

BASIC MORPHOLOGICAL CHARACTERISTICS OF THE RANA (*PELOPHYLAX*) SYNKLEPTON *ESCULENTA* COMPLEX IN RELATION TO LEGAL REGULATIONS IN SERBIA

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Abstract — The main purpose of this paper is to provide an analysis of the basic morphological characteristics of all three taxa of this complex on the territory of Serbia in relation to legal regulations, e.g., the prescribed exploitation limitations. Altogether, 789 water frogs were collected at 22 localities in Serbia and analyzed. The two legally prescribed measures — total body length (L) and body weight (M) — were utilized for this analysis. Inasmuch as the law allows for the choice of characters, this particular formulation is harmful for the reproductively active part of the population. If the formulation of the act could be altered so as to satisfy both criteria, the presented results show that only 5% of the population would qualify for exploitation.

Key words: Morphometry, *Rana ridibunda*, *Rana lessonae*, *Rana kl. esculenta*, protection, law

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INTRODUCTION

During the second half of the 20th century, the problems of population decrease and extinction of amphibians (including green frogs) come to be recognized. The causes of these negative occurrences are numerous and often related to synergistic activities. Together with global climate change (increased ultraviolet radiation and global warming), a number of additional factors affect endangered populations of amphibians: increasing amounts of chemical and other toxic materials, contagious diseases of a widespread epidemic nature, the introduction of allochthonous species, and terrain changes in terms of habitat modification and destruction. Thus excessive exploitation becomes even more threatening for amphibian populations (Houlahan et al., 2000; Reaser, 2000; Cohen, 2001; Kiesecker et al., 2001; Young et al., 2001; Blaustein and Kiesecker, 2002; Collins and Storer, 2003; Corn, 2003; Stuart et al., 2004; Garner et al., 2005; Alford et al., 2007; Fagotti and Pascolini, 2007; Harris and Madison, 2007; Rachowitz and Briggs, 2007).

The territory of the Republic of Serbia is populated by three taxa from the Western

Palaearctic group of green frogs: *Rana (Pelophylax) ridibunda* Pallas, 1771; *Rana (Pelophylax) lessonae* Camerano, 1882; and their hybrid *Rana (Pelophylax) kl. esculenta* Linnaeus, 1758. The disposition, condition, and viability of their populations have been the subject of several investigations to date. The temporal characteristics and spatial distribution of endangering factors remain largely unknown (Đorđević, 1900a, 1900b; Karaman, 1948; Džukić, 1968; Spasić-Bošković et al., 1999; Džukić et al., 2001, 2003; Krizmanić, 2008).

Legal regulations in Serbia dealing with the problems of preserving these species are relatively scarce and are all set forth in the Act on Controlling Exploitation and Trade of Protected Wild Plant and Animal Species (Službeni glasnik RS No. 31/05). The given act defines criteria for permissible exploitation/catching of individuals of this complex in Serbia. Article 12 of this act states precisely: "It is forbidden to collect, utilize, or put in traffic individuals of protected species, as follows: ... 2. a frog weighing less than 50 g or more than 120 g; and/or a frog shorter than 9 cm or longer than 15 cm". Permits are issued for protected species in the fresh/raw condition (unprocessed) (Article 14, line 2).

However, legal regulations in Serbia (Službeni glasnik RS No. 31/05) do not require the precise identification of specimens, so persons engaged in trafficking do not have to pay attention to this issue. Field research and evidence obtained by collectors (Krizmanić, 1998) indicate that *R. ridibunda* is the main species caught. Moreover, the populations of *R. lessonae* are also notably endangered. In places known as areas of exploitation of these particular frogs for many decades (Gornje Podunavlje, Koviljsko-Petrovaradinski Rit, Obedska Bara, parts of Deliblatska Peščara along the Danube), the presence of *R. lessonae* is either already established or expected.

The main purpose of this paper is to provide an analysis of the basic morphological characteristics of all three taxa of this complex on the territory of Serbia in relation to legal regulations, e.g., the prescribed exploitation limitations.

