of anatomical anomalies was the same for experimental and control regimes. No significant differences between control and experimental chicken eggs were detected (Maffeo et al., 1984). In contrast to low frequencies, high frequencies have a significant impact on the development of living organisms. It is known that the equivalent of cell-phone frequencies (900 MHz) has a negative effect on reproductive organs and tissues of mammals, and also destroys the balance of reproductive hormones (Ozguner et al., 2005). While studying the effects of chicken embryo exposure to high frequency irradiation, there was a significantly higher mortality and change in somatic structure in irradiated groups in comparison with the control group. As an instrument for generating an electromagnetic field in such experiments, the Agilent 9310A RF (9 KHz-3 GHz) was used. Consequently, the study concluded that the effects of electromagnetic radiation had a detrimental effect on the development of the chicken embryos during the early incubation period (Shafey et al., 2011; Jyoti, 2014; Faeghi, 2015).

Similar conclusions have come from scientists from post-soviet countries. Affecting of quail embryos 5 days before and during incubation with a microwave GSM 900 MHz irradiation leads to increased embryonic mortality and decreased quail derivability (Tsybulin, 2016). As a high-frequency generator, the commercial model of the Nokia 3120 mobile phone was used in this study. Birds' (chickens', quails') eggs were the model object in these studies as it is a convenient and accessible material, which, moreover, develops beyond the mother's body. The effect of irradiation was also studied on plant organisms, namely on the beans, seeds of which were exposed to irradiation prior to sowing. It turned out that pre-sowing processing of seeds of agricultural crops by electromagnetic fields during a certain period of time positively affected the development and growth of plants. The frequency which acts on the organisms of plants stimulating the effect of more rapid growth is equal to 22 MHz (Poghosyan et al., 2018). As these animals inhabit urbanised territories as well as natural habitats, using both water and terrestrial areas for different aspect of their life, they suffer from a great variety of harmful factors. In particular, they constantly feel the impact of electromagnetic fields that are generated in cities by human-made powerful devices. Therefore, the main aim of this work was a preliminary study of the effects that are caused in amphibians of urbanised territories by imitating high-frequency irradiation that is equal to the levels currently taking place in our cities (mostly generated by cell phone stations). The object of this experiment was the spawn of grass frogs, *Rana temporaria* Linnaeus, 1758 (Anura, Ranidae).

The main aim of the work was a preliminary study of the effects of electromagnetic field irradiation commonly used in city radio stations (22 MHz) on the ontogenesis of the European grass frog, particularly on mortality level and linear growth of its tadpoles.

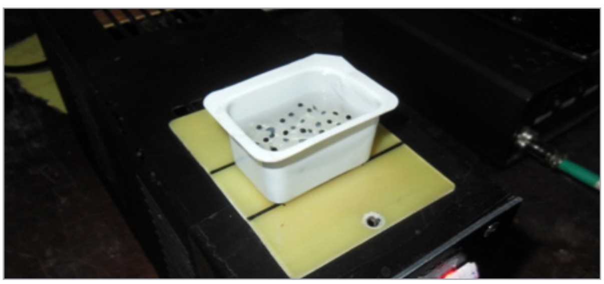
Methods

Irradiation device description

The device consists of two blocks, connected by a cable with manufactured plugs at its ends (Fig. 1). The first block is a power supply, powered by a 220 V AC voltage and a frequency of 50 Hz. The second block, the irradiator itself, is a metal case in which electronic components are located. In the upper part of the irradiator block, there is a textolite table, not covered with a copper layer, for irradiation of the material (seeds, spawn, etc.). The table is secured by two screws on the textolite surface. A rectangular hole is cut out underneath the irradiation source for unobstructed passing of electromagnetic radiation to the irradiated material (Fig. 2). The block is equipped with a metal handle, a milliammeter and light indicators of the irradiator work. The power supply is equipped with the main switch and three switches of output voltage. The device is equipped with a 1.5 A fuse.

