Academy of Romanian Scientists
Annals Series on Biological Sciences

Volume 1, No. 1, 2012, pp. 59 - 68
Online Edition ISSN 2285 - 4177

ORIGINAL PAPER

Aspects of chromatic polymorphism in green frogs from Gheorghiu pond (Dorohoi, Botoşani county, Romania)

Received for publication, November, 1, 2011.
Accepted, May, 15, 2012

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Abstract.
It was investigated the chromatic polymorphism within a green frog population from Gheorghiu pond (Botoşani County). The population is composed of two species belonging the complex *Rana esculenta, Rana ridibunda* (Pall.) and *Rana esculenta* (L.). Using the method of determination of chromatic morphs proposed by Ishcenko (1978) there were identified 7 dorsal morphs and 4 ventral in *Rana ridibunda* (Pall.) and a number of 6 dorsal and 2 ventral morphs in *Rana esculenta* (L.). The analysis of chromatic correlations showed a higher chromatic variability in *Rana ridibunda* (Pall.), species in which there were delimited 18 distinct morphs, comparing to only 6 morphs in *Rana esculenta* (L.).

Key words*: Rana ridibunda, Rana esculenta*, chromatic variability

1. Introduction

Skin colour in Amphibians, in the absence of poison glands, ensures the function of camouflage of the animal within the specific habitat. So, principal colours are green and brown, with their different nuances. More, chromatic variability appears prioritary in dorsal region, and the ventral region shows a more reduced chromatic variability. Prevalent aquatic species show some ventral colour variation, function of water transparency or of the abundance of aquatic macrophytes.

Skin colour represents a morphological feature with adaptative character, being determined, on one hand, by the specific pattern of distribution of pigments within skin cells, and, on second hand, by the action of environmental factors. The distribution of pigments is relatively various and that is why there are an important number of morphs, i.e. intrapopulational polymorphism.

External factors implied in the determination of amphibian colour are the light, temperature and moisture, and the receptors for these stimuli are the eyes and external

receptors from the tegument. Through the cutting of optic nerve in Bufo bufo or through nuclear removing, the secondary response (change of colour) is suspended (Rowlands Anne, 1954). Hogben and Slome (1931) showed that the most important factor affecting the adults of Anura is the moisture, determining the darkening of colour. This assumption is sustained by other investigators, but only referring to predominantly aquatic species. For the genus Bufo, the moisture has no effect upon pigmentary system (Rowlands Anne, 1950). The same situation is in the case of the genus Hyla which, living in a dry habitat, is not sensitive face to moisture (Besson, 1947).

In the case of forest red frogs, skin colour doesn't show major changes; only a certain change of coulour intensity may be produced, under the action of environmental factors. Along different stages of annual cycle, dorsal colour of these frogs presents essential variations (Cârlig, 2002).

It is generally accepted the fact that chromatic changes of Amphibians are controlled by neurohumoral system, and according to the theories of colour control, in this process are implied one or two hormones: intermedin and adrenalin (Hogben et. al., 1931; Parker, 1948). The hormone produced by intermediary lobe of hypophysis (intermedin) determines the dispersal of melanine, so this hormone is also called melanophore-dilatator. In frogs, cauterization of any portion of pituitary stem, or extirpation of the hypophysis determines permanent expansion of melanophores and, implicitly, skin discoloration (Rowlands Anne, 1954; Stugren, 1965).

In our previous papers (Mîndrescu, 2007; Mîndrescu et. al., 2008; Mîndrescu et. al., 2009) there were investigated cytogenetically, acoustically and biochemically the green frogs from Rana esculenta complex and it was observed an interspecific, intraspecific and even individual variability. Following the same general goals, in this paper we intended to improve the knowledge of the biodiversity in green frogs, through the estimation of chromatic variability in Rana ridibunda (Pall.) and Rana esculenta (L.), using adult specimens caught in Gheorghiu pond located in Dorohoi city (Botoşani County).