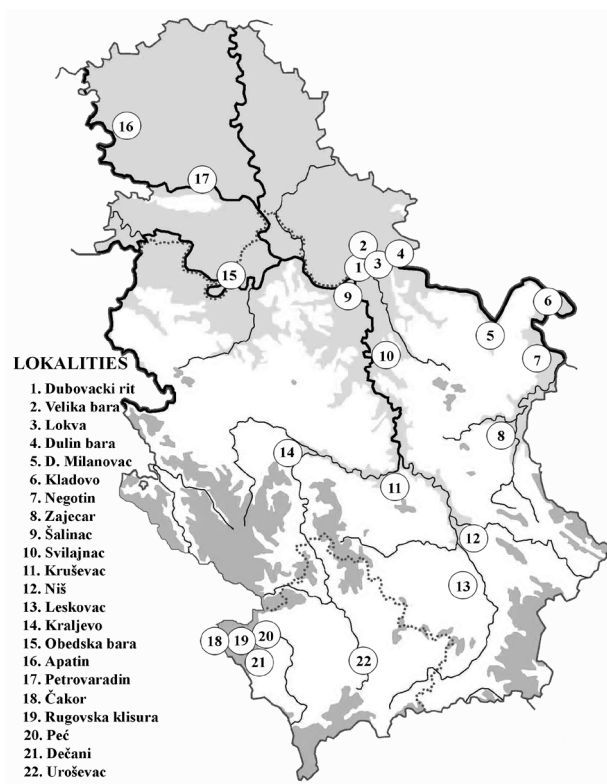


Fig. 1. Map of localities.

MATERIALS AND METHODS

At 22 localities throughout the entire territory of Serbia, a total number of 789 individuals belonging to all three taxa of the *Rana synklepton esculenta* complex were analyzed. A list of localities with UTM markings, together with the number of female and male individuals, is provided in Table 1 (Appendix), while the layout of localities in a particular research area is specified in Fig. 1.

The two legally prescribed measures (Službeni glasnik RS No. 31/05) — total body length (L) and body weight (M) — were utilized for this analysis. In order to establish characteristics of geographical division of the measured values, pooling of specific population samples was conducted. This operation was necessary for the following reason: although separate taxa (requiring separate exploitation permits) are specified by the legal regulations, it should be noted that collectors, merchants,

Table 1. Localities (the name of the locality is given first, followed by UTM data and the number of females and males).

1. Dubovački Rit (Deliblatska Peščara), UTM EQ16, 13+7
2. Velika Bara (Deliblatska Peščara), UTM EQ26, 15+15
3. Lokva (Deliblatska Peščara), UTM EQ26, 20+11
4. Dulin Bara (Deliblatska Peščara), UTM EQ26, 17+13
5. Donji Milanovac, UTM EQ82, 24+6
6. Kladovo, UTM FQ24, 13+17
7. Negotin, UTM FP29, 11+19
8. Zaječar, UTM FP06, 13+17
9. Šalinci, UTM EQ04, 17+13
10. Svilajnac, UTM EP19, 11+14
11. Kruševac, UTM EP33, 14+16
12. Niš, UTM EN79, 15+15
13. Leskovac, UTM EN74, 4+17
14. Obrva, UTM DP64, 3+7
15. Obedska Bara, UTM CQ63, 19+0
16. Apatin, UTM CR45 38+11
17. Petrovaradin, UTM DR11, 111+51
18. Čakor, UTM DN22, 16+5
19. Rugovska Klisura, UTM DN22/32, 31+17
20. Peć, UTM DN42, 26+31
21. Dečani, UTM DN41, 13+10
22. Uroševac, UTM EM19, 21+12.

exporters, processors, and even state officials (in charge of controlling the activity of persons involved in the chain of green frog trafficking are unable to recognize the taxa (or sexes) and treat them as cumulative objects or simply “green frogs”. For this reason, analysis of legal regulations is possible only with the pooled data for all three taxa, regardless of sex structure and only conditionally considering the taxonomic groupings.

Where this was possible, analyses separated according to specific taxa were conducted for the purpose of contrasting (highlighting) the existing differences. Pooled populations in areas north of the Sava and Danube were processed in samples 1, 2, 3, 4, 9, 15, 16, and 17, while samples from localities 5, 6, 7, 8, 10, 11, 12, 13, 14, 18, 19, 20, 21, and 22 were processed as ones pooled from areas south of the Sava and Danube (Table 1, Appendix).

Descriptive statistics were used for

morphometric characters: mean values, standard deviation of the means (SE), and range (max and min). For comparison of size categories, analysis of correlations between body length (L) and mass (M) was conducted. The Statistica for Windows (version 6) software package (Statsoft Inc., 2001) was used for statistical data processing.

RESULTS AND DISCUSSION

Tables 2 to 4 present data on body length (L) and body mass (M) of 789 analyzed individuals from all three taxa of this complex present in pooled population samples from the entire territory of the Republic of Serbia.

