

Ecological conditions and phenotypic characteristics of *Austropotamobius torrentium* (Schrank, 1803) in the Subotica and Toplik Rivers

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Abstract

Austropotamobius torrentium (Schrank, 1803) lives in two watercourses, namely Subotica (Sana) and Toplik (Tilava). The influence of the ecological conditions of these rivers on individuals of this species is analyzed based on 22 morphometric parameters. The assessment of abiotic conditions was done through the measurement of physical and chemical water parameters and the analysis of the composition of phytobenthos and macrozoobenthos. Sampling was carried out in June, September, and October 2021. Water quality at the investigated localities (on the basis of abiotic and biotic parameters) is oligo to betamesosaprobic. A total of 37 crayfish were analyzed (20 from Subotica and 17 from Toplik). Female crayfish have shown significant differences in the head width (HEW) and abdominal thickness (ABH). Males differed significantly in a number of parameters, including the weight (W), head shape (HEL and HEW), rostrum length (ROL), carapace width (CPW), and abdominal shape (ABH and ABL). Higher values of the indicated parameters have been found in the males from the Subotica River. The obtained results are influenced by different ecological conditions of their habitats. The Subotica River is a larger watercourse in the lowland belt with a large number of microhabitats, and the Toplik is a small river in the mountain belt, with uniform sediment and a small number of ecological niches of the analyzed species. The general ecological conditions in the water of both watercourses are also affected by

aquaculture facilities, where wastewater has a direct influence on the distribution of *Austropotamobius torrentium* individuals in the watercourses of the Subotica and Toplik Rivers. For more detailed and complete data, it is necessary to analyze a larger number of individuals in both populations, and to include additional morphometric-meristic parameters and molecular-genetic analyses.

Key words: stone crayfish, water quality, fish farm, morphometric characteristics.

Introduction

Austropotamobius torrentium (Schrank, 1803) is a species of freshwater crayfish in the Astacidae family. It dominates the Black Sea basin in central and south-eastern Europe (Holdich, 2002; Kouba et al., 2014), and also inhabits parts of the Adriatic basin. According to previous research (Streissl and Hödl, 2002), this species prefers lower water temperatures, sediment large stones, and watercourses at higher altitudes (hilly to mountainous). Due to the increasing degradation of watercourses, the threat to its habitat has been emphasized for many years (Füreder et al., 2010; Maguire et al., 2017). Consequently, it is considered an endangered species and is listed in Appendix III of the Berne Convention, in Appendix II and V of the Habitats Directive (92/43 / EEC), it is listed as a data deficient species (DD) on the IUCN Red List of Threatened Species (Füreder et al., 2010). It is registered throughout the entire Balkan peninsula, i.e., in Bosnia and Herzegovina (Karaman, 1963; Trožić-Borovac, 2011; Roljić et al. 2022), Croatia (Maguire et al., 2017), Slovenia (Bedjanič, 2004), Serbia (Simić et al., 2008), Republic of Macedonia (Slavevska-Stamenković et al., 2017), Montenegro (Simić et al., 2008; Rajković, 2012), Albania (Mrugala et al., 2017), Greece (Vlach, & Valdmanová, 2015), Bulgaria (Hubenova et al., 2010), and Turkey (Harhõglu & Güner, 2006). In Bosnia and Herzegovina and Croatia, this is the most widespread species of crayfish from the Astacidae family. Morphometric characteristics vary depending on changes in environmental conditions (Sint et al., 2007). Considering its global status, it is of great importance to review and determine the parameters which will contribute to a deeper understanding of biology of this species.

Material and methods

The Subotica River is a right tributary of the Gomjenica River (the Sana River). The spring is located in the locality Strmec at an altitude of 520 m. GPS

coordinates of the studied area are 44.783092°N, 16.960986°E elevation 195 m (Fig. 1). The Toplik stream is a right tributary of the Tilava River. The sampling location is in the town of Toplik and starts from the spring downstream and the Toplik pond. GPS coordinates of the part of the stream where the crayfish were found are 43.83622° N and 18.231966° E, elevation 551 m (Fig. 1). There is a fish farm upstream on the Subotica River, and a smaller fish farm on Toplik is located at the research level.