Fig. 1
Outside view of the irradiation device

Fig. 2
A place where the frogs' spawn is situated for the irradiation time



The irradiating block consists of two parts. The first part is a pre-requisite quartz generator on a radio lamp of 6Z52P. The second part of the block is the oscillation amplifier (power amplifier) on the radio lamp GU-19-1. From a quartz oscillator, a signal at a frequency of 22,000 kHz falls on a power amplifier (a radio lamp SU-19-1). In the anode chain of this lamp, there is a vibrational circuit (coil), arranged on the quartz oscillator frequency (22,000 kHz). During the resonance of the circuit, high frequency oscillations (40–50 W) are emitted, with a frequency of 22,000 kHz. Thus, the spawn in a plastic box is irradiated with this generated oscillation.

Experiment

Our experiment was conducted from the end of March to the middle of August. Grass frog spawn (tadpole stage №17-20) (Objects of biological development, 1975) was collected from a spring near Didorivka lake (N 50.375118; E 30.499945) in Holosiivskyi National Nature Park during spawning at the end of March 2017. No special permissions from local authorities were required for sampling and conducting of the experiment. Eggs were transferred to a plastic container (25 cm^3) in portions of 22 pieces for further irradiation after mixing. The plastic box with each portion of eggs was placed on the device so that the eggs were situated inside the electromagnetic field for a certain period of time. Then the material was placed in 10 boxes: A, B, C, D (series 1); K1, K2 (control); A (ob), B (ob), C (ob), D (ob) (series 2) with the same water temperature of 12 °C. Irradiation time of eggs in each box were as follows: A – 0.5 min, B – 5 min, C – 10 min, and D – 20 min (1 irradiation session for series 1). Boxes A (ob) – D (ob) were irradiated twice (Table 1), repeating the previously mentioned times on the day after the first session (2 irradiation sessions in total for series 2). Control groups were not exposed to electromagnetic influences (0 irradiation sessions). Two control groups were necessary in order to confirm a slight discrepancy in the appearance of morphological anomalies in the absence of irradiation during the experiment. The manifestation of anomalies is considered to be background if the percentage of abnormal individuals does not exceed 5%. This figure may be higher if the sample consists of more than 100 individuals (as in our case) (Borkin et al., 2012). A total amount of 264 eggs (Nstart) was put inside each box. All boxes were placed in the same room, so they were exposed to the same temperature fluctuations. All of them were supplemented with water aerators to provide sufficient level of oxygen and lamps. Water was renewed weekly. After hatching, tadpoles were fed with boiled dandelion leaves and boiled carrot, and after tadpole stage №45, they were fed with boiled eggs and dry fish food Tetra Min (Tetra©). After the first signs of appearing limbs, all boxes were transferred outside the room to the natural sunlight to stimulate metamorphosis as it was quite cold in the room and the tadpoles' growth was a bit slower than it was expected (Marushchak et al., 2018).

Table 1

Time of eggs irradiation in each box and amount of energy spent on it

Box code	Irradiation time (min)	Irradiation energy (J)
1	2	3
A	0.5	567
B	5	5,670
C	10	11,340
D	20	22,680
A (ob)	(0.5 + 0.5) = 1	1,134
B (ob)	(5 + 5) = 10	11,340
C (ob)	(10 + 10) = 20	22,680
D (ob)	(20 + 20) = 40	45,360
K1	0	0
K2	0	0

Measurements

Tadpoles' total length was used to study the irradiation effects. The samples for measuring (3 active specimens were randomly caught from each box) were taken every 4–5 days before the first limbs' appearing. Length measuring and stage determination were made on fixed material (70% ethanol), and the photos were taken from Leica 2.0 microscope. Until the first signs of metamorphosis beginning, the number and percentage of tadpoles in each box were counted every 4–5 days to measure the death rates.

On 10/06/2017, several tadpoles in each box grew the hind limbs. This date was considered a moment of the start of metamorphosis. To assess the effect of exposure, the following parameters were counted (Table 5):

1. the number of tadpoles at the beginning of metamorphosis (NMetSt);
2. the percentage of tadpoles that survived to the beginning of metamorphosis (MetSt), MetSt=(NMetSt*100)/Nstart;
3. the number of individuals that underwent metamorphosis (NMetFin);
4. the percentage of individuals that underwent metamorphosis (from the initial number of eggs) (MetFin1), MetFin1=(NMetFin*100)/Nstart;
5. the percentage of individuals that underwent metamorphosis (from those that entered metamorphosis) (MetFin2), MetFin2=(NMetFin*100)/NMetSt;
6. the percentage of abnormal individuals among those that underwent metamorphosis (Abnorm).