When values were separated by taxa (Table 2), the highest mean values (both characters) were found in *R. ridibunda*, the lowest in *R. lessonae*. Results of the t-test show a significant difference of total body length (L) between the taxa *R. ridibunda* and *R. lessonae*

Table 2. Values of body mass (M) (measured in grams) and total body length (L) (measured in mm) from localities 1–15.

	N	Mass (M)				Total length (L)			
		min	max	8	SD	min	max	8	SD
<i>R. ridibunda</i>	245	2.8	187.8	41.5	27.7	32.8	110.6	69.5	16.0
<i>R. lessonae</i>	27	8.82	187.0	30.8	33.4	43.6	117.3	62.7	15.5
<i>R. kl. esculenta</i>	124	4.0	95.5	36.9	19.7	33.9	102.5	68.9	12.6
All three taxa	396	2.8	187.8	39.3	26.1	32.8	117.3	68.9	15.1

Table 3. Values of body mass (M) (measured in grams) and total body length (L) (measured in mm) in a total sample pooled from localities 16 and 17 (AP Vojvodina).

	N	Mass (M)				Total length (L)			
		min	max	8	SD	min	max	8	SD
Vojvodina	211	4.0	95.5	20.1	14.2	33.1	101.5	52.2	11.8

Table 4. Values of body mass (M) (measured in grams) and total body length (L) (measured in mm) in a total sample pooled from localities 18 - 22 (AP Kosovo and Metohija).

	N	Mass (M)				Total length (L)			
		min	max	8	SD	min	max	8	SD
Kosovo and Metohija	182	4.9	96.0	33.4	16.9	37.2	101.0	63.2	13.2

($t= 2.1$, $p<0,05$, $df = 270$) and between *R. lessonae* and *R. kl. esculenta* ($t= 2.22$, $p<0,05$, $df = 149$). The differences between *R. ridibunda* and *R. kl. esculenta* were not significant ($t= 0.36$, $p<0,05$, $df = 367$).

These findings suggest that differences between taxa caught in these areas may have significant meaning for both exploitation and the drafting of regulatory laws. In comparing samples pooled from the most distant northern and southern areas of Serbia (Tables 3 and 4), a significant difference is evident between the resulting mean values ($t= -4.39$; $p<0,05$, $df = 443$). Also, in comparing population samples pooled from two different parts of Serbia, namely areas north and south of the Sava and Danube watercourses (Table 5), we notice significant differences between the mean values of character (L) based on the t-test ($t= -3.705$; $p<0,05$, $df = 487$).

In order to evaluate the legal regulations and permissible total length values and establish shortcomings of the aforementioned act (Službeni glasnik RS, No. 31/05), the average size of individuals of all three taxa of the *Rana* synklepton *esculenta* complex was estimated at the stage of sexual maturity. On a total population sample of 182 subadult individuals collected from the Vojvodina area, samples from Apatin, Koviljsko-Petrovaradinski Rit and Deliblatska Peščara were analyzed through dissection and examination of gonads. Within these samples, the highest value of total body length of an individual with undeveloped gonads was determined

and the average value calculated ($\bar{X}= 49.7\pm 2.6$).

It is important to emphasize that such a value is approximate, considering that it excludes differences between taxa and sexes. In this case, it is utilized only as a general indicator of the condition of green frog populations in these areas (regardless of taxonomic and sex structure). It should be noted that these parameters are highly variable. For example, the average body length of female *R. ridibunda* at the stage of sexual maturity in Greece is 62 mm to 66 mm (Kyriakopoulou-Sklavounou and Loumbourdis, 1990; Kyriakopoulou-Sklavounou et al., 2007). Both attainment of the stage of sexual maturity and body length strictly depend on ecological conditions (Ivanova, 2002). In northern populations, body length of *R. ridibunda* on reaching the stage of sexual maturity may attain up to 72.5 mm (Ivanova, 2002). Since our sample contains all three taxa and both sexes, we believe that this approximate value can characterize the condition of the adult group within the sample of populations analyzed in this research. In event of eventual deviation from the values presented here, its impact on the estimates of proportional representation of certain categories is miniscule. In fact, it may even have a negative effect. In order to evaluate the values prescribed by law, three categories were formed: subadults (< 50 mm), adults (50 mm to 90 mm), and adults (> 90 mm).

Within the total sample, 23.5% of individuals

Table 5. Values of total body length (L) (measured in mm) in populations on territory of Serbia north and south of the Sava and Danube (total of 789 individuals).

