Zoodiversity, **55**(4): 285–294, 2021
DOI 10.15407/zoo2021.04.285

UDC 595.384.1(497.6)

NEW FINDINGS OF WHITE CLAWED CRAYFISH, *AUSTROPOTAMOBIUS PALLIPES* (DECAPODA, ASTACIDAE), AND PECULIARITIES OF ITS SPATIAL DISTRIBUTION IN NERETVICA (BOSNIA AND HERZEGOVINA)

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New Findings of White Clawed Crayfish, *Austropotamobius pallipes* (Decapoda, Astacidae), and Peculiarities of its Spatial Distribution in Neretvica (Bosnia and Herzegovina). Afanasyev, S., Lietitska, O., Golub, O., Iarochevitch, O., Mudra, K., Marushevska, O. — At the territory of Bosnia and Herzegovina *Austropotamobius pallipes* (Lereboullet, 1858) is present mainly in the Neretva basin, partly in the Una and Tsetina river basins (Trožić-Borovac et al., 2012). This species was registered at elevations from 180 to 1024 m a. s. l., but most habitats are located at elevations from 300 to 600 m a. s. l. Based on indirect data, one can state that more than 2/3 of the crayfish population in the Neretva basin is concentrated in the Neretvica basin. Our studies showed that the crayfish are very irregularly distributed in the Neretvica basin. The crayfish are almost absent at well-flushed habitats without CPOM (deposits of coarse particulate organic matter, e. g., fallen leaves), as well as at habitats with monotonous boulders or gravel-sand bottom. The study of the size structure of the crayfish population showed the presence of juvenile crayfish with a size of less than 5 cm in the Neretvica River course. The most balanced structure of the crayfish population represented by juvenile as well as adult species is observed in Gorovnik.
Key words: *Austropotamobius pallipes*, habitats, spatial distribution, Neretvica, Neretva (Bosnia and Herzegovina).

Introduction

White clawed crayfish, *Austropotamobius pallipes* (Lereboullet, 1858), is the most ancient species in the family Astacidae. In Miocene, this species had a wide distribution throughout Europe (Maguire, 2002; Maguire et al., 2004). At present, its habitats got decreased. At present the western boundary of the species distribution has shifted from Portugal to north-western Spain; northern one — to Scotland, southern one — to Spain, and eastern ones — to Austria and Montenegro. Their habitats are decreased in Austria, Corsica, Germany, Lichtenstein, and Montenegro. It is expected that during the last 10–15 years in Great Britain, France, and Italy, the abundance of this species has decreased by 50–80 % (Souty-Grosset et al., 2006). It has been assessed by IUCN as "endangered" under criterion A2ce.

This species has been listed under the EU Habitats Directive Annex II and V and therefore requires the designation of special areas of conservation for its protection. It has also been listed under Appendix III of the Bern Convention. Despite its protected status, the biological, ecological, abundance, and diversity of this species is not well-studied. For Bosnia and Herzegovina, there are only a few studies: Entz, 1914; Karaman, 1929, 1963; Šandra and Petrusek, 2008; Trožić-Borovac, 2011; Trožić-Borovac et al., 2012. There is insufficient knowledge to understand the biology, behavioral patterns, feeding and habitats of the crayfish, their role in the river ecosystem, what is needed to develop a program of measures