2. Material and methods

There were caught 100 specimens belonging to *Rana esculenta* complex [78 specimens of *Rana ridibunda* (Pall.) and 22 of *Rana esculenta* (L.)] from Gheorghiu pond located in south-estern area of Dorohoi city.

The capture of frog specimens was performed with a special net. It was observed that using a colored floating piece before the opening of net determined an increase of capture eficiency.

All the specimens were photographed dorsally and ventrally; after this operation, the specimens were released back in their natural habitat. In order to avoid the repetated examination of newly caught frogs, all the individuals were

marked before releasing. For frog marking it was used banderole methods (Elmberg, 1989; Rice şi Taylor, 1993, cited by Cogălniceanu, 1997).

For the analysis of chromatic polymorphism of caught green frogs there were used the photographs made with a digital camera Lumix Panasonic (DMC – LS3) with a resolution of 5 Mp.

Interpretation of tegumentary chromatics was performed using the method proposed by Ishcenko (1978) and taken over by Nicoară (2004). According to this methodology, dorsal and ventral morphs of the Amphibians belonging to *Rana esculenta* complex are the following:

1. *Maculata* (M) – characterized by the presence of some dark spots with a diameter of 2-3 or 6-7 mm. Their number and location usually vary;
2. *Hemimaculata* (hm) – presents the same dark spots as the previous morph, but their number is usually smaller, 2-6;
3. *Burnsi* (B) – in the specimens of this morph the spots lack integrally or partially;
4. *Punctata* (P) – dorsal region has intense pigmentation, with small points;
5. *Hemipunctata* (hp) – the characteristic of pigmentation of this morph is more weakly pronounced as compared to the precedent morph. The number of points is smaller, then their dispersion is bigger;
6. *Striata* (S) – for the representatives of this morph it is characteristic the presence of a green dorso-median strip. In principle, it could not represent an independent morph, creating combinations with other morphs.

For the ventral area, the variability of pigmentation determines two main morphs:

7. *Albicollis-albiventris* (AC/AV) – the lack of the pigment;
8. *Nigricollis-nigriventris* (NC/NV) – total and uniform pigmentation, both of the oropharyngian region, and of the abdominal region;

In this two ventral morphs there are possible variants:

9. *Nigricollis-albiventris* (NC/AV) – the pigmentation is present only in the region of gizzard;
10. *Albicollis/nigriventris* (AC/NV) – the pigmentation is present only on the abdomen

In the case of each population of green frogs it was calculated the frequency of each dorsal and ventral morph, as well as the frequence of chromatic correlations between dorsal and ventral morphs.

The results of observations upon the chromatic polymorphism in species of *Rana* investigated in Gheorghiu pond (Botoşani County) are presented in Tables 1 – 6 and in Figures 1-4.

3. Results and discussions

Searching dorsal chromatic variability within frog sample from species *Rana ridibunda* (Pall.) it was stated the presence of 7 different morphs, from which 5 represent basic morphs and 2 are complex morphs (Table 1).

Table 1. Frequency of dorsal morphs in *Rana ridibunda* (Pall.) from Gheorghiu pond

	maculata	hemi-maculata	hemi-punctata	burnsi	striata	striata-maculata	striata-hemimaculata
Total specimens	10	21	1	2	9	15	20
Frequency	12.82%	12.82%	1.28%	2.56%	11.53%	19.23%	25.64%

Dorsal morph with highest frequency within the population of *Rana ridibunda* (Pall.) is *hemimaculata* (26.92%), followed by *striata-hemimaculata* (25.64%) and *striata-maculata* (19.23%). The most weakly represented within the population there are dorsal morphs *burnsi* (2.56%) and *hemipunctata* (1.28%).
As concerning the variability of ventral chromatic in the population of *Rana ridibunda* (Pall.) from Gheorghiu pond, all the four morphs were identified (Table 2).

