MORPHOLOGICAL VARIABILITY AND SEXUAL DIMORPHISM OF NOBLE CRAYFISH Astacus astacus FROM THE BALKANA LAKE

RAJKO ROLJIĆ¹, VERA NIKOLIĆ², NEBOJŠA SAVIĆ³
¹Genetic Resources Institute University City, University of Banja Luka, Banja Luka, Bosnia and Herzegovina
²Faculty of Biology, University of Belgrade, Belgrade, Serbia
³Faculty of Agriculture University City, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

ABSTRACT
This paper presents the information about morphological variability and sex dimorphism of the Noble crayfish (Astacus astacus) in the area of the Balkana Lake in Mrkonjic Grad. The crayfish were caught by hand made baited traps from October 9th 2018 until May 31st 2019. A total of 58 crayfish were caught, of which 38 males and 20 females. The eight morphometric characteristics: body weight (W), body length (TBL), claw length (CLL), cephalothorax length (CFL), carapace width (CPW), abdomen length (ABL), rostrum length (ROL) and rostrum width (ROW) were measured, both in males and females. Also, the body condition was determined for all specimens. The results of morphometric characteristics partially matched into the already known range of variations. These data represent first ones for the observed area. The t-test showed that there were significant differences between the sexes in W, TBL, CLL, CFL and CPW which are explained by the emphasized sex dimorphism of the noble crayfish.

Keywords: Noble crayfish, Morphometric features, Sex dimorphism, The Balkana Lake.

INTRODUCTION
The morphometric features of crayfish are the basic criteria for specifying the taxonomic status of the species. Precise crayfish taxonomy involves the application of these methods combined with anatomical, morphological, cytogenetic, biochemical, physiological, ecological, evolutionary, and other methods (Vukovic et al., 1978). A study of morphometric features of certain crayfish species from various water ecosystems to determine their systematic position and status was conducted by numerous authors such as Trozic-Borovac et al. (2007), Trozic-Borovac (2012a, 2012b) and Rajkovic (2012). Maguire (2010) emphasizes the importance of research of morphometric feature variability in species from the genus Astacus and Austropotamobius in order to produce an efficient key for determination, given the present difficulties in determination thereof.

There is a lack of information available of morphometric features of for species Astacus astacus from numerous places within their distribution area. The Noble crayfish lives in rivers and lakes with muddy or gravel bed, and seeks for the shelter by the coast covered with aquatic vegetation, or digs the holes (Maguire, 2010). This species is present and dominate in Bosnia and Herzegovina (Trozic-Borovac, 2011) and neighboring countries such as Serbia (Simic et al., 2008), Croatia (Maguire & Gottstein-Matoce, 2004), Montenegro (Rajkovic, 2012) and Kosovo and Metrovia (Zivic et al., 2014).

A significance of morphometric study of crayfish from the A. astacus species is justified by the fact that it is autochthonous European species being on the IUCN list of endangered species. According to IUCN criteria the said species was assigned to the VU (Vulnerable) category for the European region (Edsman et al., 2010) and is listed on the national Red List of Bosnia and Herzegovina as vulnerable species.

This study aims to determine variability of selected morphometric features of male and female specimens of the Noble crayfish from the Balkana Lake in Mrkonjic Grad.

MATERIALS AND METHODS
The Tourist and Recreational Center “Balkana” is located in the northwest of the Municipality of Mrkonjic Grad, 4-5 km away from the city and next to the motorway Jajce- Mrkonjic Grad-Bihac (formerly known as the “AVNOJ road”). It is situated at 750 m above sea level, in the foothill of the Lisina Mountain (1,650 m altitude).

What makes this complex very special are the Balkana Lakes at the altitude of 750 m. Surface of the small lake (downstream) is 10.800 m² and surface of the big lake (upstream) is 42.300 m² (Figure 1, 2). The lakes were formed by natural depression which volume increased when a dam was constructed. The lake is separated in two parts by a lateral embankment. Principal filling of the lake is made by two smaller watercourses (Cijepalo from the right side composed of two springs - the Kovacko and Lazino spings from the left side of the thermal spring) that flows into the Big Lake and airfoil spring at
the bottom of the Big Lake. The Crna Rijeka river flows out from the Lake (Crnogorac et al., 2013).

Figure 1. The Small Lake of Balkana.

Field studies were conducted in the period from October 9, 2018 until May 31, 2019. Sampling period included three seasons of crayfish activity: autumn, winter and spring. Sampling was carried out in two locations – one location was in the Malo jezero lake and the other location was the Veliko jezero lake. Crayfish were sampled with baited LiNi traps (Westman et al., 1978). We left traps overnights and collected crayfish the next morning. Then, 58 crayfish specimens were collected. Time of collection, apparent physical defects, signs of illness and parasites were recorded for each specimen. Afterwards, sex was determined for each collected specimen and morphometric features were measured.