Anomalies were considered to be any deviation from the mean value of the indicator for this type that goes beyond the signs of variability (Prisny, 2009) and noticeable to the eye. In determining and describing the anomalies, we used the classification of Nekrsova (Nekrasova, 2008; Tytar et al., 2018) and Vershinin (Vershinin, 1989; Vershinin, 2015), as well as the guidelines of Borkin with co-authors (Borkin et al., 2012). The percentage of individuals with a certain type of anomaly (P_{as}, %) was counted after the end of metamorphosis.

Data processing was done in MS Excel (2010) and Statistica 10.0. The statistical analysis of irradiation impact on tadpoles' total length and percentage of individuals that survived until the beginning of metamorphosis was done using the multiple regression analysis in Statistica 10.0 (Ivanter, Korosov, 2000).

Results and Discussion

Multiple regression analysis showed that the number of irradiation sessions was more likely to have an impact on the increased body length of tadpoles (Table 2). The average body size of the individuals from the

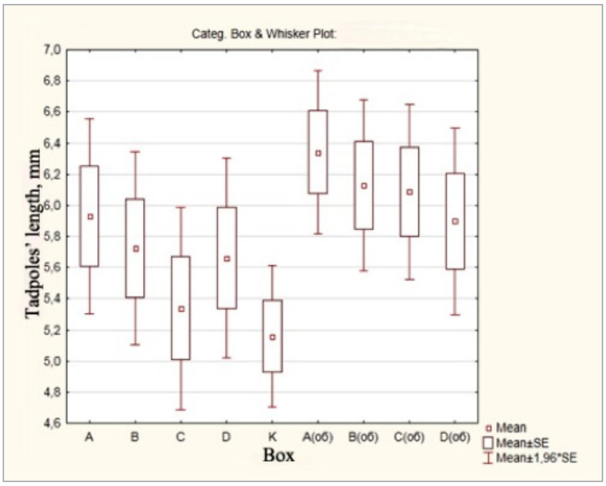
Table 2

Results of Multiple regression analysis, that demonstrates the impact of irradiation sessions' number on tadpoles' length

	b*	Std. Err.	b	Std. Err.	t(366)	*P* value
	1	2	3	4	5	6
Date	0.8388	0.0267	0.11	0.0035	31.395	< 0.01
Irradiation time	–0.0202	0.0288	–0.01	0.0075	–0.701	0.48
Number of irradiation sessions	0.1933	0.0288	0.50	0.0745	6.711	< 0.01

Fig. 3

The difference between the average tadpole size depending on treatment variant (One-Way ANOVA analysis)



A (ob) – D (ob) series was 6.120 mm, while in the A–D series, it was 5.665 mm. Control series had the lowest body length – 5.158 mm. In boxes with twice irradiated spawn, tadpoles grew significantly faster. There was no reliable difference between two control boxes; therefore, only one of them was used for statistical analysis (Fig. 3).

Taking into account the critical ontogenetic periods (Date), number of irradiation sessions and irradiation time (the same parameters, as were taken for processing of body length data) the same trend was observed (Table 3). Tadpoles' mortality was higher in the boxes, where the spawn was irradiated twice (Figure 4).

In twice irradiated boxes, tadpoles tended to grow bigger and die in bigger quantities at the same time. As it is indicated in recent researches (Poghosyan et al., 2018), high frequencies can modify the biological systems' response to the action of chemical substances and physical factors. For example, irradiation can increase the cellular membrane growth by making it more open to incoming ions. This potentially can explain the more rapid growth of tadpoles and, at the same time, as animal cells do not have cell walls, so they are less resistant to rapid growth of cellular membrane, therefore, leading to a cell damage and the death of the whole organism. As a result, more dead individuals resulted in more free space to those that survived and more protein food source. That is why the remaining individuals managed to survive and grow bigger than their siblings in series 1 or control boxes.

