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COMPARATIVE BIOMETRIC STUDY OF CRAYFISH POPULATIONS IN THE ANINA MOUNTAINS (SW ROMANIA) HYDROGRAPHIC BASINS

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SUMMARY. The paper presents data concerning the biometric aspects of two crayfish species from the rivers of the Anina Mts: the stone crayfish (*Austropotamobius torrentium*) and the noble crayfish (*Astacus astacus*). The populations of the two species are not evenly distributed within the three hydrographic basins of the investigated area: Bârzava, Caraş, and Nera. Thus, through the discriminant analysis of the measured parameters we were able to identify several parameters that proved to be highly related to the differentiation of the studied specimens into hydrographic basins; these specimens have been afterwards the subject of the ANOVA type analysis. The study revealed that, whereas the *A. torrentium* populations from the Caraş basin present high significantly differences in comparison to the populations analysed in the Nera basin, the *A. astacus* populations from the Caraş basin differ significantly from those of the Bârzava basin. As far as the morphometric differences between the two sexes are concerned, the most powerful and visible distinctions that have been registered for both species can be found at the level of chela, propodus length, dactylus length and width of chela. The maximum dimension of the *A. torrentium* males, as well as the average of these values, is very close to the female one, while for the *A. astacus* the differences between the sexes are much more significant.

Keywords: Anina Mountains, *Astacus astacus*, *Austropotamobius torrentium*, biometry, populations

Introduction

Freshwater crayfish residing in the Romanian aquatic ecosystems belong to the Decapoda orders; they are represented by three indigenous species and an invasive one (Pârvulescu, 2009c). Among these, two indigenous species are present in the Anina Mountains: *Austropotamobius torrentium* (Schrank 1803) and *Astacus astacus* (Linnaeus 1758) (Pârvulescu 2009a). The main purpose of the comparative study regarding the biometry of different populations is to point out the morphological

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similarities and dissimilarities between populations that are geographically separated into different hydrographic basins, as a result of diverse environmental influences (Ďuriš *et al.*, 2006; Burba *et al.*, 1999; Papadopol and Diaconu, 1987; Gutiérrez-Yurrita *et al.*, 1996; Streissl and Hödl, 2002). Studies regarding the three indigenous species of crayfish in Romania have also been published by Papadopol and Diaconu, in 1987.

The investigated area, i.e. the Anina Mountains, is located in southwestern Romania; it has a surface of 770 km^2 (Sencu, 1978) most of which is included in two National Parks: Semenic-Cheile Caraşului (Semenic – Caras Gorges) National Park and Cheile Nerei-Beuşniţa (Nera Gorges - Beusnita) National Park. The area of these mountains gathers three hydrographic basins: Bârzava, Caraş and Nera, basins that collect both surface water and ground water. The Caraş and the Nera rivers are the direct tributaries of the Danube River, and the Bârzava River flows into the Danube River, after the confluence with the Timiş River (Ujvari, 1972). The *A. astacus* species lives almost exclusively in the Caraş basin, with the exception of the Buhui stream and the Cândeni River. The Nera basin shows exclusively the presence of the species *A. torrentium*, while the Bârzava basin displays a mixture of populations from both species that can be found in different streams. (Pârvulescu, 2009a).

Taking into consideration the quality of the aquatic habitats, the Bârzava basin is affected by a powerful anthropic impact; on its upper stream there are three artificial dams and various villages which clearly affect the water quality and, implicitly, the quality of the aquatic fauna. The upper stream of the Caraş River presents a very low anthropic impact and registers a very high water quality, with few punctiform exceptions. In the Nera Basin the anthropic impact is scattered along the small villages situated on the stream of the rank 1 tributaries and that alter the water quality, like in the case of the Cândeni creek, where almost an entire popuation was destroyed as a result of the usage of modern detergents within a traditional wash house. (Pârvulescu, 2009b).

Materials and Methods

The biologic samples were collected in August 2008 and June - July 2009, in a total of 52 sampling stations, on all the permanent waters in the upper sector of the Bârzava, Caraş and Nera rivers. Each sampling station comprised at least 200 m of the river under investigation, with similar catching effort. The crayfish were collected using active methods, i.e. direct hand sampling from the waterbed, by checking the galleries within banks and the spaces between roots or rocks.