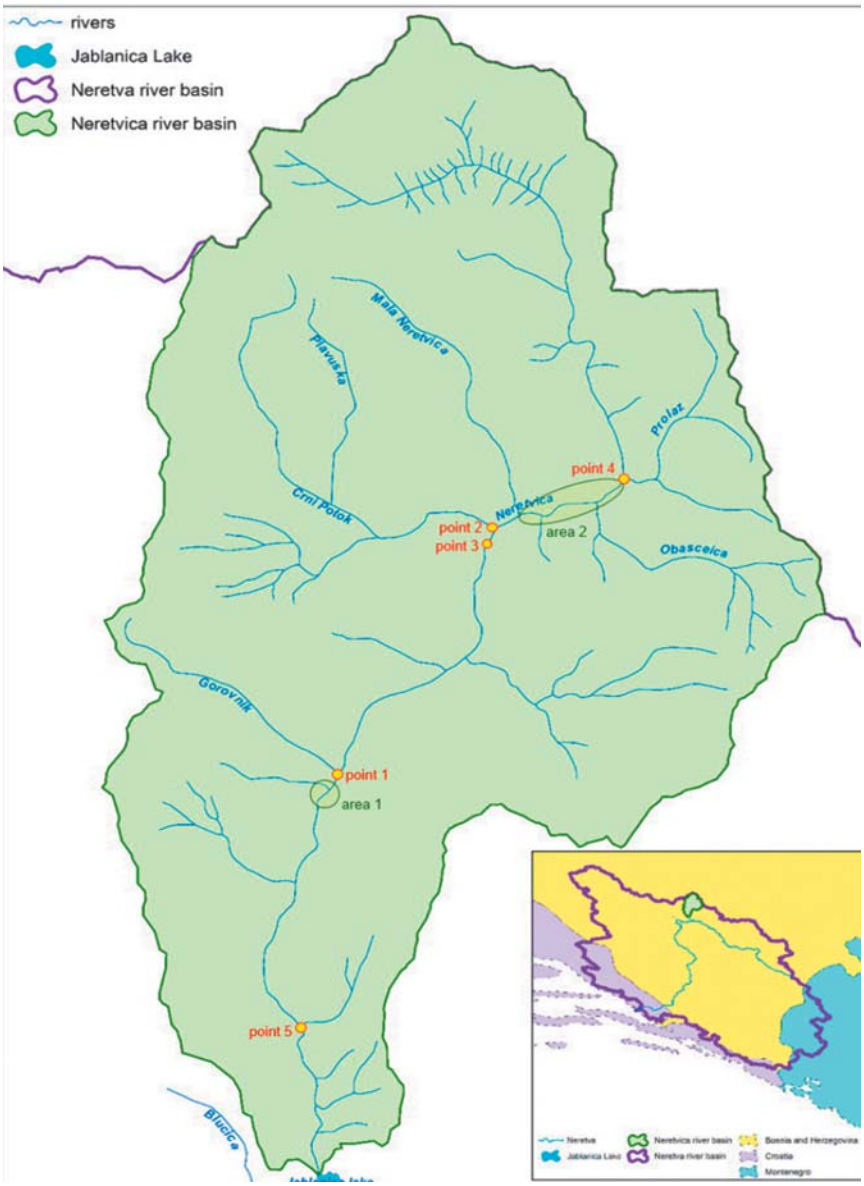

Fig. 1. Map of the research area.

Material and methods

A study of the distribution of the white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet, 1858), was conducted in the Neretvica River basin in August 2016, May and September 2017 in the frame of complex studies of water fauna of this river in connection with its possible regulation for hydropower needs (fig. 1).

The Neretvica is a river in Bosnia and Herzegovina which has a source on the slopes of Zec mountain at the altitude of 1792 m. The river flows into Jablanicko Lake (a large water reservoir formed at the Neretva River). The Neretvica is a right tributary of the Neretva River. The length of the river is 27 km; the catchment area is around 136 km^2 (in hydrological station Gorani). The average elevation of the river catchment is 376 m above sea level.

The river valley is U-shaped of the most part its length. U-shaped valley is typical for the lower course of the Neretvica. A single type of channel is typical for most parts of the river. In several places, river channel is braided, but its total length is insignificant. There are much wood debris (both trunk and brushwood) in the river channel from very upstream up to the mouth.

The four largest tributaries — Obascica, Prolaz, Mala Neretvica, Crni Potok — enter Neretvica in its middle course, and one tributary — Gorovnik enter in the lower course.