Table 3

Results of Multiple regression analysis that demonstrates the impact of irradiation sessions' number on tadpoles' mortality

	b*	Std. Err.	b	Std. Err.	t(212)	*P* value
	1	2	3	4	5	6
Date	–0.846	0.0315	–0.77	0.029	–26.818	< 0.01
Irradiation time	–0.0308	0.0330	-0.08	0.083	–0.9360	0.35
Number of irradiation sessions	–0.2594	0.0330	–7.29	0.927	–7.8657	< 0.01

Fig. 4

Differences in quantities of alive individuals at a certain moment of time before the start of metamorphosis. Control boxes show a significantly higher level of survived individuals and less rapid mortality than the irradiated ones

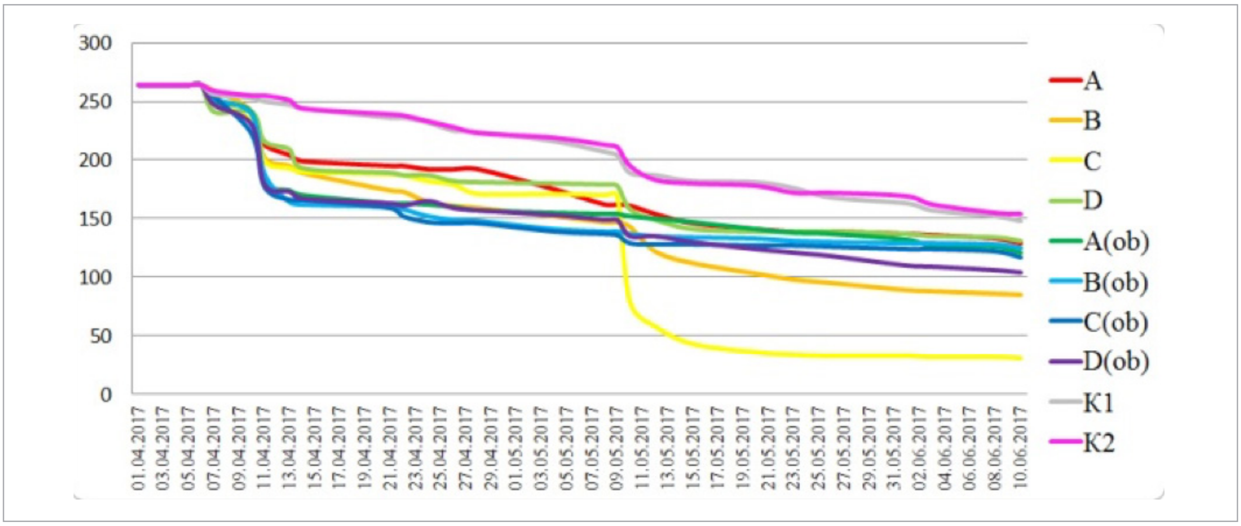


Table 4

Percentage of individuals with certain anomalies after metamorphosis

Box	Anomaly type	P_{as} (%)
1	2	3
A	none	0
B	Macrognathia (enlarged upper jaw)	1.25
	Amelia (absence of limb)	1.25
C	Macrophthalmia (enlargement of the whole eye or its parts)	3.22
D	Hemimelia (shortening of a limb)	1.78
A (ob)	Amelia	2.94
	Hemimelia	2.94
B (ob)	Scoliosis	4.76
C (ob)	none	0
D (ob)	Amelia	6.25
K1	Amelia	3.85
	Anophthalmia (absence of an eye)	3.85
K2	Amelia	9.37

After all individuals underwent metamorphosis, the types of abnormalities were determined as follows and the percentage of abnormal individuals (Table 3) was counted (from NMetFin). In no case, the percentage of abnormal individuals was higher than 5% (except D (ob) and K2, where the figures are only a bit higher (Table 4). The reason for such results is likely to be related to higher mortality in experimental groups. As noted above, tadpoles in the experimental groups grew faster, but at the same time, due to cell deformation, more tadpoles died (Fig. 4). As a result, most of the individuals with morphological anomalies simply did not survive to start metamorphosis, dying at one of the so-called critical stages of metamorphosis, which are characterised by high mortality even in healthy populations (Objects of..., 1975; Severtsova, Severtsov, 2011). Since the control groups grew under normal conditions, the manifestation of morphological anomalies in them was a little bit higher. However, it should be noted that all the obtained rates were near the mark of 5% and did not demonstrate a truly mass character of the anomaly manifestation (for example, more than 25%, as it was expected) (Borkin et al., 2012).