Trait	Populations north of the Sava and Dunav				
	N	8	min	max	SD
L	371	58.91	32.85	117.30	15.5

Trait	Populations south of the Sava and Dunav				
	N	8	min	max	SD
L	418	68.38	33.32	110.62	14.8

Table 6. Values of body mass (M) (measured in grams) in populations on territory of Serbia north and south the Sava and Danube (total of 789 individuals).

Trait	Populations north of the Sava and Dunav				
	N	8	min	max	SD
M	371	27.22	2.80	186.94	21.9

Trait	Populations south of the Sava and Dunav				
	N	8	min	max	SD
M	418	38.71	5.00	187.83	25.1

belong to the category of subadult individuals, 72.75% to the category of adult individuals up to the size prescribed by law as minimal in order to permit exploitation, and 3.67% to the category for which the law allows exploitation (Fig. 2). If body length on reaching the stage of sexual maturity is 50 mm on average, the share of adult individuals within the total sample would be 44% (as opposed to approximately 73% according to our calculations), while the share of individuals with body length within the limitations permitted for exploitation would remain the same.

When adult individuals longer than 50 mm are considered, our analysis indicates the following: adult individuals with total length of 50-90 mm are represented with 95.19% (Fig. 3), while individuals with total length of 90-150 mm are represented with 4.81% (Fig. 3).

Also, within the group of adult individuals with total body length of more than 50 mm and body mass (M) between the boundary values defined in legal regulations, 76.6% of individuals weigh less than 50 g, 22.5% weigh between 50 and 120 g, and 0.83% weigh more than 120 g, which is above the prescribed maximum body mass of permissible exploitation according to the act (Fig. 4).

It is important to emphasize that in the category of adult individuals ranging from 90 to 120 mm in body length (whose exploitation is permitted), all of the individuals (100%) weigh over 50 g. Also, body

mass is an extremely variable character and may represent a confusing parameter when it comes to determining exploitation tolerance values. To a great extent, this character depends on biological and biotic factors, and it can vary in the same individual during a short period of time.

Since the act (No. 31/05) stipulates minimal and maximal permitted values and in light of the established correlation of body length and mass (Figs. 5–7), analyses of body mass were conducted on the same population sample where total body length analyses were performed for the purpose of determining the mutual relationship of these two characters and their usefulness in defining the parameters of active protection. Analyzing the entire sample (Tables 2–6) with respect to minimal and maximal values prescribed in legal regulations, we find that in neither case do mean values of the measured populations (pooled and individualized by taxa) reach the minimal value for permitted exploitation (50 g). These results agree entirely with previously obtained data (Džukić et al., 2003). It should be noted that this character is not mentioned in the specialized literature, which in fact underlines its uncertainty.

Analysis of the correlation between body length (L) and body mass (M) of these animals shows that Pearson's coefficient of correlation within the category of subadult individuals (up to 50 mm) is not significant ($r = 0.178$, $SE \pm 0.118$; $p = 0.135$)

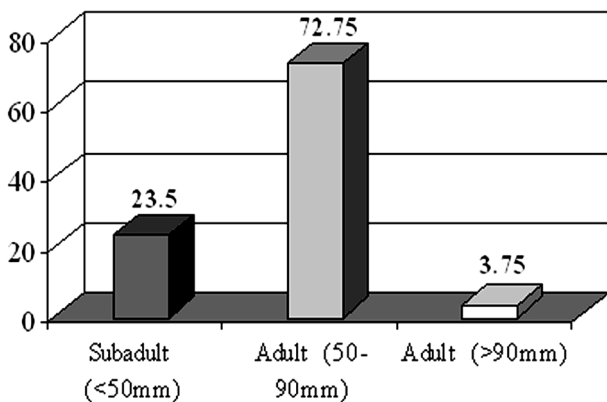


Fig. 2. Age categories in relation to total body length (L) values legally permitted for exploitation.

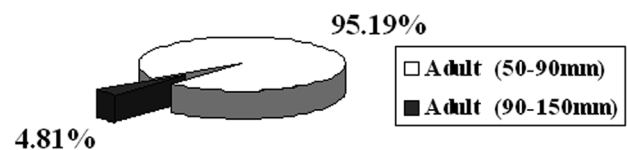


Fig. 3. Percentage of adults falling into body size categories legally permitted for exploitation.

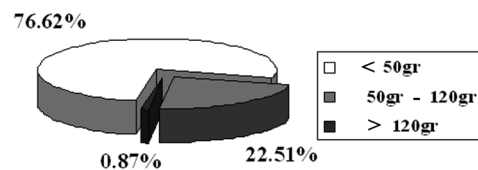


Fig. 4. Percentage of adults of *R. synklepton esculenta* falling into weight range categories legally permitted for exploitation.

