

Morphometric characteristics and condition indices of stone crayfish *Austropotamobius torrentium* (Shrank, 1803) from the Mlinska River

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Abstract

Regarded as a protected species on the IUCN list of endangered species, and classified as data deficient species, the stone crayfish (*Austropotamobius torrentium*) is one of the five European species from the Astacidae family that lives in freshwater ecosystems across Bosnia and Herzegovina. The aim of this study was to investigate the variability of selected morphometric characteristics of male and female crayfish of the *A. torrentium* species from the upper reaches of the Mlinska River. The crayfish were sampled in June 2021 when 62 individuals (22 males and 40 females) were caught. They were analyzed fresh in the field and in the laboratory. The treatment included the measurement of 12 morphometric features: total body length (TBL), weight (W), claw length (CLL), claw width (CLW), carapace length (CPL), carapace width (CPW), rostrum length (ROL), rostrum width (ROW), abdominal length and width (ABL, ABW) and telson length and width (TEL, TEW). The mean body length \pm SD was 79.05 ± 7.35 in males and 60.54 ± 10.03 mm in females. The mean recorded body weight \pm SD was 17.51 ± 8.3 g in males and 7.02 ± 4.41 g in females. Using the linear regression method, a positive correlation was found between body length and weight ($\sigma^2 R^2=0.7644$; $\phi^2 R^2=0.8222$), body weight and claws length ($\sigma^2 R^2=0.876$; $\phi^2 R^2=0.9661$), as well as carapace width and body length ($\sigma^2 R^2=0.7216$; $\phi^2 R^2=0.8411$). Using the T-test, a statistically significant difference of the analyzed morphometric parameters was found between the sexes, which

is explained by the pronounced sexual dimorphism of stone crayfish. The calculated values for Condition factor and the Crayfish constant indicate that the males are of better fitness, which is in line with the results of previous research in Europe. Data presented in this paper can serve as a basis for further research of *A. torrentium* in this area.

Key words: stone crayfish, males, females, morphometric characteristics

Introduction

A large number of authors dedicated their studies to morphometric characteristics of certain species of crayfish from the Astacidae family. Thus, Trožić-Borovac et al. (2007) provide data on morphometric characteristics of juvenile, subadult, and adult crayfish of *Austropotamobius torrentium*. Maguire (2010) emphasises the significance of applying traditional (linear) morphometry and meristic methods to differentiate crayfish from the *Astacus* and *Austropotamobius* genera. Dakić and Maguire (2016) investigated morphometry in relation to sexual dimorphism of adults belonging to the *A. torrentium* species, whereas Trožić-Borovac (2012) define morphometric and meristic characteristics significant to determination of *Austropotamobius pallipes* genus.

Study of freshwater crayfish goes beyond, by starting the application of molecular-phylogenetic methods with the aim to determine phylogenetic relationship of freshwater crayfish. Research in the field of phylogenetics of freshwater crayfish showed that genus *Austropotamobius* is older than genus *Astacus*, and species *A. pallipes* is the oldest species which spread throughout the whole Europe in Tertiary (Miocene), when barriers such as the Alps or the Pyrenees did not exist. Species *A. torrentium* started to spread in the Ponto-Caspian region later (Souty-Grosset et al., 2006; Trožić-Borovac, 2012).

Data on both the values of morphometric characters and the ecology of stone crayfish *A. torrentium* from many areas within their range are scarce. It is a widespread European species (Holdrich, 2002), which shows a tendency to reduce the surface area due to the presence of allochthonous species and loss and degradation of habitats (Holdrich et al., 2009). The stone crayfish occurs only in the source and upper parts of streams with stone bottoms (Kappus et al., 1999), although they sometimes can be found in clean rivers and lakes (Renz and Breithaupt, 2000). The above-mentioned species of crayfish is present and dominant in Bosnia and Herzegovina (Trožić-Borovac, 2011; Roljić, 2020), as well as in the neighboring countries, Serbia (Simić et al., 2008), Croatia (Maguire and Gottstein-Matočec, 2004), Montenegro (Rajković, 2012). The existing populations are the remnants of a wider historical population that was

ravaged by duck plague by the end of the 19th and during the 20th century (Budihna, 1991). Also, water pollution, regulation of watercourses, and removal of vegetation along the bank, as well as the consequences of extreme droughts, strongly affect the diversity and distribution of *A. torrentium* populations. Comparing historical and recent data, Maguire et al. (2011) found obvious decline in the number of stone crayfish populations.