No reliable correlation (Pearson correlation) was found between the percentage of tadpoles that survived to the beginning of metamorphosis (MetSt), the percentage of individuals that underwent metamorphosis

Table 5

Parameters measured and counted to assess the influence of irradiation

Box code	Nstart, (pcs)	NMetSt, (pcs)	MetSt, (%)	NMetFin	MetFin1, (%)	MetFin2, (%)	Abnorm, (%)
1	2	3	4	5	6	7	8
A	264	129	48.86	29	10.98	22.48	0
B	264	85	32.20	80	30.30	94.12	2.5
C*	264	31	11.74	31	11.74	100.00	3.22
D	264	131	49.62	56	21.21	42.75	1.78
A (ob)	264	121	45.83	34	12.88	28.10	5.88
B (ob)	264	125	47.35	21	7.95	16.80	4.76
C (ob)	264	117	44.32	61	23.11	52.14	0
D (ob)	264	104	39.39	16	6.06	15.38	6.25
K1	264	148	56.06	26	9.85	17.57	7.69
K2	264	154	58.33	32	12.12	20.78	9.32

Note*. After moving outside the room, almost all tadpoles in box C died for no obvious reason; thus, the data was not included in statistical processing.

(from the initial number of eggs) (MetFin1), the percentage of individuals that underwent metamorphosis (from those that entered metamorphosis) (MetFin2), the percentage of abnormal individuals among those that underwent metamorphosis (Abnorm) and the amount of energy spent on the irradiation. This indicates that the post-metamorphic death rates have no correlation with the amount of energy and time spent on the irradiation. The reason possibly is that the whole result of irradiation was seen during pre-metamorphic death rates and all information about external morphological anomaly manifestations was lost with dead individuals that were usually eaten by other tadpoles in the box.

Conclusions

The analysis of length and mortality changes in amphibian tadpoles under the impact of high-frequency electromagnetic irradiation was carried out for the first time. The difference between the mean lengths of tadpoles from different series was reliable and was more than 95% ($p = 0.036$). The largest size of tadpoles was found in series 2 (2 irradiation sessions). As confirmed by literary data, irradiation leads to cell growth (on an example of plants). Meanwhile, this led to higher mortality among tadpoles and this trend increased with the number of irradiation sessions. The tadpoles that survived demonstrated a simultaneous increase of linear growth. It might be not only the result of irradiation itself, but also the space per each alive individual that was increasing with each new death of its siblings. As high mortality rates in irradiated samples were significant, the result of our research is the recommendation to minimise the impact of devices forming the electromagnetic field near natural water areas (especially in urban areas). The implementation of this recommendation will help to preserve the biodiversity of drift ecosystems and reduce the anthropogenic load on the population of dead-fly amphibians, and, probably, other groups of hydrobionts. However, they show interesting trends in the influence of electromagnetic irradiation on tailless amphibians. As the higher values of death rates in irradiated samples are significant, it is suggested to minimise the electromagnetic influence near natural water areas, especially within urbanised territories, in order to save the biodiversity and reduce the anthropogenic impact on the amphibian populations.

{Gurauskiene, 2006, Eco-design methodology for electrical and electronic equipment industry}

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References

BeeBee T. J. C., Griffiths R. A. (2005) The amphibian decline crisis: a watershed for conservation biology. Biological Conservation 125: 271-285. https://doi.org/10.1016/j.biocon.2005.04.009

Borkin L. J., Bezman-Moseyko O. S., Litvinchuk S. N. (2012) Evaluation of animal deformity occurrence in natural populations (an example of amphibians). Trudy Zoologicheskogo instituta RAN 316(4): 324-343.

Faeghi P., Narimani-Rad M. and Besharat P. E. (2015) Electromagnetic field and its effect on chicken embryo. Biological Forum - An International Journal 7(1): 559-563.