As far as there is a deficit of data about the ecology of this rare species and its habitat preferences, the study was done using the screening method of the maximum number of microhabitats. The study was done using scuba diving and special crayfish traps with baits, placed in identified and potential crayfish habitats. Besides, the crayfish were registered using investigatory fish catches using electrofishing based on the BiH permit DOZ-VOLU Federal Ministry of Agriculture, Water Management and Forestry Bosnia & Hercegovina 03-3-24/3-970/17 A.S. 04.05.2017.

The following parameters were measured using a metal ruler: total (zoological) length, length of carapace, a width of a carapace in the widest place, and its weight.

The total number of the measured crayfish was 25. Besides, 7 dead crayfish were measured. Moreover, more than 20 specimens were observed in nature.

The caught crayfish were tagged using notching at the carapace (Rumyantsev, 1970). After measurements, the caught crayfish were released using accurate dorsiventral diving to avoid having air bubbles under the carapace.

Results of study

For the first time *Austropotamobius pallipes* was found in Neretvica basin during the spring survey of 2016 during scuba diving observation of mouths of tributaries of Gorovnik (fig. 1, point 1) and Crni Potok (fig. 1, point 2). Only two specimens were caught in the habitats outside of the main course. These habitats are characterized by the accumulation of fine and middle-size fractions of sand and gravel with a significant share of organic detritus. Besides, during the visual survey of the main course of Neretvica downstream of the Crni Potok one dead crayfish was found (fig. 1, point 3), and during the survey of the riparian zone near Podhum 1 (fig. 2, area 1) the remains of one more specimen were found. In traps placed in deep spots along the main course of the Neretvica from Prolaz tributary till the mouth, the crayfish were not found.

A study using the electrofishing conducted during May–September 2017 along the whole river and its tributaries allowed the identification of peculiarities of the crayfish distribution in the Neretvica river basin. It showed that the crayfish are distributed irregularly.

The maximum elevation above the sea level, where the crayfish were found (one dead specimen) was 918 m a. s. l. The first location starting from the river source, where significant concentrations of the crayfish were found was in the mouth of Prolaz Rver (coordinates 43 49′56.3″ N 17 52′58.3″ E) (fig. 1, point 4). The average elevation is 753 m a. s. l. It is located 11 km downstream from the source. The form of the valley is U-shaped. The riverbed type is classified as a single channel type. Flow types are turbulent, broken standing waves, unbroken standing waves, and rippled.

The average width of Prolaz was 2.5 m and varied from 1.1 to 3.5 m. Bed elements included bars, rocks, and step/pools. The average depth of Prolaz was 0.15 m, with a maximum of 0.25 m. The average velocity of Prolaz was 0.2 m/s, with a maximum of 0.6 m/s (figs 2, 3).

Fig. 2. General view of the confluence of the Prolaz tributary into the Neretvica.

Distribution of bottom habitats in the places of the crayfish catch: megalithal — 30 %, macrolithal — 20 %, me-solithal — 20 %, micro-lithal — 8 %, psam-mal — up to 3 %, CPOM (deposits of coarse particulate organic matter, e. g. fallen leaves) — 12 %. An assessment by hydrobiological parameters using the Methodology of the field protocol) (Afana-syev, 2002), showed that the water quality in the river corresponds to the category "very clean" and "high biological status" in all seasons. The value of TBI and BBI indexes is 10 points. Saprobic index of Pantle&Buck calculated by macrozoobenthos is 1.2–1.5, which corresponds to α-oligosaprobic zone.

Found at this location 4 specimens (one female) had a length from 6.3 to 8.2 cm and were quite regularly distributed along the location, preferring the habitats with the depths of 0.25–0.3 m and the slowest flow. One more crayfish of the size 4.2 cm was found in the mouth of the tributary.

Further downstream the Neretvica till the confluence with the Crny Potok (fig. 1, area 1), a few crayfish were found in the main river course and at the mouths of the tributaries of Obascica and Mala Neretvica using electrofishing. In total, 5 crayfish were found.