Table 2. Frequency of ventral morphs in *Rana ridibunda* (Pall.) from Gheorghiu pond

	Albicollis-albiventris	Albicollis-nigriventris	Nigricollis-albiventris	Nigricollis-nigriventris
Number of specimens	59	12	4	3
Frequency	75.64%	15.38%	5.12%	3.84%

As concerns the frequency, the morph *albicollis-albiventris* separates clearly from the other ventral morphs, reaching a frequency of 75,64 % (59 specimens from the total of 78 presenting this morph). The lowest frequency was presented by ventral morph *nigricollis-nigriventris*: only 3 specimens from the 59 expressed this phenotype.

The situation of dorso-ventral chromatic combinations in *Rana ridibunda* (Pall.) population may be presented as follows: 7 distinct morphologic groups are generated by dorsal morphs, from which 5 are combined with several ventral morphs (between 2 and 4), and 2 are combined only with one type of ventral morph. In this last case are implied dorsal morphs *hemipunctata* and *burnsi,* combined only with ventral morph *albicollis-albiventris*.

Considered in its whole, in all the population of *Rana ridibunda* (Pall.) there are expressed 18 distinct dorso-ventral phenotypes (Table 3, Figure 1).

Table 3. Frequency of dorso-ventral chromatic combinations in *Rana ridibunda* (Pall.) population from Gheorghiu pond

	Number of specimens	Frequency
Maculata+albicollis-albiventris (M+AA)	9	11.53%
Maculata+albicollis-nigriventris (M+AN)	1	1.28%
Hemimaculata+albicollis-albiventris (hm+AA)	18	23.07%
Hemimaculata+albicollis-nigriventris (hm+AN)	2	2.56%
Hemimaculata+nigricollis-nigriventris (hm+NN)	1	1.28%
Hemipunctata+albicollis-albiventris (hp+AA)	1	1.28%
Burnsi+albicollis-albiventris (B+AA)	2	2.56%
Striata+albicollis-albiventris (S+AA)	3	3.84%
Striata+albicollis-nigriventris (S+AN)	4	5.12%
Striata+nigricollis-nigriventris (S+NA)	1	1.28%
Striata+nigricollis-nigriventris (S+NN)	1	1.28%
Striata-maculata+albicollis-albiventris (S-M+AA)	9	11.53%
Striata-maculata+albicollis-nigriventris (S-M+AN)	3	3.84%
Striata-maculata+nigricollis-albiventris (S-M+NA)	2	2.56%
Striata-maculata+nigricollis-nigriventris (S-M+NN)	1	1.28%
Striata-hemimaculata+albicollis-albiventris (S-hm+AA)	17	21.79%
Striata-hemimaculata+albicollis-nigriventris (S-hm+AN)	2	2.56%
Striata-hemimaculata+nigricollis-albiventris (S-hm+NA)	1	1.28%

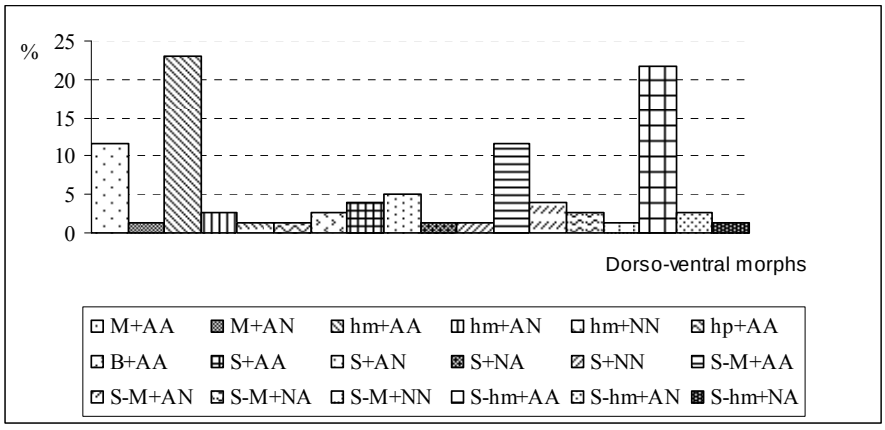

Figure 1. Frequency of dorso-ventral chromatic combinations in *Rana ridibunda* (Pall.) from Gheorghiu pond