Ivanter E. V., Korosov V. A. (2000) Introduction to the quantitative biology. Petrozavodsk: 320 c.

Jyoti, Kohli R. K. and Bagai U. (2014) Effect of mobile phone frequency radiation on early development of chick embryo. International Journal of Science, Environment and Technology 3(3): 1273 - 1280. http://www.ijset.net/journal/359.pdf

Maffeo S. Morton W. Miller and Edwin l. Carstensen (1984) Lack of effect of weak low frequency electromagnetic fields on chick embryogenesis. Journal of anatomy 139(4): 613-618.

Marushchak O., Nekrasova O., Voitenko V., Oskyrko O., Zhytnyk D. (2018) Electromagnetic field influence on peculiarities of Rana Temporaria Linnaeus, 1758 (Anura, Ranidae) ontogeny. In: Abstract book of Second International Conference «Smart Bio», Kauna, Lithuania, 3-5 May 2018: P. 162.

Nekrasova O. D. (2008) Classification of amphibians' anomalies. Proceedings of Ukrainian herpetological society 1: 55-58.

Objects of ontogenetic biology. - Moscow: Nauka, 1975: 580.

Orizaola G., Dahl E. and Laurila A. (2014) Compensatory growth strategies are affected by the strength of environmental time constraints in anuran larvae. Oecologia 174(1): 131-137. https://doi.org/10.1007/s00442-013-2754-0

Ozguner F., Altinbas A., Ozaydin M., Dogan A., Vural H., Kisioglu A. N., Cesur G. and Yildirim N. G. (2005) A novel antioxidant agent caffeic acid phenethyl ester (CAPE) prevents shock wave-induced renal tubular oxidative stress. Urological Research 33(3): 239-243. https://doi.org/10.1007/s00240-005-0470-x

Poghosyan G., Mukhaelyan Z. and Vardevanyan P. (2018) Combined effect of cadmium ions and millimeter range electromagnetic waves on growth and antioxidant enzymes activity of wheat seedlings. In: Abstract book of Second International Conference «Smart Bio», Kaunas, Lithuania, 3-5 May 2018: P. 129.

Prisny, Yu. A. (2009) Classification of morphological anomalies of coleopteran insects (Coleoptera). Scientific bulletins of Belgorod State University. Series: Natural Sciences. 9(11): 72-81.

Tytar V. M., Nekrasova O. D., Marushchak A. Yu. (2018) Ecological and Geographical Gis-Analysis of Anomalies in Amphibians of Ukraine. In The Second International conference "Amphibian and reptiles anomalies and pathology: methodology, evolutionary significance, monitoring and environmental health", KnE Life Sciences: 42-48. https://doi.org/10.18502/kls.v4i3.2101

Vershinin V. L. (1989) Morphological abnormalities in urban amphibians. Ecology 3: 58-66.

Vershinin V. L. (2015) Bases of methodology and methods of research of anomalies and pathologies of amphibians. Ekaterinburg: Publishing house of the Ural University: 80 p.

Romaniuk M. S., Mandzinets S. M., Bura M. V., Sanahurskii D. I. (2011) Influence of low-intensity laser radiation on Na +, K +-ATP-phase activity of weathefish embryos during early embryogenesis. Photobiology and experimental photomedicine 1: 76-83.

Shafey T. M., Aljumaah R. S., Swillam S. A., Al-mufarrej S. I., Al-abdullatif A. A. and Ghannam M. M. (2011) Effects of short term exposure of eggs to magnetic field before incubation on hatchability and post-hatch performance of meat chickens. Saudi Journal of Biological Sciences 18: 381-386. https://doi.org/10.1016/j.sjbs.2011.06.004

Severtsova E. A., Severtsov A. S. (2011) Critical periods in R. arvalis embryogenesis. Part 1: Linear size of the embryo. Experimental embriology 42(5): 378-389. https://doi.org/10.1134/S1062360411020111

Tsybulin O. (2016) Impact of microwave radiation gsm 900 mhz standard on the embryonic development of Japanese quail. Odesa National University Herald 21, 2(39): 152-162. https://doi.org/10.18524/2077-1746.2016.2(39).81206