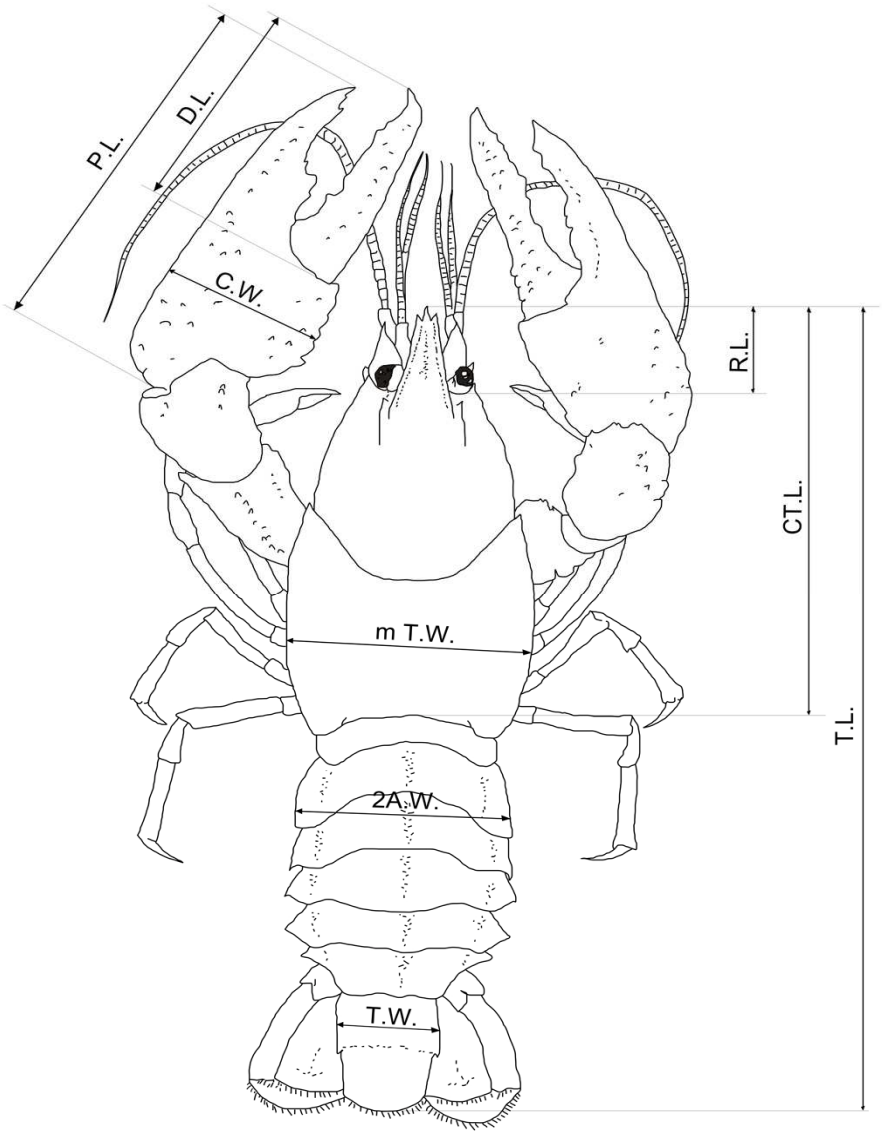

Fig. 1. The biometric parameters measured during the biometric studies for the crayfish from the Anina Mountains

The crayfish were identified *in situ* according to their morphological features, sexed and measured. Subsequently, the specimens were set free exactly in the same location where they had been captured. We have labeled as population all the specimens belonging to one species within each of the three hydrographic basins. For the biometric measurements we have used an electronic Black and Decker caliper with the maximum width of 150 mm and an accuracy of 0.01 mm. The measured parameters (see figure 1) are only those distances which allow the firm grip of the measuring tool. The morphological characteristics measured were: TL – total body length, CTL - cephalothorax (shell) length, mTW - maximum thoracic (shell) width, RL - rostrum length (for the value that indicates the length of the rostrum we subtracted from the value of the cephalothorax length (CTL) the value of the post-orbital cephalothorax length), 2AW – second abdominal segment width, TW – width of telson, PL – propodus length, DL – dactylus length and CW – width of chela. We have measured only the specimens that did not present any sign that both chelae have been regenerated. All specimens have been measured by the same person. The specimens that were smaller than 50 mm total body length have not been measured.