The importance of ecological and morphometric research of this crayfish, species *A. torrentium*, is justified by the fact that it belongs to an autochthonous European species, which is on the IUCN list of endangered species, classified as Data Deficient, a category of species that are not sufficiently studied and are threatened to be extinct due to changed life conditions caused by anthropogenic influence.

As preliminary studies determined the presence of this species in Mlinska River (Roljić, 2020), our intention was to describe its morphometric characteristics. This research aims to determine whether there are differences between the morphometric characteristics of males and females and to observe the condition status of the captured individuals in order to estimate the possible pressure on the population of stone crayfish.

Material and Methods

Description of the research location

Between Jošavka Donja and Dubrava Stara, northern of Dubrava Nova location, in a rolling-upland country area, there is a relatively dense spring catchment area of the Mlinska River. It consists of two source branches: left, from Matino brdo at 275 m height above sea level, and right, from Crna stijena at 340 m altitude. A permanent watercourse is formed on 275 m above sea level, while the mouth into Jošavka is on up to 240 m altitude, so considering the total length of watercourse, the river does not have a large absolute, nor an average drop (Rajčević and Crnogorac, 2011). A riverbed is originally preserved and is sometimes used for irrigation in the upper watercourse of the Mlinska River. It belongs to watercourses that need to be ecologically evaluated. GPS coordinates of the studied area: 44.742492° N 17.45392° E, elevation 252 m.

Methodology

Collection of crayfish samples was performed during June, 2021. Crayfish samples were collected manually, using LiNi traps with baits and nets (Westman et al., 1978). All crayfish were caught during the night, in the period between 8.00 p.m. until 8.00 a.m. To identify freshwater crayfish from the

Astacidae family, the illustrated key was used (Maguire, 2010). In order to learn about the fundamental biological characteristics of crayfish, the following measurements were made: total body length (TBL), claw length (CLL), claw width (CLW), carapace length (CPL), carapace width (CPW), rostrum length (ROL), rostrum width (ROW), abdominal length (ABL), first abdominal pleural width (ABW), telson length (TEL) and telson width (TEW), and apart from these parameters, values of body weight (W) were measured.

Apart from these parameters, values of two condition indices are to be checked: Condition factor (Ricker, 1975) according to the formula:

$$CF = \frac{W}{TBL^3}$$

where we have: CF - Condition factor (g/mm^3), W - crayfish weight (g) and TBL - total length of the individual (mm), and decapods constant (Adegboye, 1981) according to the formula:

$$CC = \frac{W}{TBL \times CPL \times CPW}$$

where we have: CC – decapods constant (g/mm^3), W – total weight, TBL -total length, CPL - carapace length and CPW - carapace width.

A caliper (brand: Stainless Hardened) with a precision of 0,02 mm was used to determine the morphometric characteristics, and a technical scale of "Kern" type (Kern PFB Version 2,2) with max. weighing of 1,200 g with and precision of 0,01 g was used to measure the body weight. The measured values of morphometric characteristics were statistically processed by using the statistical program Statistica 6 (minimum, maximum, mean values, standard deviation and coefficient of variation), interpreted and compared with available data from the literature.

Results and Discussion

Of 62 analyzed specimen it was found that 22 (or 35.48%) were male, and 40 (or 64.52%) were female (sex ration close to 1:1.82; $\chi^2 = 5.23$, $p < 0.022$). This value significantly differs from the theoretical 1:1 value (Aydin et al., 2015).

Having the information on the sex ratio in population is significant because it tells us about the health and stability of the population (Jurković, 2016). Unbalanced sex ratio may be caused by biased sampling (Deniz et al., 2010),

but also several biological factors, such as: unequal sex ratio after hatching, differentiation of immigration, and emigration of individuals of different sex. Uneven sex ratio can be a consequence of differences in the activity between different sex (Dakić & Maguire, 2016), differences in the age of reaching sexual maturity, but also different life expectancy of individuals of different sexes.

The population of stone crayfish at the locality of research has relatively stable age structure. At the research location in the Mlinska River, the most common was the group whose length varied between 60-80 mm by 50%. The longest male was 88.16 mm long, and the longest female was 86.45 mm long, which is in accordance with the range of 70-80 mm for stone crayfish, mentioned in the literature (Obradović, 1988), as well as with the values recorded by Trožić-Borovac et al. (2007) and Rajković (2012). The heaviest male weighed 32.41 g and the heaviest female weighed 16.94 g. Higher average weight of males can be ascribed to the fact that males have larger claws, which contributes to their body weight in comparison to females (Rajković, 2012).