Fig. 1. Location of the investigated localities

Physico-chemical water parameters as well as the phytobenthos and macrozoobenthos composition were analyzed. The physical and chemical parameters were measured in the field with an oximeter (Oxi 3205 Set 3 2BA103 WTW) and in the laboratory at the Faculty of Science and Mathematics in Banja Luka. The following parameters were analyzed: water temperature, oxygen concentration, saturation, nitrogen, phosphorus, BOD5, and electrical conductivity. Sampling of phytobenthos was carried out in the Subotica River using standard methodology, and further treatment of samples and determination was carried out in the laboratory of the Faculty of Science and Mathematics in Banja Luka. Sampling of macrozoobenthos was carried out using a net with a 0.5 mm diameter mesh. For the purposes of water quality evaluation, the Pantle-Buck saprobic index was applied ($SI = \sum (h-s)/h$). Sampling was done during September and October 2021 (the Subotica River) and from June to September 2021 in the Toplik River. Based on the obtained abiotic and biotic data, in order to determine the degree of similarity/ difference between sites, the Bray-Curtis index of similarity was applied (software Primer 5). Twenty one morphological characteristics were recorded for each crayfish (Sint et al. 2005). After the measurements, the individuals were returned to the watercourse. All data were normalized for size

by dividing them with the corresponding postorbital length (POL) and only normalized values were used in the analysis. The Fulton's condition factor was checked according to the formula: $FCF = W/TL^3$ and the decapods constant according to the formula: $CC = W/(TL \times CPL \times CPW)$. A calliper with a precision of 0.02 mm was used to measure all morphometric characteristics. Technical scales (Kern PFB Version 2.2) with a precision of 0.01 g was used to measure the body weight. The values obtained for morphometric parameters were analyzed by using the IBM SPSS Statistics 25 software and Primer 5.

Results and discussion

A high concentration of oxygen was determined in the Subotica (9.83 mg/l) and Toplik Rivers (9.80 mg/l). All analyzed parameters (TP, TN, nitrates, nitrites) were within the limits for clean waters with a small inflow of allochthonous organic matter (Table 1.).

Tab. 1. Presentation of the values of the physical/ chemical parameters of water at the investigated localities of the Subotica and Toplik Rivers

Parameter	Unit	Toplik	Subotica	Quality category
Electrical conductivity	$\mu\text{S}/\text{cm}$	280	488	I and II
Dissolved O ₂ concentration	mg/l	9.80	9.83	I
Saturation O ₂	%	90.2	95.1	I
BOD5	mg/l	1.25	1.89	I
pH		8.01	8.36	I
Total phosphorus TP	(g/m ³ P)	-	<0.01	I
Nitrite	(g/m ³ N)	-	0.004	I
Nitrate	(g/m ³ N)	-	0.9	I

The phytobenthos composition analysis showed the dominance of Bacillaryophicea with 10 taxa which indicates a good ecological condition, based on the value of the saprobic index (SI). The presence of Cyanobacteria and Chlorophyta in the Subotica River is the result of pollution and influx of allochthonous organic matter. These species are a food source for herbivores in the water. The composition of the macrozoobenthos of the Subotica and Toplik Rivers is dominated by the preimaginal stages of the Ephemeroptera and Trichoptera (ET group). The dominance of the amphipod *Gammarus balcanicus* (Schäferna, 1922) was registered in the samples of the

Toplik stream. According to the saprobic index values ($S=1.8$ and 1.72), parts of the watercourse in the investigated sections are in the oligo to

betamesosaprobic category (II quality category) based on the Regulation on the classification and categorization of watercourses (Sl. Glasnik RS 41/01). By applying the Bray-Curtis index of similarity, a high degree of similarity (82.44%) between the research sites was determined.

