PHENES OF WATER FROGS (PELOPHYLAX) AS THE INDICATORS OF WATER BODIES’ CONTAMINATION IN PRE-CARPATHIANS, ROZTOCHIA, LESSER AND WESTERN POLISSIA

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The results of phenotypic variability of water frogs (Pelophylax) analysis depending on internal and external influence factors are presented. 345 individuals of three species (Marsh Frog, Pool Frog and Edible Frog) were taken for the analysis from water bodies of different level of anthropogenic influence. The hydrochemical composition of water from the investigated aquatic ecosystems was analyzed, and the classification of water bodies after their water quality was done. The expression rates of nine phenotypes depending on the species of frog, its age stage and water quality class were established. It was found out that all the analyzed phenes show a significant dependence on the habitat conditions. The expression rates of middorsal stripe, as well as the coloration of femur and tibia depend mostly on water bodies’ contamination level. The increasing level of chemical contamination changes the ratios of alternative variants of middorsal stripe, and coloration of femur and tibia. New variants in coloration of femur and tibia that have never been described were noticed. Pelophylax individuals from the most contaminated water bodies (Cholgyni, Perekalky and Zhovtantsi) showed the highest variability by the analyzed phenes.

Keywords: Pelophylax esculentus complex, polymorphism, hydrochemistry, middorsal stripe, femur and tibia coloration.

INTRODUCTION

The adaptive polymorphism of amphibians can be used during the research of their population reactions on the changes of habitat conditions. Adaptive polymorphism is the existence of two or more genetically and phenetically distinct forms which dominate under different conditions (temperature, light, chemical composition of aquatic habitat, presence of predators, population density, etc.) [18].

Phenetically distinct forms (phenes) are the discrete (sharply detached from others), elementary (simple, indivisible without of quality loss), alternative (existing in two or more variants) hereditary traits that are the result of genes–environment interactions during the ontogenesis. There is described a number of phenes with different expression rates for Anura [6].
MATERIALS AND METHODS

The material was collected in 2011–2016 according to the generally adopted in batrachology method of dip-netting [2] in the water bodies of Pre-Carpathians, Roztocchia, Lesser and Western Polissia (Fig. 1).

Fig. 1. Localization of the investigated water bodies

There are three species of water frogs on the territory of Ukraine: Marsh Frog – *Pelophylax ridibundus* [Pallas, 1771], Pool Frog – *Pelophylax lessonae* [Camerano, 1882 «1881»] and their hybrid – Edible Frog – *Pelophylax esculentus* [Linnaeus, 1758]. There were sampled 345 individuals of *Pelophylax* (Table 1); species differentiation of the frogs is confirmed by genetic analysis [11].

Table 1. The number of *Pelophylax* individuals sampled in the investigated water bodies

| Water bodies     | *Pelophylax ridibundus* | *Pelophylax lessonae* | *Pelophylax esculentus* |
|------------------|-------------------------|-----------------------|-------------------------|
| Piscohe          | 26                      | 21                    | 34                      |
| Svitiaz          | -                       | 7                     | 4                       |
| Luky             | 4                       | 5                     | 12                      |
| Nyzhankovychi    | 75                      | -                     | -                       |
| Velykyi Lubin    | 34                      | 9                     | 1                       |
| Ivano-Frankove   | 16                      | -                     | -                       |
| Cholyni          | 22                      | 24                    | 12                      |
| Zhovtantsi       | 19                      | -                     | 1                       |
| Perekaliky       | 20                      | -                     | -                       |
The description of phenotypes of water frogs was made on the following features taking into account their alternative variants: middorsal stripe, dorsal maculation, ventral coloration, throat coloration, femur and tibia coloration, presence of snout stripes, presence of yellow spots on the thigh, dorsal coloration and eyes color [2, 6, 8, 10, 16].

General background of the coloration was determined according to “Colour scale” [4] under the daylight illumination. Since general background of coloration of the same individual can change depending on humidity, temperature and illumination [5], all sampled frogs were kept for several hours in the identical conditions before their coloration description.

The hydrochemical samples were taken according to the generally adopted methods [1]. The samples analysis was carried out in the certified chemical laboratory of Lviv Research Station of Institute of Fisheries of NAAS. Such indices as pH, water hardness, total mineralization, NH₄, NO₂⁻, NO₃⁻, PO₄³⁻, Cl⁻, SO₄²⁻, hydrogen carbonate content, dissolved oxygen, dichromate oxidability, and biological oxygen consumption were determined [9].