Tab. 1. Descriptive statistics - mean value, standard deviation, ranges of measured morphometric characteristics for all specimens of species *A. torrentium* from the Mlinska River

| | Male (n=22) | | | | | Female (n=40) | | | | | T-test |
|-----|-------------|-------|-------|------|-------|---------------|-------|-------|-------|-------|--------|
| | Mean | Min | Max | SD | CV | Mean | Min | Max | SD | CV | |
| TBL | 79.05 | 62 | 88.16 | 7.35 | 9.30 | 60.54 | 39.52 | 86.45 | 10.03 | 16.57 | p<0.00 |
| W | 17.51 | 5.6 | 32.41 | 8.30 | 47.40 | 7.02 | 1.6 | 16.94 | 4.41 | 62.82 | p<0.00 |
| CLL | 28.36 | 16.52 | 37.78 | 5.91 | 20.84 | 21.87 | 12.47 | 36.02 | 6.89 | 31.50 | p<0.00 |
| CLW | 12.58 | 8.05 | 17.57 | 2.93 | 23.29 | 10.23 | 5.5 | 19.97 | 3.76 | 36.75 | p<0.01 |
| CPL | 32.34 | 20.78 | 37.41 | 5.44 | 16.82 | 27.75 | 18.1 | 51.08 | 6.49 | 23.39 | p<0.01 |
| CPW | 18.80 | 15 | 23.00 | 2.6 | 13.83 | 15.12 | 9.23 | 26.2 | 3.68 | 24.23 | p<0.00 |
| ROL | 7.86 | 6.9 | 9.47 | 0.88 | 11.20 | 7.07 | 4.26 | 10.11 | 1.57 | 22.10 | p<0.03 |
| ROW | 6.05 | 5 | 7.99 | 0.86 | 14.21 | 5.01 | 3.1 | 7.73 | 1.19 | 23.75 | p<0.00 |
| ABL | 30.33 | 22.42 | 42.01 | 4.14 | 13.65 | 17.27 | 8.76 | 28.52 | 6.13 | 35.50 | p<0.00 |
| ABW | 18.42 | 14.34 | 21.99 | 2.52 | 13.68 | 13.01 | 7.36 | 23.92 | 3.39 | 26.06 | p<0.00 |
| TEL | 11.69 | 9.9 | 13.84 | 1.18 | 10.09 | 9.12 | 6.33 | 11.79 | 1.46 | 16.01 | p<0.00 |
| TEW | 9.96 | 8.46 | 11.46 | 0.76 | 7.63 | 7.34 | 4.81 | 10.63 | 1.44 | 19.62 | p<0.00 |

Using the T-test, a statistically significant difference of the analyzed morphometric parameters has been found between the sexes (Table 2), which is explained by the pronounced sexual dimorphism of this type of stone crayfish. Sexual dimorphism was found, with higher mean values for most males (Table 1), due to allometric growth of adult individuals (Jelić, 2014), recorded in previous morphological studies of crayfish (Grandjean et al., 1997; Grandjean & Souty-Grosset, 2000; Sint et al., 2005, 2006, 2007; Trožić-Borovac et al., 2007; Maguire

& Dakić, 2011; Rajković, 2012; Đuretanić, 2019). Maguire's (2002) statements support our data in which statistically significant differences were established between males and females concerning body weight, width of the first abdominal pleura, length and width of the right claws and that represents the main sex-dimorphic character.

The results obtained for the length-weight (TBL/W, W/CLL) and width-length (CPW/TBL) relationships, along with some descriptive statistics, are given in Table 2. Correlation tests have shown that there are significant positive correlations between the measured parameters for both males and females.

Tab. 2. Descriptive statistics and estimated parameters of weight-length and biometric relationships for both sexes of *A. torrentium* from the Mlinska River. (TBL, total length (mm); W, weight (g); CLL, chela length (mm); CPW, carapace width (mm))

| Drainage area | Relation | | Sex | |
|---------------------|----------|---------|---------------------|---------------------|
| | | | M | F |
| Mlinska River Basin | TBL/W | y | $0.9881x^{-60.602}$ | $0.3989x^{-17.131}$ |
| | | R^2 | 0.7644 | 0.8222 |
| | | Correl. | 76.44 | 82.22 |
| | W/CLL | y | $0.6666x^{+16.683}$ | $1.5352x^{+11.101}$ |
| | | R^2 | 0.8760 | 0.9661 |
| | | Correl. | 87.60 | 96.61 |
| | CPW/TBL | y | $0.3x^{-4.9167}$ | $0.337x^{-5.2799}$ |
| | | R^2 | 0.7216 | 0.8411 |
| | | Correl. | 72.16 | 84.11 |