At the confluence of the Crny Potok and Neretvica, a significant concentration of the crayfish was found (coordinates 43°49′33.9″ N 17°51′20.8″ E) (fig. 1, point 2; fig. 4). The average elevation is 699 m a. s. l. It is located 13.5 km downstream the source, in the mouth of the Crny Potok. The form of the valley is a U-shaped. The riverbed type is classified as a single channel type. This site is located in the narrowed part of the Crny Potok canyon. There is a bluff / vertical waterfall with a height of 4–5 m in spring and 2–3 m in autumn. Under the waterfall, there is a deepening with a depth of up to 4 m (in spring) and 2 m (in autumn) (figs 4, 5).

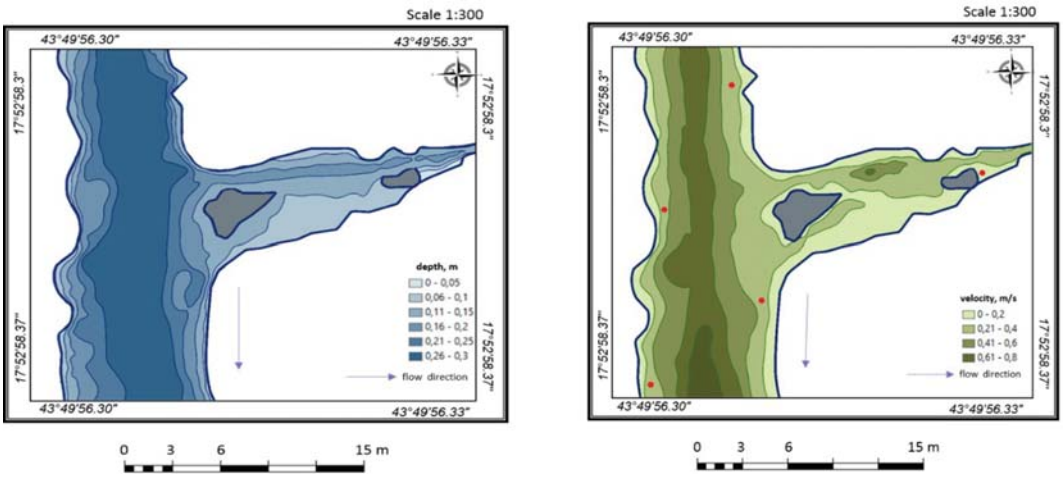

Fig. 3. Isobath map (depth, m) and isopach map (velocity, m/s) (the locations of the crayfish catches are marked with red dots).

Fig. 4. Habitat with the highest concentration of the crayfish in Neretvica at the confluence with the Crni Potok.

In the deepening under the waterfall in the Crni Potok, only 3 specimens were found; their size was 5, 8 and 6 cm. Directly in Neretvica River, the biggest concentration of the crayfish was found at the confluency with the Crny Potok. Distribution of bottom habitats at the locations of the crayfish catches was as follows: HS (hygropetric sites (water layer on solid substrates) — 10 %, megalithal — 35 %, macrolithal — 20 %, mesolithal — 10 %, microlithal — 5 %, psammal — up to 5 %, CPOM (deposits of coarse particulate organic matter, e. g. fallen leaves) — 15 %. The assessment by hydrobiological parameters showed that the water quality in the river corresponds to the category "very clean" and high biological status during all seasons. The value of TBI and BBI indexes is 10 points. Saprobic index of Pantle & Buck calculated by macrozoobenthos is 1.4–1.62, which corresponds to α-oligosaprobic and β-mesosaprobic zones.