In order to show which of the measured parameters are relevant for the morphological differences, we have processed the data with the help of the discriminant analysis, a technique used to classify cases into categorical-dependent values that helps us obtain pairs of parameters that are significantly different within the lot of the measured data. The means, the standard deviations, the minimum and the maximum as well as the ANOVA tests have also been measured. In order to do this statistical analysis and to create the diagrams, we used the Statistica StatSoft Inc. software (version 7.00 for Windows).

Results

During the investigations performed in the summers of 2008 and 2009 in the rivers of the Anina Mountains, 115 specimens from the *A. torrentium* species and 73 specimens from the *A. astacus* species have been measured. The species are not evenly distributed in all the three hydrographic basins, according to the already published data (Pârvulescu, 2009a). Thus, for the *A. torrentium* species the most representative streams have been the streams of the Nera basin. The species was also found in the Caraş and Bârzava basins. As far as the *A. astacus* species is concerned, the most representative have been the streams from the Caraş basin. The species was also found in the Bârzava basin, especially in the reservoirs. The biometric data have been summarized in Tables 1 and 2.

According to the data from Tables 1 and 2, we can notice that the clearest differences between the sexes are represented by the dimensions of the chelae. However, we cannot use this difference alone if we are to correctly make a distinction between the sexes. Many and diverse situations may appear in nature when the chela may be abnormally well-developed: regeneration after breakage, malformations etc (Pârvulescu *et al.*, 2009).

Table 1.

Biometric data (mm) for the *A. torrentium* species, measured during the summer campaign of 2008-2009, in the Anina Mts. (N = number of specimens).

	Austropotamobius torrentium			
	Male (N=57)		Female (N=58)	
Parameter	Min*-Max	Mean ± SD	Min*-Max	Mean ± SD
TL	50.0-94.0	**69.07** ± 10.862	50.53-93.34	**67.16** ± 10.403
CTL	23.7-48.9	**34.5** ± 6.01	24.6-44.37	**31.46** ± 5.512
RL	4.8-9.1	**6.78** ± 1.072	4.78-9.6	**6.66** ± 1.057
mTW	11.78-27.9	**18.72** ± 3.76	12.3-24.02	**17.04** ± 2.907
2AW	6.17-20.9	**15.33** ± 2.737	11.5-25.43	**16.97** ± 3.486
TW	5.9-13.7	**9.42** ± 1.789	5.93-14.1	**9.37** ± 1.884
PL	16.6-49.9	**29.95** ± 8.021	15.79-33.56	**22.59** ± 4.578
DL	10.08-28.5	**17.23** ± 4.75	8.91-20.45	**13.21** ± 2.757
CW	7.8-19.6	**13.05** ± 3.235	6.75-13.2	**10.05** ± 2.036
Sex ratio	**1:1.017**			

* *Specimens smaller than 50 mm in total length have not been measured*

Table 2.

Biometric data for the (mm) *A. astacus* species, measured during the summer campaign 2008-2009, in the Anina Mts. (N = number of specimens).