Morphometric features such as: total body length (TBL), claw length (CLL), carapace length (CPL), carapace width (CPW), abdomen length (ABL), rostrum length (ROL), rostrum width (ROW) and values of body weight (W) were analyzed.

The fit/fitness coefficient was also calculated: Fulton’s Conditions Factor (Ricker, 1975) and Crayfish Constant (Adegboye, 1981).

Fulton’s Conditions Factor (FCF):

\[ FCF = \frac{W}{TBL} \]

Crayfish Constant (CC):

\[ CC = \frac{W}{TBL \times CPL \times CPW} \]

Where: W – total weight, TBL – total length

Each specimen was measured with caliper to the nearest 0.02 mm and weight to the nearest 0.01 g with technical balance (type “Kern” PFB Version 2.2). Obtained values of morphometric features were processed statistically (minimum, maximum, mean value, standard deviation and variation coefficient) by applying statistical program Microsoft Office Excel 2007, interpreted and compared to available data from related literature.

Database with photos was created and each specimen was captured from dorsal and ventral side. Such photos can serve to analyze body color and to identify re-collected specimen.

RESULTS

There were 58 analyzed specimens of which 38 (in particular 65.52%) were male and 20 (in particular 34.48%) were female (sex ration close to 1.9 : 1) (Figure 3).

Figure 3. *Astacus astacus* – noble crayfish (male left, female right).

Measurement results of morphometric parameters of the Noble crayfish specimen from the Balkana Lake are given in tables as mean value, minimum (min), maximum (max), standard deviation (SD) and coefficient of variation (CV) (Table 1, 2).

The values obtained for male specimens show that the average body weight was 27.99 g, an average body length was 85.57 mm, the claw length was 36.25 mm, the cephalothorax length was 41.57 mm, the carapace width was 23.94 mm, the abdomen length was 36.78 mm, the rostrum length was 11.19 mm and the rostrum width was 5.5 mm. Based on these values,
the standard deviation (SD) was obtained and had the highest value for body length (17.46), slightly smaller value for the total body length (17.2), the claw length (9.63), the abdomen length (9.03), the cephalothorax length (7.82), the carapace width (5.82), and the least value was for the rostrum length (2.75) and width (0.38). The only steady feature at male specimens (CV < 10%) showed a morphometric feature (ROW) (CV = 6.87%). CFL (CV = 18.8%) falls into a low variable (10% - 20%). Most of the analyzed morphometric features were moderately variable (CV 20% - 30%, Table 1). Morphometric feature W (CV = 62.37%) at male specimens is the only feature that shows high variability (CV > 30%) (Table 1).

Table 1. Descriptive statistics - mean value, standard deviation, ranges of measured morphometric characteristics for males of species Astacus astacus from the Balkana lake.

| Statistical parameters | Mean | Min | Max | SD  | CV      |
|------------------------|------|-----|-----|-----|---------|
| W                      | 27.99| 5.8 | 72  | 17.46| 62.37   |
| TBL                    | 85.77| 56.35| 114.14| 17.2| 20.1    |
| CLL                    | 36.25| 18.8| 56.71| 9.36| 26.56   |
| CFL                    | 41.57| 28.16| 51.79| 7.82| 18.8    |
| CPW                    | 23.94| 13.7| 39.1 | 5.82| 24.3    |
| ABL                    | 36.78| 18.9| 43.37| 9.03| 24.55   |
| ROL                    | 11.19| 7.39| 15.81| 2.75| 24.54   |
| ROW                    | 5.5  | 5.08| 6   | 0.38| 6.87    |

The values obtained for female specimens show that the average body weight was 17.09 g, an average body length was 74.34 mm, the claw length was 24.81 mm, the cephalothorax length was 31.34 mm, the carapace width was 18.56 mm, the abdomen length 32.15 mm, the rostrum length was 11.54 mm and the rostrum width was 5.46 mm.

Based on these values, the standard deviation (SD) had the highest value for the total body length (16.38) and the body weight (11.54), slightly smaller for the abdomen length (8.78), the cephalothorax length (8.0), the claw width (6.64) and the carapace width (4.64) while the least value was for the rostrum length (1.99) and the width (0.58). The observed morphometric features are moderately variable (CV 20% - 30%), while a morphometric feature W (CV = 63.17%) was the only highly variable (CV > 30%). None of analyzed features (Table 2) was a steady morphometric feature in female samples (CV < 10%).