RESULTS AND DISCUSSION

The variability of feature is determined by the genetic spectrum of reaction norm and factor that influences on the expression rate of the feature. For the determination of influence level of the analyzed factors (water body type, species of frog and its age stage), it was necessary to carry out the group comparison. Kruskal-Wallis test was used to find out the dependence between the factor and the feature [7]. It lets us compare the medians of more than two sample groups. The test can check the null hypothesis saying that the medians of the feature in the populations (sample groups) are equal. The higher value test shows, the more significant feature is. So, using this non-parametric test we found out the dependence of features from the analyzed factors. Test results with the confidence levels are presented in Table 2.

| Feature                      | Water body |              | Species |              | Age      |
|------------------------------|------------|--------------|---------|--------------|----------|
|                              | H          | p            | H       | p            | H        | p        |
| Middorsal stripe             | 33.113     | 0.0000       | 4.414   | 0.1100       | 4.119    | 0.1275   |
| Dorsal coloration            | 16.413     | 0.0003       | 38.185  | 0.0000       | 1.235    | 0.5394   |
| Dorsal maculation            | 57.086     | 0.0000       | 34.714  | 0.0000       | 14.710   | 0.0006   |
| Ventral coloration           | 22.604     | 0.0000       | 4.607   | 0.0999       | 84.583   | 0.0000   |
| Throat coloration            | 40.160     | 0.0000       | 2.027   | 0.3629       | 36.831   | 0.0000   |
| Snout stripes                | 19.380     | 0.0130       | 2.368   | 0.306        | 12.017   | 0.0025   |
| Thigh yellow spots           | 29.175     | 0.0000       | 26.298  | 0.0000       | 4.303    | 0.1163   |
| Eyes color                   | 9.870      | 0.0072       | 34.387  | 0.0000       | 7.501    | 0.0235   |
| Femur and tibia coloration   | 11.809     | 0.0027       | 0.276   | 0.8713       | 1.582    | 0.4534   |

Comments: the significant values of factors influencing on the feature expression are shown in bold

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Table 2. The dependence of factor influence on the feature expression (Kruskal-Wallis test)

| Feature                      | Water body |              | Species |              | Age      |
|------------------------------|------------|--------------|---------|--------------|----------|
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Примітка: жирним виділено достовірність значень факторів, які значимо впливають на прояв аналізованих ознак
According to the table the water body type influences on the expression of all the analyzed phenotypic features to the certain extent. Middorsal stripe as well as femur and tibia coloration are the phenes which expression rates significantly depend on physicochemical peculiarities of habitat. The status of such phenes as dorsal coloration and yellow spots on the thigh depend on both species of frog and water body conditions. Ventral coloration, throat coloration, and snout stripes are the phenes which status is defined by the individual age stage and habitat conditions. Eyes color and dorsal maculation of the frogs depend on all three given factors.

**Fig. 2.** The distribution of middorsal stripe variants in water frogs from the study plots

Middorsal stripe has four variants: clearly seen, slightly seen, intermittent, absent. The comparison of middorsal stripe variants of water frogs from the study plots points out the individuals without the stripe in water bodies of Perekalky, Zhovtantsi, Velykyi Lubin, Cholgyne and Nyzhankovychi (Fig. 2). It doesn’t depend on the species of frog and its age as we have ascertained already (Table 2), so the dominant factor causing such a response of the organism is physicochemical conditions of its habitat.

The next phene that shows significant dependence from habitat conditions is femur and tibia coloration. It has three alternative variants: continuous cross stripes, intermittent cross stripes and spots. The detailed analysis of the maximal variability of this feature gives us the following distribution: Perekalky, Velykyi Lubin, Cholgyne, Piscoche, and Nyzhankovychi (Fig. 3).

**Fig. 3.** The distribution of femur and tibia coloration variants in water frogs from the study plots
Water frogs are the most aquatic ones among the amphibians of Ukraine; they remain in water after the breeding. So that physicochemical characteristics of water play an important role in the amphibian vital activity. There is no single index to characterize water quality in total therefore optimal is to use a number of them. Studied aquatic ecosystems of Pre-Carpathians, Roztochia, Lesser and Western Polissia were classified according to the generally adopted method of ecological evaluation of the surface waters quality [9]. As a result of chemical analysis of water we have got the classification of investigated water bodies by their water quality (Table 3).

Table 3. Classification of the investigated water bodies by their water quality

| Territory | Luki* | Piscoche* | Svitiaz* | Nyzhankovychi | Velykyi Lubin | Ivano-Frankove | Cholgyi | Zhovtantsi | Perekalky |
|-----------|-------|-----------|----------|---------------|---------------|---------------|---------|------------|-----------|
| Water quality class | II    | II        | II       | II            | II            | II            | III     | III        | III       |
| Water quality category | 2     | 2         | 2        | 3             | 3             | 3             | 4       | 4          | 4         |
| Category name by purity degree (contamination) | Clean | Clean enough | Slightly contaminated |
| Trophics (dominating type) | Mesotrophic | Mesoeutrophic | Eutrophic |
| Saprobity | α’-olygosaprobc | β’-mesosaprobc | β”-mesosaprobc |

Comment: * – the results of chemical analysis are taken from Sytnyk et al [13–15]
Примітка: * – результати хімічного аналізу взяті з робіт Ситника зі співавторами [13–15]

The lakes of Western Polissia (Luki, Piscoche, Svitiaz in Shatsk National Nature Park) have similar chemical composition of water and have been referred to clean category. The water bodies of Nyzhankovychi, Velykyi Lubin (Pre-Carpathians), and Ivano-Frankove (Roztochia) are fish ponds so they belong to clean enough ones according to the results we can say also that the absence of middorsal stripe isn’t peculiar to Pool Frog. The literature affirms that middorsal stripe can assist in individual variability of the phenes in the water bodies of different categories by water purity degree.