	Astacus astacus			
	Male (N=39)		Female (N=34)	
Parameter	Min*-Max	Mean ± SD	Min*-Max	Mean ± SD
TL	61.34-127.34	**99.73** ± 17.978	55.85-108.8	**86.8** ± 14.543
CTL	27.06-68.46	**51.46** ± 10.878	27.82-54.07	**42.98** ± 8.022
RL	5.21-18.8	**12.1** ± 2.671	6.45-12.6	**10.49** ± 1.779
mTW	15.45-49.97	**28.82** ± 7.621	14.42-29.31	**23.24** ± 4.63
2AW	13.17-31.09	**22.93** ± 4.31	12.34-28.26	**21.97** ± 4.655
TW	7.34-18.14	**13.23** ± 2.414	6.83-14.7	**11.38** ± 2.278
PL	20.65-85.5	**47.79** ± 16.925	18.92-42.7	**32.22** ± 8.13
DL	11.69-48.36	**28.08** ± 10.181	10.99-25.6	**19.2** ± 4.778
CW	9.13-32.67	**20.53** ± 6.555	8.19-20.5	**14.43** ± 3.708
Sex ratio	**1:0.871**			

* *Specimens smaller than 50 mm in total length have not been measured*

In order to compare the populations we have resorted to the discriminant analysis that allows to find the most powerful correlated sets of values that could differentiate the specimens from different hydrographic basins. Thus, the correlation

matrix for the *A. torrentium* (Table 3) shows that the most powerful discriminative character is represented by the groups of values propodus length – dactylus length (PL-DL), propodus length – width of chela (PL-CW) and dactylus length – width of chela (DL-CW).

Table 3.

Correlation matrix between the measured parameters for the *A. torrentium* specimens in the Anina Mts. in 2008-2009.

correl.	TL	CTL	RL	mTW	2AW	TW	PL	DL	CW
TL	1.00								
CTL	0.91	1.00							
RL	0.82	0.81	1.00						
mTW	0.95	0.94	0.81	1.00					
2AW	0.84	0.73	0.71	0.76	1.00				
TW	0.75	0.70	0.82	0.73	0.68	1.00			
PL	0.83	0.89	0.73	0.92	0.55	0.62	1.00		
DL	0.82	0.88	0.76	0.90	0.56	0.65	**0.98**	1.00	
CW	0.82	0.87	0.72	0.90	0.56	0.62	**0.97**	**0.95**	1.00

The correlation matrix for the *A. astacus* (Table 4) shows that the most powerful discriminative character is represented by the same groups of values that are relevant for the previous species and by the total length – cephalothorax length (TL-CTL).

Table 4.

Correlation matrix between the parameters measured for the *A. astacus* specimens in the Anina Mts. in 2008-2009.

correl.	TL	CTL	RL	mTW	2AW	TW	PL	DL	CW
TL	1.00								
CTL	**0.97**	1.00							
RL	0.89	0.89	1.00						
mTW	0.92	0.93	0.84	1.00					
2AW	0.92	0.86	0.78	0.82	1.00				
TW	0.80	0.76	0.76	0.72	0.73	1.00			
PL	0.92	0.92	0.83	0.88	0.77	0.72	1.00		
DL	0.91	0.92	0.83	0.86	0.76	0.73	**0.98**	1.00	
CW	0.90	0.90	0.80	0.85	0.77	0.69	**0.97**	**0.96**	1.00

In our studies, for the comparison of these populations, we have used ratio among these parameters. Therefore, for both species we have measured the proportion between propodus and dactylus length, propodus length and chela width, dactylus length and chela width and also total length and cephalothorax length (table 5).

Table 5.

Rapports that show a discriminative character for the crayfish specimens measured in the Anina Mts.

Austropotamobius torrentium		*Astacus astacus*	
Ratio between parameters:	Abbreviation	Ratio between parameters:	Abbreviation
-	-	TL and CTL	**TL/CTL**
PL and DL	**PL/DL**	PL and DL	**PL/DL**
PL and CW	**PL/CW**	PL and CW	**PL/CW**
DL and CW	**DL/CW**	DL and CW	**DL/CW**

For our comparison between different populations we have used the ANOVA test, in order to underline the differences between specimens of the same sex, from different hydrographic basins. The individuals of *A. torrentium* species, that inhabit mainly the Nera basin, have also been found well represented in high number of individuals in two streams of the Caraş basin and in five streams of the Bârzava basin. According to the analysis of the diagrams from Figures 2 A, B and C the female populations of crayfish within the three investigated basins are not significantly different. However, we may notice a greater resemblance between the populations of the Nera and of the Bârzava basins.