According to the values of the calculated indices, it is obvious that the males are in better condition, which corresponds to findings of the research by Lindquist & Lahti (1983) and Streissl & Hödl (2002). Furthermore, according to the proposal of Streissl & Hödl (2002), it is possible that this difference is caused by larger claws in males in comparison to females. Research conducted by Streissl & Hödl (2002) reveals a relationship between the condition of the stone crayfish body and characteristics of their habitat. These statements coincide with our results, if we take into account the characteristics of the above-mentioned location. The sampling locality is situated in the natural part of the stream, separated from the inhabited area, without any anthropogenic impact. Indisputably, the life of stone crayfish *A. torrentium* is directly dependent on the specific characteristics of the aquatic ecosystem. Heterogeneity of habitat conditions, quality of the waterbody substrate as well as the absence of contaminants are prerequisites for the existence and survival of the species (Troschel, 1997).

Tab. 3. Values for the condition factor for stone crayfish specimen

| Statistical parameters | | CF | | | |
|------------------------|-------|-------|-------|-------|-------|
| Sex | Mean | Min | Max | SD | CV |
| M | 0.033 | 0.021 | 0.050 | 0.009 | 27.27 |
| F | 0.028 | 0.017 | 0.045 | 0.007 | 25 |
| Statistical parameters | | CC | | | |
| Sex | Mean | Min | Max | SD | CV |
| M | 0.367 | 0.158 | 0.786 | 0.149 | 40.60 |
| F | 0.258 | 0.087 | 0.487 | 0.1 | 38.76 |

Conclusion

Morphometric features of the Stone crayfish in the Mlinska River were monitored. Twelve morphometric features and two fit factors were analyzed on 62 specimens (22 males and 40 females). The values obtained for morphometric features of the stone crayfish in the area of Čelinac partially match the known scope of variability and represent the first data for the investigated area. Presence of statistically significant difference among adult male specimens compared to females is justified by emphasized sex dimorphism in the stone crayfish. Data presented in this paper can serve as a basis for further research of stone crayfish in this area.

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Морфометријске карактеристике и кондициони индекси поточног рака *Austropotamobius torrentium* (Shrank, 1803) из Млинске ријеке

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Сажетак

Заштићена врста, поточни рак (*Austropotamobius torrentium*) налази се на IUCN листи угрожених врста, класификована као недовољно позната врста, једна је од пет европских врста из породице Astacidae које живе у слатководним екосистемима Босне и Херцеговине. Циљ овог рада био је испитати варијабилност одабраних морфометријских карактеристика мужјака и женки поточног рака врсте *A. torrentium* из горњег тока Млинске ријеке. Ракови су узорковани током јуна 2021. године и тада су ухваћене 62 јединке (22 мужјака и 40 женки). Ракови су испитивани у свјежем стању на терену и у лабораторији. Обрада је обухватила мјерење 12 морфометријских карактера: укупна дужина тијела (TBL), тежина (W), дужина клијешта (CLL), ширина клијешта (CLW), дужина карапакса (CPL), ширина карапакса (CPW), дужина рострума (ROL), ширина рострума (ROW), дужина и ширина трбуха (ABL, ABW) и дужина и ширина телзона (TEL, TEW). Просјечна дужина тијела \pm SD износила је $79,05 \pm 7,35$ mm код мужјака и $60,54 \pm 10,03$ mm код женки. Просечна забиљежена тежина тијела \pm SD била је $17,51 \pm 8,3$ g код мужјака и $7,02 \pm 4,41$ g код женки. Примјеном методе линеарне регресије утврђена је позитивна корелација између дужине тијела и тежине тијела (σ $R^2=0,7644$; ρ $R^2=0,8222$), тежине тијела и дужине клијешта (σ $R^2=0,876$; ρ $R^2=0,9661$), као и ширине карапакса и дужине тијела (σ $R^2=0,7216$; ρ $R^2=0,8411$). Применом Т-теста утврђена је статистички значајна разлика анализираних морфометријских карактера између полова, што се објашњава израженим полним диморфизмом код поточног рака. Израчунате вредности за Фултонов кондициони фактор и Константу декаподног рака указују да су мужјаци у бољој кондицији, што је у складу са резултатима досадашњих истраживања у Европи. Подаци представљени у овом раду могу послужити као основа за даља истраживања врсте *A. torrentium* на овом подручју.

Кључне ријечи: поточни рак, мужјаци, женке, морфометријске карактеристике

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