Dorso-ventral phenotypes which present the biggest frequencies in the population of *Rana ridibunda* (Pall.) were: *hemimaculata* with *albicollis-albiventris* (23.07%), *striata-hemimaculata* with *albicollis-albiventris* (21.79%), *maculata* with *albicollis-albiventris* and *striata-maculata* with *albicollis-albiventris* (having the same frequencies – of 11.53%). The lowest frequency (1 %) was recorded in dorso-ventral combinations *maculata* with *albicollis-nigriventris*, *hemimaculata* with *nigricollis-nigriventris*, *hemipunctata* with *albicollis-albiventris*, *striata* with *nigricollis-albiventris*, *striata* with *nigricollis-nigriventris*, *striata-maculata* with *nigricollis-nigriventris* and *striata-hemimaculata* with *nigricollis-nigriventris*.

It is to be observed that dorso-ventral phenotypes presenting highest frequencies are based on totally depigmented ventral phenotype (*albicollis-albiventris*), that sustain the hypothesis considering the equilibrium trend within the population toward this ventral phenotype.

Analysing dorsal chromatics in the specimens of the species *Rana esculenta* (L.) it was observed the phenotypization of 6 morphs, from which 5 are basic (*maculata, hemimaculata*, *hemipunctata*, *burnsi* and *striata*) and one is complex (*striata-hemipunctata*), (Table 4).

Table 4. Frequency of dorsal chromatic morphs in *Rana esculenta* (L.) from Gheorghiu pond

	maculata	hemi-maculata	hemi-punctata	burnsi	striata	striata-hemipunctata
Number of specimens	1	1	10	2	3	5
Frequency	4.54%	4.54%	45.45%	9.09%	13.63%	22.72%

According to data presented in Table 4, the highest frequency was recorded by *hemipunctata* morph, followed by complex morph *striata-hemipunctata*. The lowest frequency was presented by the morphs *maculata* and *hemimaculata* (each 4.54%). It was noticed that *Rana esculenta* (L.) population presents a weakly pigmented dorsal phenotype, oriented toward the expression of dorsal stripes, with low density points and combinations between these morphs.

Ventrally there were phenotypized only two distinct morphs: *albicollis-albiventris* and *nigricollis-nigriventris* (Table 5).

Table 5. Frequency of ventral chromatic morphs in *Rana esculenta* (L.) from Gheorghiu pond

	Albicollis-albiventris	Nigricollis-nigriventris
Number of specimens	21	1
Frequency	95.45 %	4.54 %

Analysis of ventral phenotypes frequency indicates very clearly the dominance of the entirely depigmented morph, which is expressed in the population with a frequency of 94.45 %.

Our observations evidenced a small number of chromatic dorso-ventral combinations within *Rana esculenta* (L.) population from Gheorghiu pond (Table 6).

Table 6. Frequency of dorso-ventral chromatic combinations in *Rana esculenta* (L.) from Gheorghiu pond

	Number of specimens	Frequency
Maculata+nigricollis-nigriventris (M+NN)	1	4.54%
Hemimaculata+albicollis-albiventris (hm+AA)	1	4.54%
Hemipunctata+albicollis-albiventris	10	45.45%
Burnsi+albicollis-albiventris (B+AA)	2	9.09%
Burnsi+albicollis-albiventris (B+AA)	3	13.63%
Striata-hemipunctata+albicollis-albiventris (S-hm+AA)	5	22.72%

From the six phenotypes expressed in *Rana esculenta* (L.) population, the highest frequency recorded the phenotype *hemipunctata* with *albicollis-albiventris*, present in almost half of the investigated specimens, and the lowest frequency (4.54 %) was recorded by phenotypes *maculata* with *nigricollis-nigriventris* and *hemimaculata* with *albicollis-albiventris*. Probably the small number of investigated specimens influenced our statements upon chromatic variability in this species.