For *A. torrentium* species the males have been captured in a rather small number of specimens in the Bârzava basin; that is why, for the data analysis only the specimens captured in the Nera and Caraş basins have been taken into account. They display statistically significant differences in the case of the ratio PL/CW (p=0.01971) (Fig. 3).

The *A. astacus* species was found in the Anina Mountains only in the Caras and Bârzava basins; in the upper part of the Bârzava River the species was discovered especially in reservoirs. After analyzing the distinct diagrams for the two sexes, we can state that the female populations from the two investigated basins are significantly different concerning the ratio between propodus length and chela width, as well as between dactylus length and chela width (Figs 4 A and B).

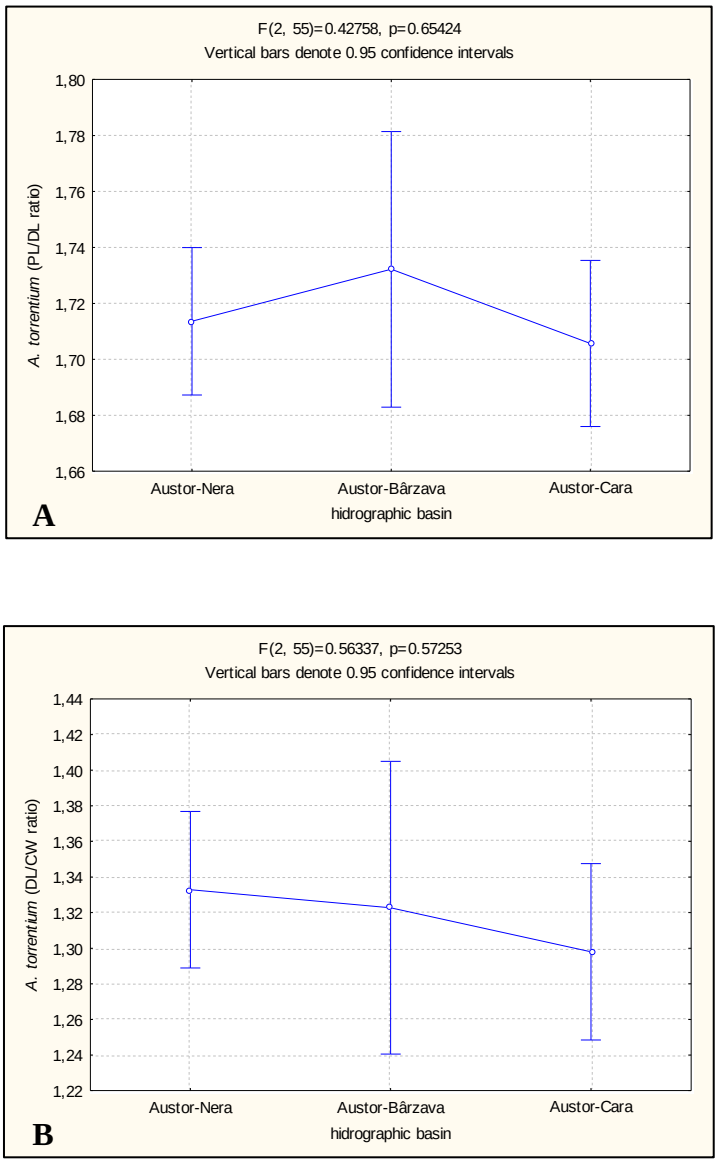

Fig. 2. ANOVA tests for the female specimens of *A. torrentium* from Nera, Bârzava and Caraş hydrographic basins: **A –**for the ratio PL/DL; **B** –for the ratio DL/CW;