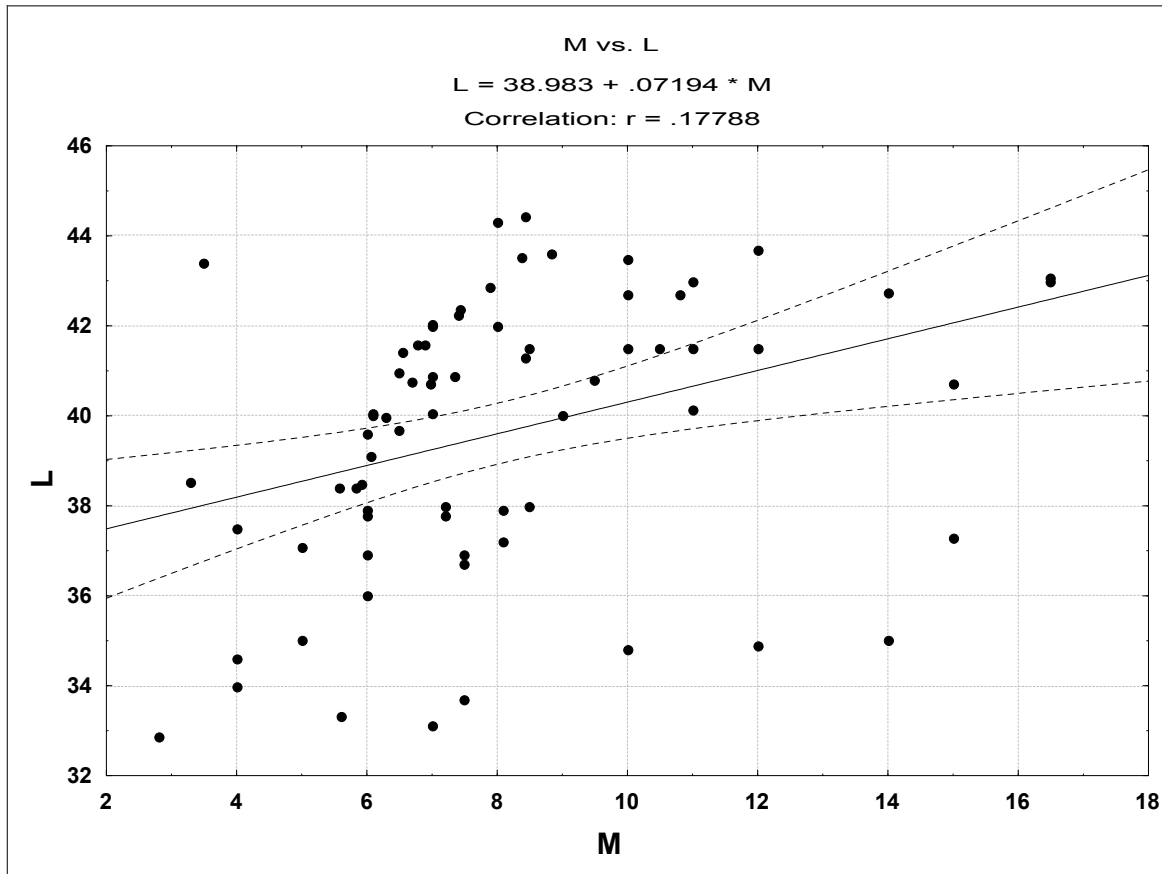


Fig. 5. Correlation between total body length (L) and body mass (M) in subadults ($L < 50$ mm).

(Fig. 5). However, in the other two categories, a statistically significant positive linear coefficient of correlation was determined. Within the category of adult individuals whose length remains below the lower limit of tolerable exploitation (from 50 to 90 mm), the correlation coefficient is significant ($r = 0.94$, $SE \pm 0.152$; $p = 0.000$) (Fig. 6). The correlation coefficient within the category of individuals whose length exceeds the upper limit of tolerable exploitation is somewhat lower than the previous one, but it remains statistically significant: $r = 0.85$, $SE \pm 0.103$; $p = 0.000$ (Fig. 7).

The estimated body length of individuals on attainment of sexual maturity is 50 mm. Body mass at this point is exceptionally variable. Comparing the mass range of adult individuals according to length

categories (Table 7) reveals significant differences between groups (Table 8).

Analysis of adult individuals which satisfy legal regulations regarding body mass and body length shows the following: 81.4% of individuals whose body mass is permitted for exploitation are shorter than the required length minimum of 90 mm, while 18.6% of these individuals are within the range permitted for exploitation (90 to 150 mm).