Besides that in the population are recorded only 6 distinct phenotypes, none of dorsal morphs forms groups of variability with ventral morphs. Each dorsal morph is combined chromatically with only one ventral morph (Figure 2).

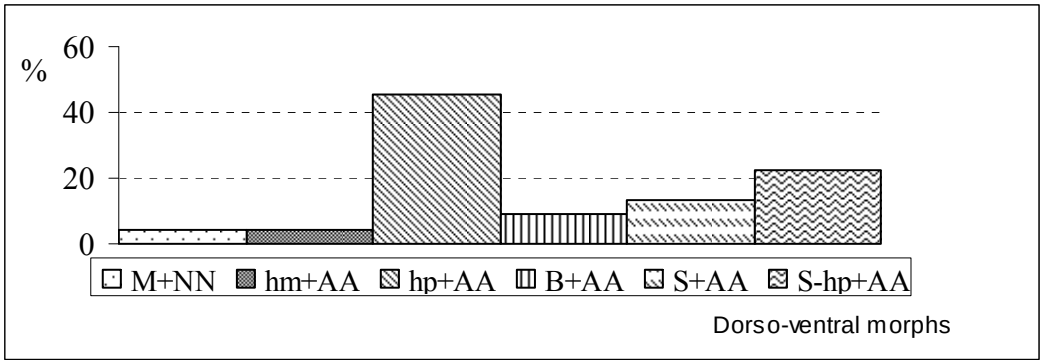

Figure 2. Frequency of dorso-ventral chromatic combinations in *Rana esculenta* (L.) from Gheorghiu pond

It is confirmed in this case the trend of population to evolve toward a entirely depigmented ventral morph. From the six distinct phenotypes which were

identified, 5 are combinations with the morph *albicollis-albiventris*, situation characteristic to more than 95 % from all the specimens.

Comparative analysis of green frog populations from Gheorghiu pond, using as the single criterion the expression of dorsal morph, allows the division of that population in 8 distinct groups. From these, 5 morphs are common to both species (*maculata*, *hemimaculata*, *hemipunctata*, *burnsi* şi *striata*), 2 morphs are presesnt only in *Rana ridibunda* (Pall.) (*striata-maculata* şi *striata-hemimaculata*), and only one morph (*striata-hemipunctata*) appears only in *Rana esculenta* (L.). Hence, dorsal chromatic variability is bigger in *Rana ridibunda* (Pall.) as compared to *Rana esculenta* (L.). The observation is also correct in the analysis of ventral morph. In the population of *Rana ridibunda* (Pall.) all the four ventral morph are phenotypically expressed, but in *Rana esculenta* (L.) population there are expressed only two morphs (*albicollis-albiventris* and *nigricollis-nigriventris*).

In figures 3 and 4 it is presented a comparation of frequency of dorsal and ventral chromatic morphs in the two species.