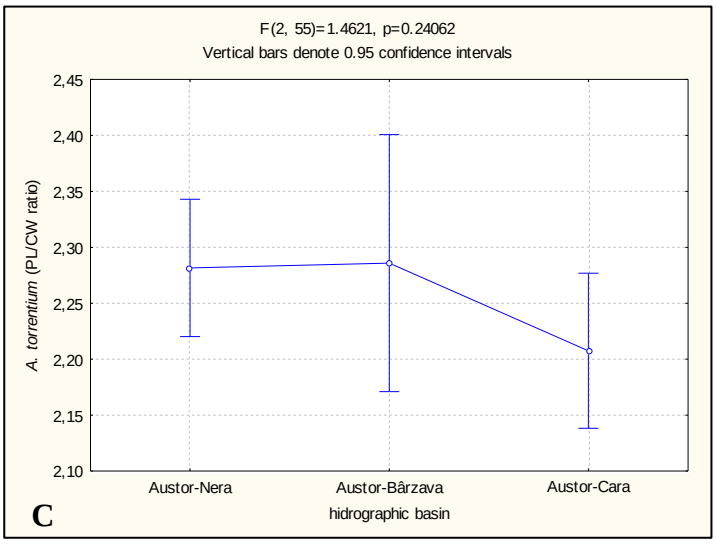

Fig. 3 (continued). ANOVA tests for the female specimens of *A. torrentium* from Nera, Bârzava and Caraş hydrographic basins: **C**- for the ratio PL/CW

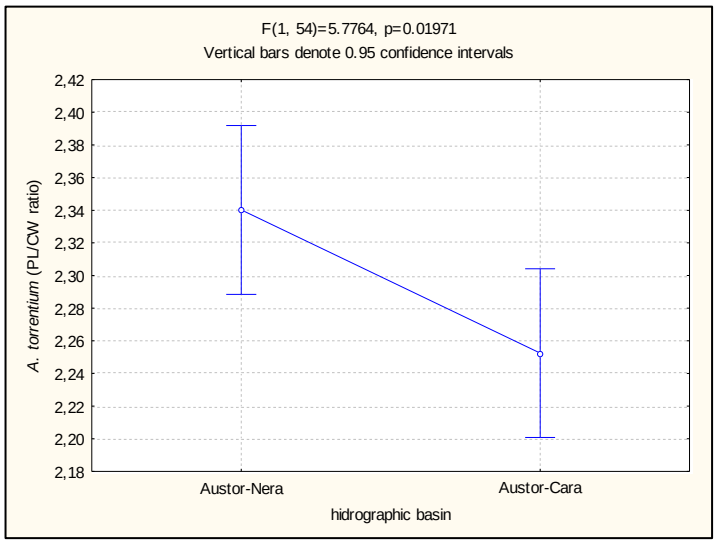

Fig. 4. ANOVA tests for the male specimens of de *A. torrentium* from Nera and Caraş hydrographic, for the ratio PL/CW