As far as such phenes as middorsal stripe and femur and tibia coloration depend on the conditions of amphibian habitat (Table 2) we carried out the analysis of variability of the phenes in the water bodies of different categories by water purity degree.

Clearly seen variant of middorsal stripe is the most common phene among the amphibians of all investigated Pelophylax species. We didn’t find any individual of Pool Frog (Pelophylax lessonae) without it. Fig. 4 shows the variability of middorsal stripe depending on water quality. Obviously, the clearly seen middorsal stripe is the most numerous in clean and clean enough water bodies. The frequency of slightly seen and absent variants of middorsal stripe is increasing when the contamination level of water is increasing too. With the increasing of chemical contamination individuals of Pelophylax esculentus complex are more polymorphic by middorsal stripe with the increasing of rare variants portion of this feature. This tendency was confirmed for all three species. According to the results we can say also that the absence of middorsal stripe isn’t peculiar to Pool Frog. The literature affirms that middorsal stripe can assist in individual...
firmness to drought and heavy metals permeability [3, 17, 19]; it also says that the same feature can increase skin permeability for sodium ions that results in decreasing of oxygen intake and decreasing of excitation threshold [19].

![Graph](image)

**Fig. 4.** The expression rate of middorsal stripe in water frogs from the water bodies of different water quality classes

The next phenotypic feature that was found out to be dependent on habitat conditions is femur and tibia coloration. This feature has to be analyzed on perpendicularly bended hind limbs only. Intermittent cross stripes on femur and tibia is the most characteristic variation of the feature among the amphibians of all investigated *Pelophylax* species (*Pelophylax ridibundus* – 50 %, *Pelophylax lessonae* – 49 % and *Pelophylax esculentus* – 55 %).

![Graph](image)

**Fig. 5.** The expression rate of femur and tibia coloration in water frogs from the water bodies of different water quality classes

CONCLUSIONS

1. The dependence of expression rates of all the analyzed phenotypes on physicochemical conditions of the habitat was found out. Dorsal coloration and presence of yellow spots on the thigh depend on the species of frog. Ventral coloration, throat color-
ation and presence of snout stripes are the phenes which status is defined by the individual age stage. Eyes color and dorsal maculation depend on three factors: habitat conditions, the species of frog, and the individual age stage.

2. The expression rates of middorsal stripe depend mostly on habitat conditions of amphibians. Most often the individuals with clearly seen middorsal stripe occur. With the increasing of chemical contamination frogs become more polymorphic, i.e., the number of rare variants of the feature increases (slightly seen stripe and intermittent one). The presence of different variants of this phene is typical for Pelophylax lessonae (clearly seen, slightly seen and intermittent stripe).

3. Physicochemical conditions of habitat is a dominant factor in causing the expression rate of femur and tibia coloration. In the consequence of increasing of water contamination the frequency of individuals with spots on femur and tibia is increasing too, while in clean and clean enough waters more common are frogs with intermittent cross stripes. The combination of different variants of the feature as well as its absence in Pelophylax ridibundus and Pelophylax lessonae was noticed.

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ФЕНИ ЗЕЛЕНИХ ЖАБ (PELOPHYLAX) ЯК ІНДИКАТОРИ ЗАБРУДНЕННЯ ВОДОЙМ ПРИКАРПАТТЯ, РОЗТОЧЧЯ, МАЛОГО ТА ЗАХІДНОГО ПОЛІССЯ

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Представлено результати аналізу фенотипної мінливості земноводних групи зелені жаби (Pelophylax) у залежності від внутрішніх і зовнішніх факторів впливу. Для аналізу відібрано 345 особин трьох видів – жаби озерної, жаби ставкової та жаби їстівної з водойм різного антропогенного навантаження. Проаналізовано гідрохімічний склад води досліджуваних гідроекосистем і проведено класифікацію водойм за якістю води. Для дев’яти фенотипів встановлено залежність у прояві станів ознак від виду, віку та класу якості води. Усі аналізовані фени у своєму прояві мають значну залежність від умов середовища проживання земноводних. Встановлено, що прояв станів дорозомедіальної смуги, а також забарвлення стегна та гомілки залежать переважно від ступеня забруднення водойм. Зі збільшенням хімічного забруднення змінюється співвідношення альтернативних станів дорозомедіальної смуги та забарвлення стегна й гомілки. Зафіксовано не описані раніше прояви забарвлення стегна та гомілки. Представники групи Pelophylax із найбільш забруднених водойм (Чолгині, Перекалки та Жовтанці) проявляли найбільшу мінливість аналізованих фенів.

Ключові слова: Pelophylax esculentus complex, поліморфізм, гідрохімія, дорозомедіальна смуга, забарвлення стегна та гомілки.

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