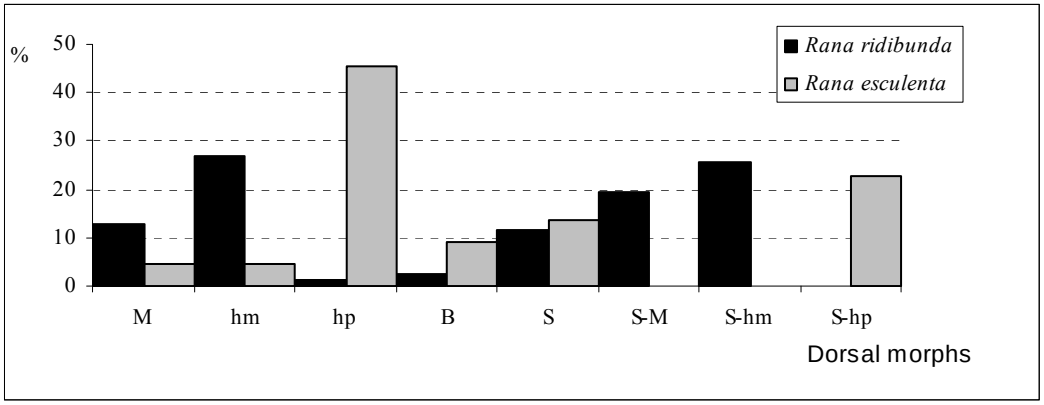

Figure 3. Frequency of chromatic morphs in the two species of green frogs

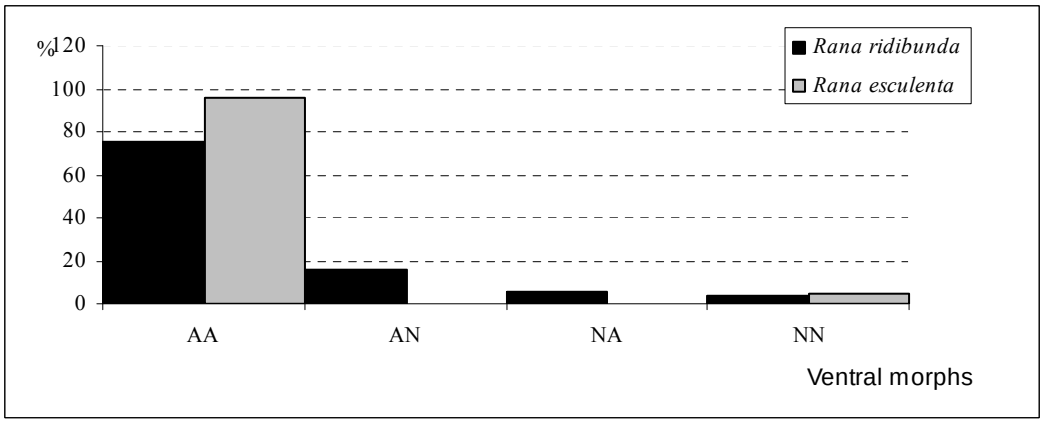

Figure 4. Frequency of chromatic morphs in the two species of green frogs

As concerns the phenotypes formed by dorso-ventral combinations, the analysis of the 100 specimens sampled from Gheorghiu pond leads to identification of 20 different phenotypes. From all these, only 4 are present both in *Rana ridibunda* (Pall.) and in *Rana esculenta* (L.); they are: *hemimaculata* with *albicollis-albiventris*, *hemipunctata* with *albicollis-albiventris*, *burnsi* with *albicollis-albiventris* and *striata* with *albicollis-albiventris.* From them, the most are characteristic to *Rana ridibunda* (Pall.) – 14 dorso-ventral phenotypes and only two in *Rana esculenta* (L.). Hence, formation of more groups in *Rana ridibunda* (Pall.) indicates a larger phenotypic variability within this species, as compared to *Rana esculenta* (L.). The same situation results from the analysis of chromatic variability subgroups in *Rana ridibunda* (Pall.), on the basis of dorsal morphs. So, dorsal morphs *maculata, hemimaculata, striata, striata-maculata* and *striata* form chromatic comations with 2 to 4 ventral morphs, while dorsal morphs expressed in *Rana esculenta* (L.) population form such combinations only with one ventral morph. A good example in this sense is offered by dorsal morph striata, presented both in *Rana ridibunda* (Pall.) and in *Rana esculenta* (L.). While this dorsal morph forms 4 subgroups of chromatic variability in *Rana ridibunda* (Pall.) (*striata* with *albicollis-albiventris*, *striata* with *albicollis-nigriventris*, *striata* with *nigricollis-albiventris* and *striata* with *nigricollis-nigriventris*), in *Rana esculenta* (L.) the same morph makes combinations only with ventral depigmented morph (*striata* with *albicollis-albiventris*).