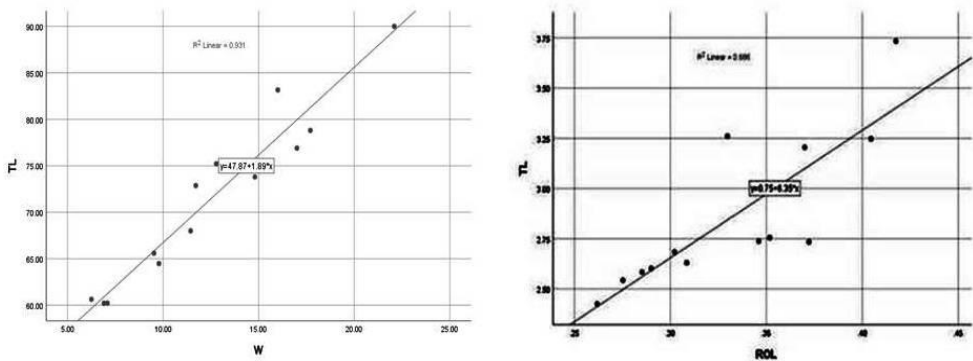
For the purposes of morphometric analysis, 20 individuals from the Subotica River were used (14 ♂ and 6 ♀), while seventeen individuals were analyzed from the Toplik River, ten males (58.22%) and seven females (41.78%). Due to the gender dimorphism being significant ($p < 0.01$) for most parameters (Grandjean et al., 1997), the first step of the analysis was to compare the measured morphometric parameters between the sexes. Based on the comparison of all females (13 individuals) and all males (24 individuals) from both watercourses, a strong statistically significant difference ($p < 0.01$) was found in the total body weight (TL), cheliped length (CLL), cheliped shape (CPL, CFL, CLW, CLH), abdominal width (ABW), rostrum length (ROL), rostrum width (ROW), and telson shape (TEL and TEW), and in value of the condition index. According to the T-test results, the females from two sites/ river have shown a statistically significant difference in the head width (HEW) and abdominal height (ABH) (Table 2.). The average body length (TL) in the females from the Subotica River was 78.44 ± 7.60 mm and the maximum length was 90 mm. The female individuals from the Toplik River had a slightly shorter body length (65.60 ± 6.17 mm), while the maximum value was 75.21 mm. Higher weight values were measured in the individuals from Subotica. The fitness index values do not differ statistically (slightly higher values in the females from Toplik), which indicates similar ecological conditions in the examined watercourses.

Tab. 2. Descriptive statistics of morphometric parameters of female *Austropotamobius torrentium* from the Subotica and Toplik Rivers for which a significant difference and fitness index was determined

Par.	Subotica				Toplik				Sig
	Mean	Min	Max	SD	Mean	Min	Max	SD	
TL	78.44	68	90	7.6	65.61	60.21	75.21	6.17	>0.05
W	16.51	11.44	22.09	3.5	9.41	6.25	12.78	2.51	>0.05
HEW	12.59	10.23	13.9	1.24	8.87	6	13.53	3.48	<0.01
ABH	18.37	9.3	34.06	11.5	7.71	7.1	9.01	0.72	<0.01
FCF	0.000033	0.000024	0.000037	0.000005	0.00003	0.00003	0.00004	0.000003	>0.05
CC	0.0003	0.00024	0.00036	0.000048	0.0003	0.0002	0.0003	0.000033	>0.05

Abbreviations used are measured morphometric parameters (Par.); total length (TL); weight (W); head width (HEW), and abdominal height (ABH).

A significant correlation in the female crayfish was found for the body length and weight, TL: W $r = 0.985$ $p < 0.01$ as well as for TL:ROL $r = 0.828^{**}$ $p < 0.01$ (Graph 1.).