Males and females differ significantly (p < 0.05) in five morphometric features W (p = 0.009), TBL (p = 0.013), CLL (p = 0.000), CFL (p = 0.025) and CPW (p = 0.001) (Table 3). There is no statistically significant difference (p > 0.05) for ABL (p = 0.195, ROL (p = 0.403) and ROW (p = 0.441) (Table 3). By comparing the mean values of the abdomen length and rostrum width and length among the sexes, the Noble crayfish males have a higher mean value for the previously mentioned morphometric features compared to females, but that difference is not significant in statistical terms.

Table 2. Descriptive statistics - mean value, standard deviation, ranges of measured morphometric characteristics for females of species Astacus astacus from the Balkana lake.

| Character | Mean | Min | Max | SD  | CV      |
|-----------|------|-----|-----|-----|---------|
| W         | 17.09| 6.5 | 48  | 11.54| 67.5    |
| TBL       | 74.34| 46.1| 105.6| 16.38| 22.03   |
| CLL       | 24.81| 12.24| 37.44| 6.64| 26.75   |
| CFL       | 31.34| 19.19| 40.8 | 8.0 | 25.52   |
| CPW       | 18.56| 11.6| 41.47| 8.78| 27.3    |
| ABL       | 32.15| 17.92| 41.47| 8.78| 27.3    |
| ROL       | 11.54| 8.35| 14.0 | 1.99| 17.25   |
| ROW       | 5.46 | 4.4 | 6.0  | 0.58| 10.66   |

The correlation between total body length and weight of males (90.43%) and females (81.68%) was calculated (Figure 4). Which shows that body weight increases as the body length increases.

Figure 4. The ratio of body length and body weight of males and females of Noble crayfish.

The correlation between body weight and the claw length in both males, females is made and we notice a positive correlation, which means that the claw growth accompanies the body weight. The correlation coefficient is 93.42% in males and 93.14% in females (Figure 5).

Based on the correlation coefficient by applying regression analysis, it is observed that the coefficient is highly significant in statistical terms between the carapace width and the body length. The correlation coefficient is 97.76% and 92.2% in females (Figure 6).
Figure 5. Relation of body weight and claw length in males and females of Noble crayfish.

Figure 6. The ratio of body length and width of a carapace in males and females of Noble crayfish.

The least CC is 0.228 and the highest CC is 0.401 (for females). The mean value is 0.309 for females and 0.386 for males. The least FCF is 0.022 and the highest is 0.039 (for females). The mean value is 0.029 for females and 0.037 for males (Table 4).

Table 4. Values for condition factor for Noble crayfish specimen.

| Statistical parameters | FCF       |
|------------------------|-----------|
| Sex                    |           |
| M                      | 0.037     |
| F                      | 0.029     |
| Min                    | 0.026     |
| Max                    | 0.055     |
| Mean                   | 0.039     |
| SD                     | 0.007     |
| CV                     | 22.64     |

DISCUSSION

Sex ration between male and female was 1.9:1 (males : females). A higher degree of male presence in the sample can be correlated with the fact that five female specimens with brooding eggs were sampled during the study period, pointing out to a season of a lower female activity (Rajkovic, 2012). Having information on the sex ratio in population is significant because it tells us about the health and stability of the population (Jurkovic, 2016).

Population of the noble crayfish at the observed location has relatively stable age structure. At the explored location on the Balkana Lake, the largest group was 101 - 120 mm long with a 60% share in population. The longest male body was 114.4 mm, and the longest female body was 105.6 mm that corresponds to the range from 80 to 120 mm applicable to the Noble crayfish mentioned in the related literature (Obradovic, 1988), and values recorded by Trozic-Borovac et al. (2012b) and Rajkovic (2012). The heaviest male was 72 g and the heaviest female was 48 g. The higher average male weight can be attributed to the fact that males has bigger claws that contributes to their higher body weight compared to females (Jurkovic, 2016).

Morphometric features of males and females from the A. astacus species found in the Balkana Lake considerably differ in statistical terms (p < 0.05), where higher mean values were noticed for most of male features (Table 3). Obtained results can be correlated with the expressed sex dimorphism of crayfish from this species, where males are bigger than females (Trozic-Borovac et al., 2012b; Rajkovic, 2012).

According to values of calculated factors we notice that males are more fit. A study of the Noble crayfish specimen fit factor in the Praca river supports the foregoing (Trozic-Borovac et al., 2012b).

CONCLUSION

Morphometric features of the Noble crayfish (Astacus astacus) in the Balkana Lake were monitored.

Seven morphometric features and two fit factors were analyzed on all 58 specimens (38 males and 20 females).

Obtained values for morphometric features of the Noble crayfish in the area of Mrkonjic Grad partially match the known scope of variability and represent first data for the investigated area.

Presence of statistically significant difference among adult specimens in body mass, total body length, the claw length, the cephalothorax length and the carapace width at males compared to females is justified by emphasized sex dimorphism in the Noble crayfish.

Data presented in this paper can serve as a basis for further research of Astacus astacus in this area.

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