From the information presented above results that green frogs from Gheorghiu pond show a trend to evolve towards ventral depigmented morphs. In *Rana ridibunda* (Pall.) it may be oberved also a tendency to promote of some dorsal pigmented morphs (*hemimaculata*), or combined with stripped forms (*striata-hemimaculata*), and for *Rana esculenta* (L.) – dorsal morphs weakly pigmented (*hemipunctata*) or combined with stripped forms (*striata-hemipunctata*).

Conclusions

1. Our investigations on the chromatic polymorphism in a population of green frogs from Gheorghiu pond (Botoşani County) allowed identification of 20 distinct dorso-ventral phenotypes, from which 4 are present both in *Rana ridibunda* (Pall.), and in *Rana esculenta* (L.). 14 are specific only to *Rana ridibunda*, and 2 are present only in *Rana esculenta*.

2. Chromatic polymorphism is more intense in *Rana ridibunda* (Pall.) than in *Rana esculenta* (L.).

3. It was observed a tendency of phenotypization of the ventral morph entirely depigmented (*albicollis-albiventris*).

4. Phenotypization of dorsal and ventral chromatic morphs represents an adequate answer of frog populations to the constraints imposed by a lot of

external factors, finally resulting in the improvement of that characters which secure camouflage, i.e. a higher protection and survival.

References

Besson S., (1947) - *Les Cellules Pigmentaires du Tegument extern chez la Grenouille*, Bulletin de la Societe des Sciences de Nancy, tom VI, nr. 4, 97 – 100.

Cârlig Tatiana, (2002) - *Particularităţile biologice şi comportamentul speciei Rana dalmatina Bonap. (Amphibia, Anura) în Codrii Centrali*. Ph.D.Thesis, Chişinău.

Cogălniceanu D., (1997) - *Practicum de ecologie a amfibienilor*, Editura Universităţii, Bucureşti, 122.

Hogben L.T., Slome D., (1931) - *The pigmentary effector System. VI. The dual character of endocrine coordination in amphibian colour change*, Proc. Roy. Soc. B. vol. 108, 10.

Mîndrescu, C., (2007) - *Aspects regarding the sound emission at Rana ridibunda (Pall.)*, Studii şi Cercetări Ştiinţifice, seria Biologie Animală, Universitatea din Bacău, vol. XIII.

Mîndrescu, C., Ghiorghiţă, G., (2008) - *The preliminary cytogenetic investigations in Rana ridibunda (Pall.) and Rana esculenta (L.) from the north of Moldavia*, Analele Ştiinţifice ale Univ. „Al. I. Cuza", Iaşi, serie nouă, Genetică şi Biologie moleculară, Tom IX, fasc.3, 65 – 71.

Mîndrescu, C., Ghiorghiţă, G., Artenie, V., (2009) - *Biochemical investigations on Rana ridibunda (Pall.) and Rana esculenta (L.)*, Analele Ştiinţifice ale Univ. „Al. I. Cuza", Iaşi, serie nouă, Genetică şi Biologie moleculară, Tom X, fasc. 3, 53 – 61.

Nicoară Andreea, (2004) - *Morphometric study of the juveniles belonging to complex Rana esculenta from Ciric River's Basin (Iaşi)*, Analele Ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi, seria Biologie animală, Tom L, 257 – 266.

Parker G.H., (1948) - *Animal Colour Change and their Neurohumors*, Cambridge University Press, Great Britain.

Rowlands Anne, (1950) - *The influence of water and light upon the pigmentary system in the common frog, Rana temporaria*, The Journal of Experimental Biology, 27 (3), p. 446 – 460, England, http://jeb.biologists.org/content/27/3.toc.

Rowlands Anne, (1954) - *The influence of water and light and the pituitary upon the pigmentary system of the common toad (Bufo bufo bufo)*, The Journal of Experimental Biology, 31 (2), 151 – 160, http://jeb.biologists.org/content/31/2.toc.

Stugren B., (1965) - *Ecologie generală*, Editura Didactică şi Pedagogică Bucureşti, 216.