Graph 1. Correlation of morphometric parameters (TL:W and TL:ROL) of the female crayfish from the Subotica and Toplik Rivers

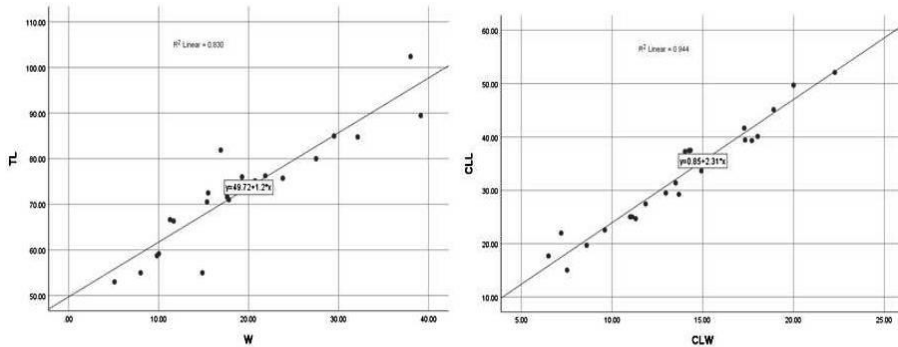
The males from the Subotica River had a larger average body length (75.79 ± 13.06 mm), compared to those from the Toplik River (68.40 ± 8.05 mm). The difference in the body length is not statistically significant. However, the male individuals from the two studied watercourses have shown a statistically significant difference ($p < 0.01$) in the weight, head width (HEW), abdominal height (ABH), and telson length (Table 3). Additionally, the males from Toplik have had a higher fitness index value, while all other parameters have higher values in the males from the Subotica River. The fitness index values, though, have not shown a statistically significant difference. The individuals differed significantly in the length of the rostrum (ROL), head length (HEL), areolar length (ARL), width of the carapace at the hind edges (CEW), and abdomen length (ABL).

Tab. 3. Descriptive statistics of morphometric parameters of male *Austropotamobius torrentium* from the Subotica and Toplik Rivers for which a significant difference and fitness index was determined

Par.	Subotica				Toplik				Sig
	Mean	Min	Max	SD	Mean	Min	Max	SD	
TL	75.79	53	102	13.06	68.4	54.9	76.2	8.05	>0.05
W	21.49	5.1	39.1	10.56	16	9.81	21.85	4.45	<0.01
ROL	8.77	6.8	10.9	1.46	8.26	5.59	10	1.16	<0.05
HEL	16.71	10.8	23.3	3.22	15.25	12	18.61	2.49	<0.05
HEW	12.21	8.6	15.7	1.87	11.79	6.15	15.37	3.45	<0.01
ARL	13.67	7.9	16.7	2.42	12.05	8.99	14.17	2.1	<0.05
CEW	14.09	9.7	17.6	2.24	12.28	9.01	15.11	2.15	<0.05
ABH	13.71	7.4	34.13	7.84	7.41	5.38	11.02	1.91	<0.01
ABL	30.69	21	49	6.65	23.16	19.97	25	2.08	<0.05
TEL	10.64	7.9	13.3	1.56	9.66	5.2	12.02	1.85	<0.01
FCF	0.0003	0.00017	0.00037	0.000049	0.00036	0.0004	0.00005	0.000003	>0.05
CC	0.0005	0.000031	0.000055	0.000008	0.000049	0.00005	0.000008	0.000033	>0.05

Abbreviations used are measured morphometric parameters (Par.); total length (TL), weight (W), rostrum length (ROL), head length (HEL), head width (HEW), areolar length (ARL), width of the carapace at the hind edges (CEW), abdominal height (ABH), abdomen length (ABL), and telson shape (TEL).

A high correlation in the male crayfish has been found between the total body length and weight (TL:W, $r=0.911$, $p<0.01$) and for the claw length CLL and claw width CLW $r=0.972$ $p<0.01$ (Graph 2.).