The results shown here highlight a number of significant points related to the legal basis of permitted exploitation. In terms of the minimal size of 90 mm permitted for exploitation, the law has enforced a restrictive approach that satisfies the necessary prerequisites for preservation of

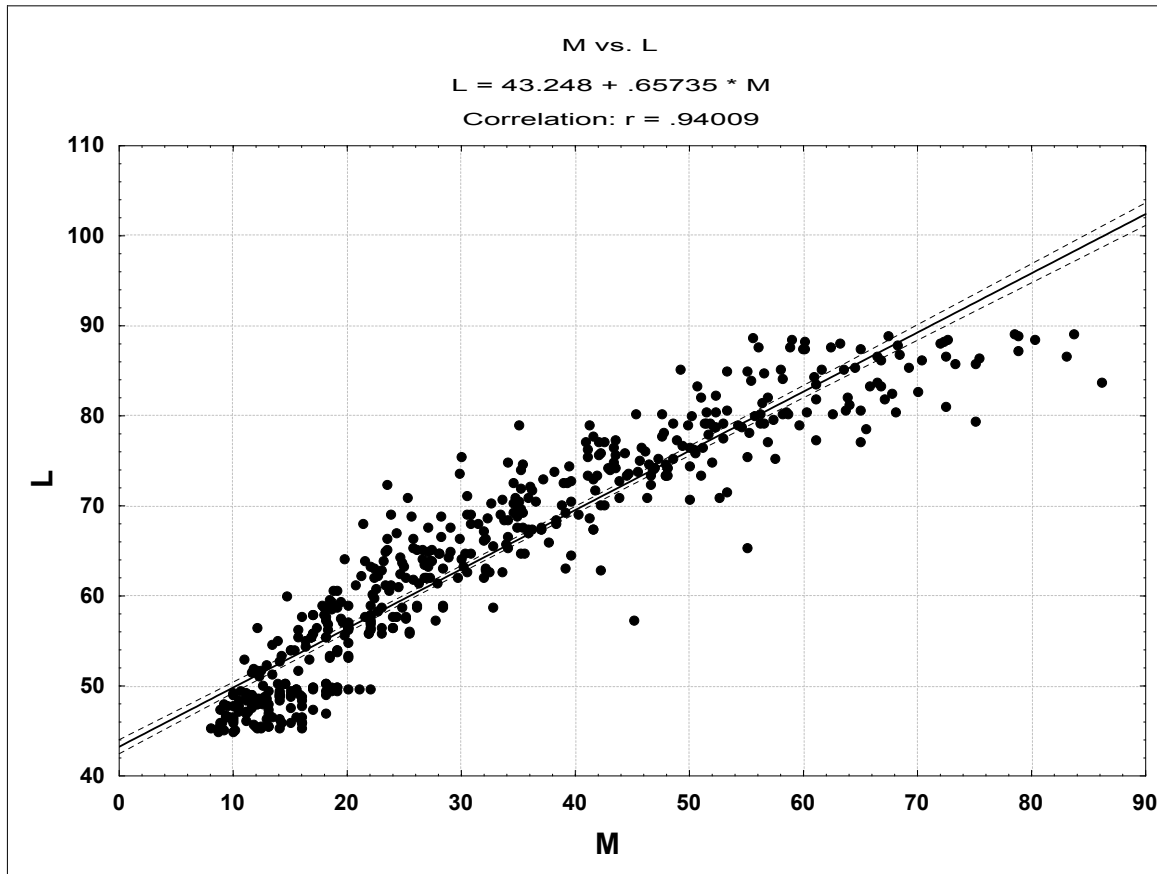


Fig. 6. Correlation between total body length (L) and body mass (M) in adults whose body length is below exploitation tolerance level as defined by law (L=50-90 mm).

the reproductively active part of the population. Moreover, there is a percentage of the reproductively active part of the population that is within the range of L values permitted for exploitation (3.67%), which we do not find to be a risk factor for preservation of these populations. However, this percentage is specified in relation to the entire analyzed sample, regardless of size structure. When related to the adult part of the population, the conditions are not any better, considering that less than 5% of individuals have the legal requirement of body length. This information shows the unfavorable state of this part of the population. The question remains as to how are the regulations are being followed in the process of exploitation. Also unclear are the condition of entire populations after many decades of unregulated exploitation (Džukić, 2003) and

the consequences of interactions between disturbed population relations and other endangering factors.