According to the results of the scuba diving here, more than 20 specimens lived there. In general, the crayfish were concentrated at the edges of the hope and behind the rock in the habitats with the slow flow and a large amount of plant detritus. Besides, some specimens sit at vertical surfaces of boulders and rocks, covered by the moss *Fontinalis,* as well as hiding behind boulders directly downstream the confluence. Having place bites made of chicken meat placed for 12 hours, 11 specimens were caught (fig. 6). The smallest one was 7 cm in length and the biggest one — 8.3 cm. The sex ratio was 0.83 with male domination. At a distance of more than 50 m upstream and downstream of the confluence, the crayfish were found neither visually nor in traps.

Further for 5 km, the crayfish were found very rarely in the Neretvica River course.

The location with the highest concentration of the crayfish was found in the mouth of the right tributary of Neretvica–Gorovnik (coordinates 43°47′18.7″ N 17°49′24.9″ E), where they were found along the whole course (150 m) (fig. 1, point 1; fig. 7). The average

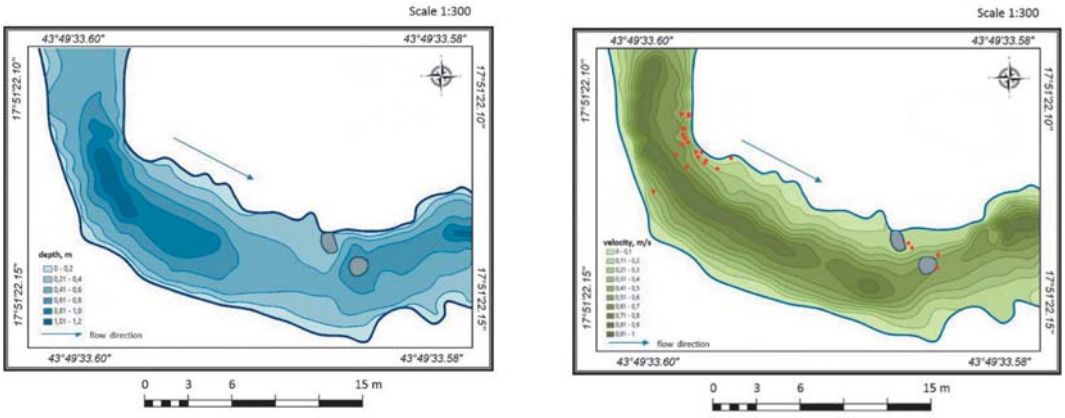

Fig. 5. Isobath map (depth, m) and isotach map (velocity, m/s) (the locations of the crayfish catches using scuba diving are marked with red dots).

Fig. 6. Extraction of the crayfish from the trap at Neretvica near the confluence with the Crny Potok.

Fig. 7. Typical crayfish habitats in the Gorovnik.

elevation is 416 m a. s. l. It is located 50 m upstream from the confluence with the Neretvica River. The form of the valley is U-shaped. The riverbed type is classified as a single channel type.

Maps of depths are created for the site of 30 m length. Flow types are broken standing waves, unbroken standing waves, and rippled. The average width was 2 m and varied from 1.5 to 7 m (fig. 8). Bed elements included bars and step/pools. The average depth was 0.15 m, with a maximum of 0.3 m. The average velocity was 0.2 m/s, with a maximum of 0.4 m/s (fig. 8). The distribution of bottom habitats in the location with the highest crayfish concentrations are as follows: megalithal — 5 %, macrolithal — 30 %, mesolithal — 20 %, microlithal — 10 %, psammal — 10 %, CPOM — 20 %.

The assessment by hydrobiological parameters using Methodology of the Field protocol showed that the water quality in the river corresponds to the category "very clean" and high biological status during all seasons. The value of TBI and BBI indexes is 10 points. Saprobic index of Pantle & Buck calculated by macrozoobenthos is 1.3–1.5, which corresponds to α-oligosaprobic zone.

The number of the crayfish in the habitats with slow flow 0.11–0.2 m/s with cobble and gravel deposits and many roots and plant detritus can reach 2–3 items per m^2. The sizes of the specimen varied from 4.3 to 8 cm. Sex ratio was 0.83 with male dominance.