Graph 2. Correlation of morphometric parameters (TL:W and CLL:CLW) of the male crayfish from the Subotica and Toplik Rivers

Morphometric characteristics are an important parameter for understanding the biology and ecology of this species. Comparison in different habitats is important for determining the parameters that condition/ limit the growth of populations of this species (Lovrenčić et al., 2020). In this study, the males were longer than the females and had larger chelipeds, and the abdomen was wider and longer in females, which was also found in the crayfish populations from other watercourses (Grandjean and Souty-Grosset, 2000; Streissl and Hödl, 2002; Pârvulescu, 2010). At the research sites, the presence of fish farms was found, which affects the spread of crayfish populations. Fish pools limit the movement and distribution of individuals along the stream. According to previous data, the species is distributed in streams with stable stony sediment, at higher altitudes, and low water temperature (Machino & Füreder, 2005; Füreder et al., 2006; Vlach et al., 2015; Kouba et al., 2014; Maguire et al., 2017), which was also confirmed by these studies. In Europe, it has been emphasized that the lotic habitats of this species are threatened by anthropogenic influences, and by the introduction of invasive species of crustaceans (Maguire et al., 2017).

Conclusion

Ecological conditions (sediment, water quality, anthropogenic influence, etc.) have a direct impact on the existence and phenotypic characteristics of the crayfish in the Subotica and Toplik River. High oxygen concentrations, low temperature, sediment, and good water quality in the studied watercourses resulted in relatively high fitness indexes in the individuals. The negative impact of fish farms on the investigated localities is visible through the development of

cyanobacteria and the presence of fish food residues and feces, which will eventually result in changes in water quality and the survival of the crayfish population. The morphometric parameters as well as the fitness index are good tools in determining the condition of the crayfish populations. However, extensive molecular phylogenetics and phylogeographic research will provide a more complete and objective overview of this species in the researched area and beyond.

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Еколошки услови и фенотипске карактеристике *Austropotamobius torrentium* (Schrank, 1803) у водотоцима Суботица и Топлик

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Сажетак

Austropotamobius torrentium (Schrank, 1803) живи у два водотока Суботица (Сана) и Топлик (Тилава). Утицај еколошких услова анализиран је кроз мјерење 22 морфометријска параметра код 37 јединки поточног рака. Пројена еколошких услова извршена је мјерењем физичких и хемијских параметара воде, те анализу састава фитобентоса и макрозообентоса. Узорковање је извршено у јуну, септембру и октобру 2021 године. Утврђени квалитет воде (на основу абиотичких и биотичких параметара) на истраживаним локалитетима је олиго до бетамезосапробан. Морфометријска анализа извршена је на 37 јединки (20 из ријеке Суботице и 17 из Топлика). Женке поточног рака из два водотока, показују значајне разлике у ширини главе (HEW) и дебљини абдомена (ABH). Мужјаци се значајно разликују по бројним параметрима, укључујући тежину (W), облик главе (HEL i HEW), дужину роstrума (ROL), ширину оклопа (CPW) и облик абдомена (ABH i ABL). Веће вриједности наведених параметара утврђене су код мужјака из ријеке Суботице. На добивене резултате утјечу различити еколошки увјети њихових станишта. Ријека Суботица је већи водоток у низијском појасу са великим бројем микростаништа, а Топлик је рјечица у планинском појасу, с уједначеним седиментом и малим бројем еколошких ниша. На опће еколошке услове у води оба водотока утичу и објекти аквакултуре, чије отпадне воде имају директан утицај на дисперзију јединки *Austropotamobius torrentium* у водотоцима Суботица и Топлик. За детаљније и потпуније податке потребно је анализирати већи број јединки у обје популације, те укључити додатне морфометријско-меристичке параметре и молекуларно-генетичке анализе.

Кључне ријечи: камени рак, квалитет воде, рибњак, морфометријске карактеристике

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