In regard to the other legally regulated element, less than one fourth of adult individuals reach the body mass permitted for exploitation, while more than three fourths remain under the lower limit of allowed body mass. It is important to note that all individuals within the permitted length range are also over the required weight (>50 g). In terms of the maximum size permitted for exploitation (150 mm), we find that such a value is unrealistic, considering that in the samples analyzed here not a single individual is even close to that size. These results are in accord with earlier research (Džukić et al., 2001, 2003). Drawing from these results, we maintain that the upper limit of body size permitted

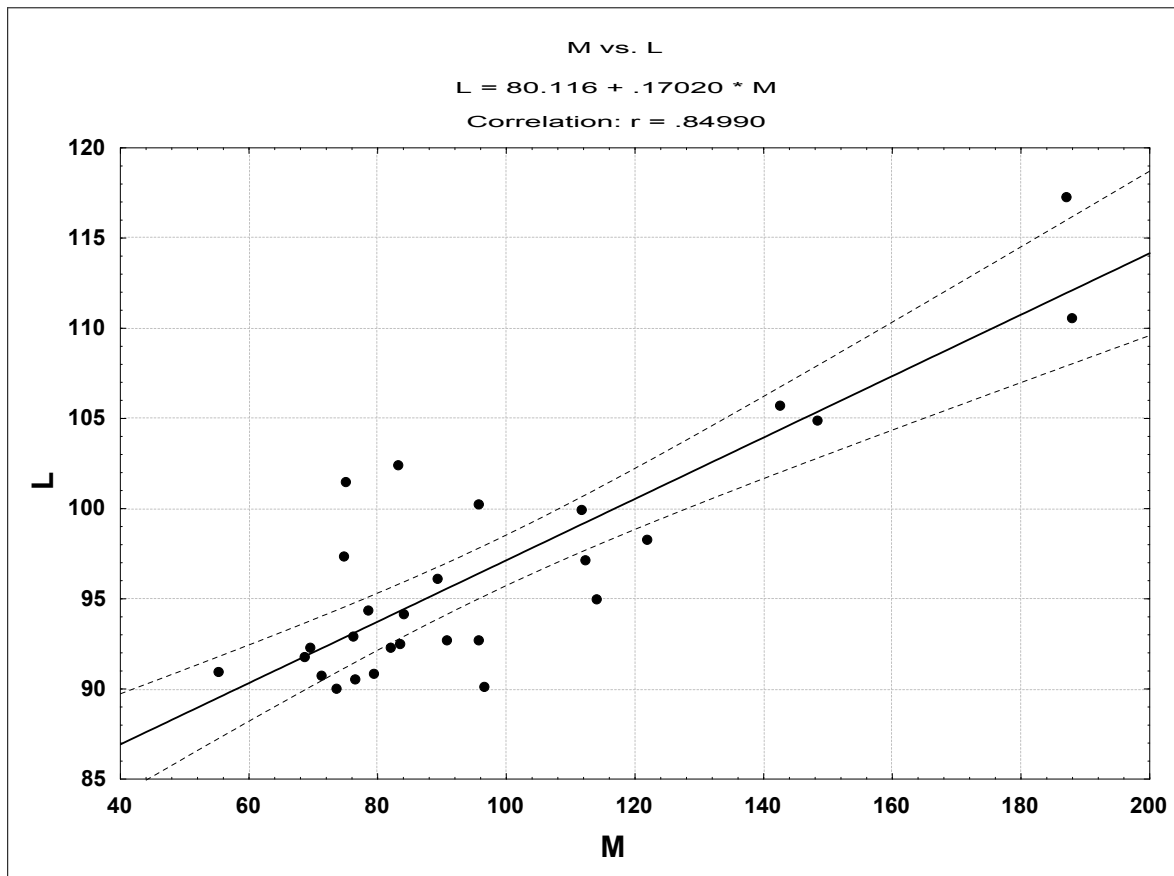


Fig. 7. Correlation between total body length (L) and body mass (M) in adults whose body length is above exploitation tolerance level as defined by law ($L > 90$ mm).