Further in the main riverbed and upstream the confluence with tributary Gorovnik only a few specimens were found using electrofishing. The crayfish habitat at the lowest elevation (268 m a. s. l.) was found in Neretvica (fig. 9). 19 km downstream its source. The form of the valley is U-shape. The riverbed type is classified as a single channel type.

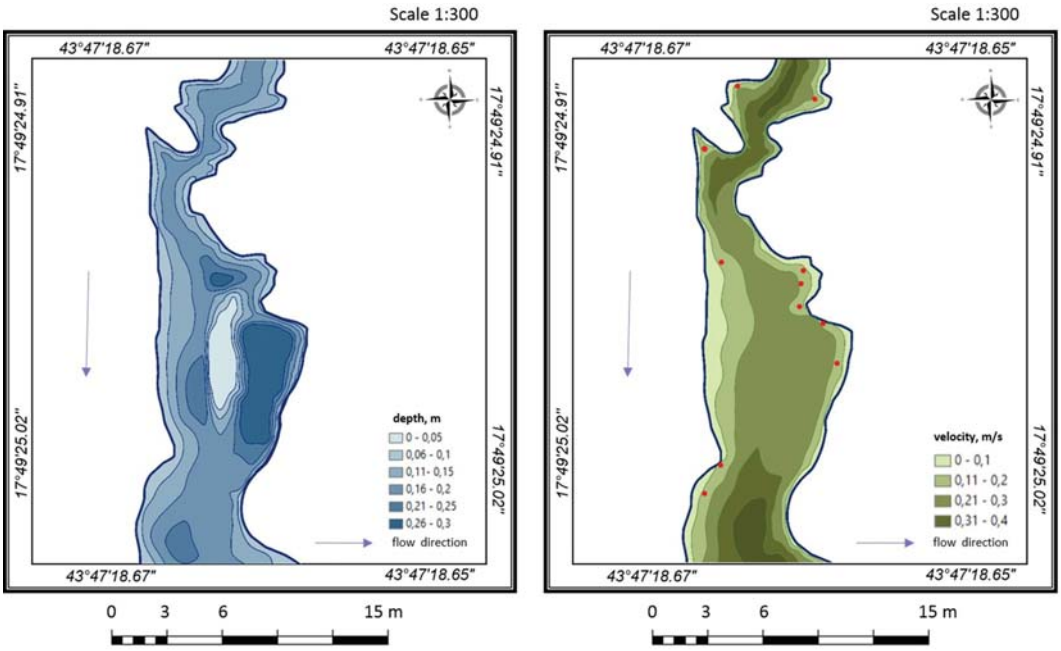

Fig. 8. Isobath map (depth, m) and isotach map (velocity, m/s) — autumn 2017.

Maps of depths are created for the site of 50 m length. Flow types are turbulent, broken standing waves, unbroken standing waves, and rippled. The average width was 8 m and varied from 5.5 to 12 m. Bed elements included bars, rocks, and step/pools. The average depth was 0.35 m, with a maximum of 0.6 m. The average velocity was 0.4 m/s, with a maximum of 1.2 m/s (figs 10).

Distribution of bottom habitats at the locations with the crayfish are as follows: megalital — 5 %, macrolithal — 30 %, mesolithal — 25 %, microlithal — 15 %, psammal — 15 %, CPOM — 10 %. Assessment by hydrobiological parameters showed that the presence of Lyngbya and Oscillatoria, as well as the increase of the number of Oligochae-

Fig. 9. Lowest habitat of the crayfish in the Neretvica.