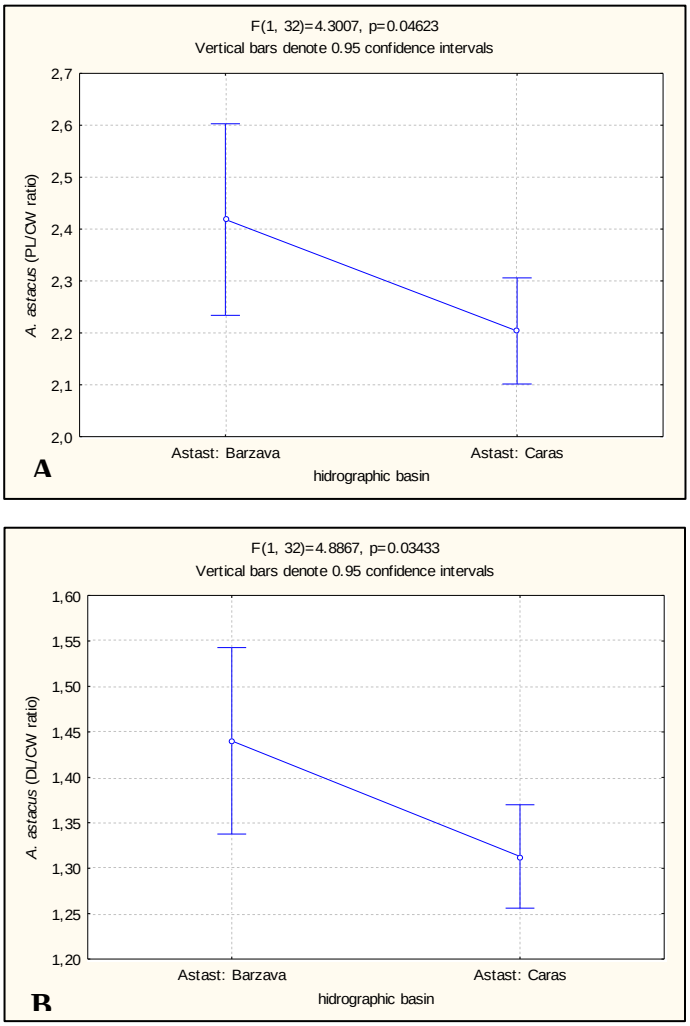

Fig. 5. ANOVA tests for the female specimens of *A. astacus* from the Bârzava and Caraş hydrographic basins: **A** –for the ratio PL/CW; **B** –for the ratio DL/CW

The *A. astacus* males analyzed in the Bârzava basin have been captured in a much smaller number, impossible to analyze statistically.

Discussions

Taking into account the obtained results, we can formulate several sentences regarding the biometric aspects observed in the measured specimens during the summer campaigns of 2008 and 2009 in the Anina Mts.

>For *Austropotamobius torrentium*

1. The maximum dimension of the specimens regarding total length (TL) varies between similar limits for males and females: the largest male specimen has a total body length of 94.00 mm, while the largest female has a total length of 93.34 mm. In comparison with the results existing in the literature the size of the biggest male is with 11.9% larger, while the size of the female is with 16.75% larger the than those measured by Papadopol and Diaconu (1987), on a population from the Nera hydrographic basin (on the Şuşara river).

2. The body characteristics that have registered the greatest differences between the sexes proved to be the parameters of the chelas: the propodus length (PL) being 24.57% smaller for the females than for the males, the dactylus length (DL) being 23.33% smaller for females than for males and the chela width (CW) being 22.98% smaller for the female population than for the male population

3. The average values that have turned out to be higher for the male population are the total length (TL), the cephalothorax length (CTL), the rostrum length (RL), the maximum thoracic width (mTW) and the telson width (TW). For the second abdominal segment width (2AW), the average value of the measured results favors the female population.

>For *Astacus astacus*

1. The maximum dimension of the total length (TL) favors the male population, the largest specimen measuring 127.34 mm, whereas the largest female measured 108.80 mm. In comparison with the results existing in the literature, the size of the larger male is with 2.79%, more reduced, while the size of the female is with 2.85% more reduced than those measured by Papadopol and Diaconu (1987) on a population from the north-eastern part of Romania (from the Bicazu Lake).

2. The body characteristics that have registered the greatest differences between sexes turned out to be the cephalothorax length (CTL) which was 16.47% smaller for the females than for the males, the maximum thoracic width (mTW) being 19.36% smaller for the females than for the males, as well as the parameters of the chelas, namely the propodus length (PL) which is 35.58% smaller for the females than for the males, the dactylus length (DL) which is 31.62% smaller for the female population than for the male population and the chela width (CW) which proved to be 29.71% smaller for females than for males.

3. All the average values measured are larger for the male population in comparison to the female population even for the second abdominal segment width where, due to the eggs, the females should be larger.

Conclusions

Concerning the difference between the populations studied in the Anina Mountains, the *A. torrentium* populations present high significantly biometric differences

between Caraş and Nera hydrographic basins. A possible explanation for the current situation can be represented by the geographical separation. Is known that *A. torrentium* occupies entirely the Nera hydrographic basin and populates only two creeks of Caraş basin.

The difference between the *A. astacus* populations, we can ascertain that the populations are significantly different in the Caraş basin as opposed to the ones measured in the Bârzava basin. In the case of the Bârzava hydrographic basin, and due to the heterogeneity of the populations, we can assume that a restocking in the area of the barrier lakes can be accomplished, probatory documents still have not yet been found.

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