for exploitation should not be over 120 mm. This standpoint is supported by information from other regions, where mean body length values in no instance surpass the minimal size stated in the act (Gubányi and Korsós, 1992; Kotlik and Šulova, 1994; Plötner et al., 1994; Günther et al., 1991; Joly et al., 1994; Ogielska et al., 1994; Gavrilović et al., 1999; Spasić-Bošković et al., 1999; Sinsch and Schneider, 1999; Борисовский et al., 2000; Budak et al., 2000; Lode and Pagano, 2000; Sinsch et al., 2002; Džukić et al., 2001; Džukić et al., 2003; Nekrasova et al., 2003; Желев and Моллов, 2004), except in the case of data for *R. ridibunda* from the territory of Turkey (Arıkan et al., 1998). Of special significance are the results of total body length comparisons between samples collected north

of Sava and Danube Rivers, and ones collected south of them. Significant differences in mean values point to the existence of an external factor acting on body size. However, the lack of adequate research makes it impossible to speculate about the possible causes of such results. Environmental factors may account for some of these changes. However, it is highly probable that the influence of selective and intensive exploitation has led to a general reduction in the mean value of total body length.

From analyses of body mass in members of the *R. synklepton esculenta* complex on the territory of Serbia and based on previously obtained results, it can be asserted that correlation of body length and body mass depends on the former (age of the individual), and that the correlation is significant

Table 7. Values of mean body-mass (M) in subadults (up to 50 mm) sorted into length categories with a 10 mm range.

	L 30-40	L 40-50
N	37	149
Min	2.8	3.5
Max	15	66.86
8	6.77	12.37
SE	0.42	0.47

Table 8. T-test values of interrelations between mean body mass values (M) of subadults (up to 50 mm) sorted into length categories. Category codes: 1 – from 30 to 40 mm; 2 – from 40 to 50 mm.

	t	df	p
1:2	-5.85	184	0.0000

in adult individuals and statistically insignificant in subadults. In subadults, body length and body mass are significantly correlated in the category up to 40 mm and the category which is nearly at the stage of sexual maturity. In the category of individuals which satisfy legal criteria in terms of body mass, less than 20% of individuals satisfy body length criteria.

CONCLUSION

In general, all individuals qualifying for exploitation on the basis of total body length (L) are in fact of the required weight. However, only 18.6% of individuals which satisfy legal body mass criteria actually attain the required body length of 90 mm. These data are important for the following reason: body mass as a character determining permissible exploitation is invalid because more than 80% of individuals which satisfy body mass criteria can be exploited without meeting length criteria. Since the act allows for the choice of characters (and ranks body mass in top position, ahead of other limitation criteria), this particular formulation is harmful for the reproductively active part of the population. If the act could be altered in order to satisfy both criteria (“...weight and length...”), our results show that only 5% of the population would qualify for exploitation. It can be concluded that the Act on Controlling

Exploitation and Trade of Protected Wild Plant and Animal Species (Službeni glasnik RS No. 31/05) satisfies protection requirements in terms of minimal body length. However, maximum length limitations are too high and should not exceed 110 mm. We note that only 1.2% of individuals within the entire sample are longer than 100 mm. Furthermore, the given act does not ensure protection of green frogs on the territory of Serbia because it permits exploitation on the basis of body mass parameters. Body mass as a parameter needs to be excluded or linked with body length. According to the act, the minimal and maximal values of body mass are 70 and 90 g, respectively, while the minimal and maximal values of body length are 90 and 110 mm. However, there are only 5.4% of individuals weighing 70-100 g and only 1.2% weighing 100 g within the entire sample.

In the light of population samples from the 22 localities processed in this study, it is clear that legal measures can satisfy the task of protecting these animals, but with certain changes and addenda. The research presented here, along with the results of earlier studies (Spasić-Bošković et al., 1999; Džukić et al., 2001; Džukić et al., 2003), provides the basis of a significant correction within existing statutes concerning the active protection of these groups. Analysis of characters such as body length (L) and mass (M) together with certain other traits under field conditions, may provide grounds for a different approach to the utilization of this renewable resource.

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ОСНОВНЕ МОРФОЛОШКЕ КАРАКТЕРИСТИКЕ RANA (PELOPHYLAX) SYNKLEPTON ESCULENTA КОМПЛЕКСА У ОДНОСУ НА ЗАКОНСКУ РЕГУЛАТИВУ У СРБИЈИ

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Циљ рада је анализа основних морфолошких карактеристика сва три таксона овог комплекса на територији Србије у односу на законом прописане дозвољене мере при излову. Анализирано је укупно 789 јединки зелених жаба са 22 локалитета на подручју Србије. У анализи су коришћене мере предвиђене Законом: укупна дужина

тела (L) и маса тела (M). Обзиром да Закон дозвољава избор карактера оваква формулација је погубна за репродуктивно активни део популације. Уколико би се формулација преиначила у обавезујућу одредницу задовољавања оба услова, у том случају би око 5% популације било дозвољено изловити.