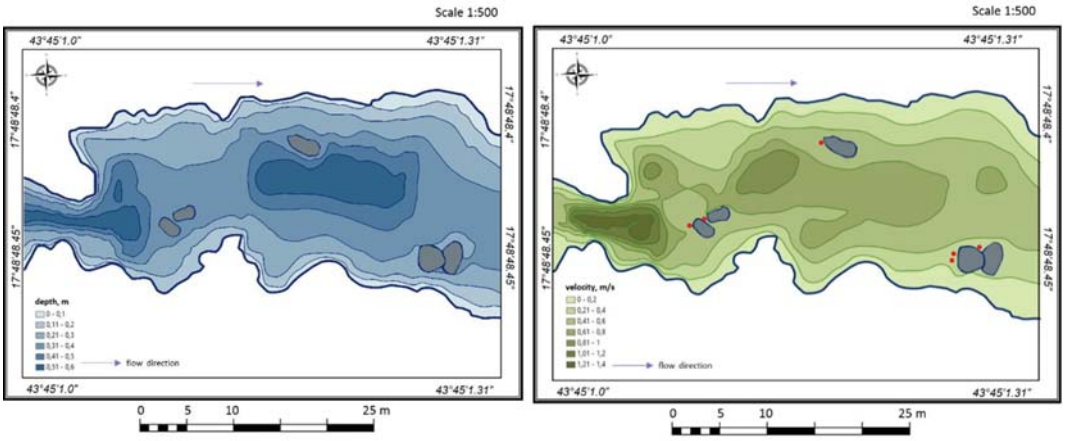

Fig. 10. Isobath maps (depth, m), isotach map (velocity, m/s) — autumn.

ta, signals the organic pollution of the river because of the fish farm located upstream. Saprobic index of Pantle & Buck calculated by macrozoobenthos is 1.6–1.8, which corresponds to β-mesosaprobic zone. Values of TBI and BBI correspond to 7–9 scores, which correspond to water quality "very clean" in spring and "clean" in autumn and 1–2 classes of eco status.

All observed specimens were found behind boulders at depths 0.2–0.3 m and velocity 0.2–0.8 m/s. Out of 6 caught species of the size varying from 6.2 to 7.5 cm, only two were females.

The size structure of the crayfish populations at different localities is presented in figure 11. The most balanced structure of the population, represented by the juvenile as well as adult species is in the Gorovnik.

Discussion of the results

In the territory of Bosnia and Herzegovina, *A. pallipes* is present mainly in the Neretva basin, partly in the Una and Tsetina River basins (Trožić-Borovac et al., 2012). This species was registered at elevations from 180 to 1024 m a. s. l., but most habitats are located at elevations from 300 to 600 m a. s. l. Water temperature, measured during surveys varied from +7 to +20 °C. Saprobic index defined by the composition of macroinvertebrates and phytobenthos shows that the crayfish is present in clean and slightly polluted waters

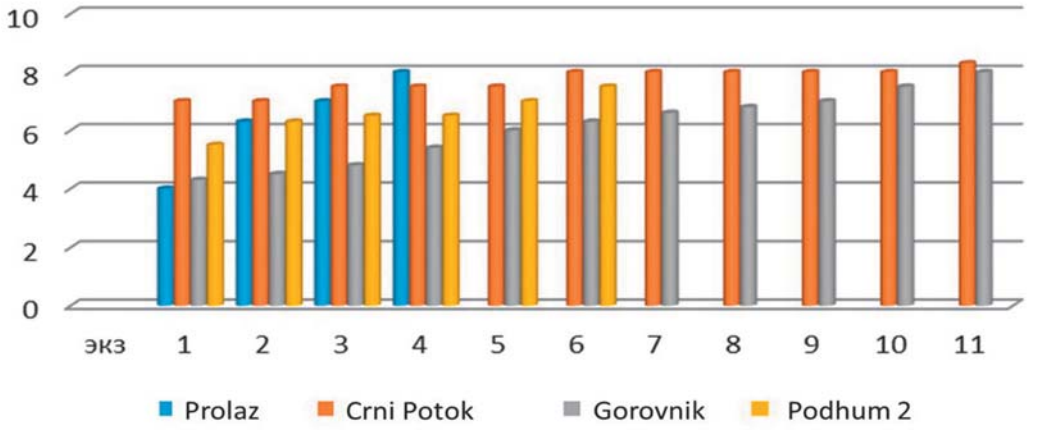

Fig. 11. Size structure of cray fish by locations.

(Trožić-Borovac et al., 2012). Based on indirect data, one can state that more than 2/3 of the crayfish population in the Neretva basin is concentrated in the Neretvica basin.

According to our data, the maximum elevation where the crayfish were found in the Neretvica River basin was 918 m a. s. l., located 8 km downstream its source, minimal — 268 m a. s. l., located 19 km downstream the source. Temperature diapason was from +5 to +12.4 °C. Depth diapason was from 0.1 to 1 m (mostly till 0.4 m). Diapason of velocities was from 0 to 0.6 m/s (mainly 0.2–0.3 m/s). Saprobic index of Pantle & Buck calculated by macrozoobenthos in the locations with crayfish concentrations was 1.2–1.8.

Our studies showed that the crayfish are very irregularly distributed in the Neretvica basin. The crayfish are almost absent at well-flushed habitats without CPOM (deposits of coarse particulate organic matter, e. g., fallen leaves), as well as at habitats with monotonous boulders or gravel-sand bottom. Significant heterogeneity of bottom habitats as a precondition for the high concentration of the crayfish is mentioned in the study of (Demers et al., 2003). In the Neretvica River, the obligatory condition of mass concentrations of crayfish is the presence of CPOM at the level of not less than 10 % from the total area of substrates as well as the presence of at least 6 types of bottom habitats. In the Neretvica River, such conditions were present at confluences with tributaries as well as at their mouths.

The study of the size structure of the crayfish population showed the presence of juvenile crayfish with a size of less than 5 cm in the Neretvica River course. The most balanced structure of the crayfish population represented by juvenile as well as adult species is observed in Gorovnik (fig. 11). Many authors state about the competition inside of one species population of many crayfish species, leading to the fact that adult species will remove juvenile ones (Stain, 1977; Lodge and Hill, 1994). The main reasons for this include cannibalism and competition for habitats, stimulating juvenile crayfish to occupy free from adult habitats (Momot, 1993; Lodge and Hill, 1994). But if this hypothesis is correct then small crayfish should be met in more diverse habitats than adults who occupy the most "comfortable" habitats. This tendency was not observed in the Neretvica basin. Moreover, quite long stretches of the river were not inhabited by the crayfish (neither adult, not juvenile ones). This confirms the presence of additional factors, affecting the distribution of the crayfish. In our study, such a factor can be the presence of predator fish. In the Neretvica basin during the ichthyological surveys 3 trout species were found, in stomachs of which remains of *A. pallipes* were found. Without discussion of the peculiarities of the quantitative distribution of the fish, one can note that in the places of the crayfish concentrations, electrofishing showed lower density of the fish compared with other locations. Besides, in the upper reaches of the Neretvica, as well as in its mouth where the crayfish was not observed, the fish abundance was much higher than in the rest of the riverbed and tributaries (except the upper reaches of the Chrny Potok, where there were very high trout concentrations and crayfish were absent). Observation of the impact of predators on the survival of the crayfish envisages that the juvenile crayfish, which is very vulnerable for predator fish, will prefer the shallow waters or the habitats with CPOM concentrations at the bottom, where they can hide (Englund, 1999; Englund and Krupa, 2000). Adult crayfish are less vulnerable to the fish and can occupy deeper waters to avoid land predators such as otter and mink. Such a spatial distribution of the crayfish was fixed in the Neretvica basin. The small up to 5 cm crayfish were found in shallow waters, where the trout enters during spawning only.

According to our research clawed crayfish were found at elevations from 268 m to 918 m a. s. l. in the Neretvica basin. The sex distribution varied from 0.33 to 0.83 in favour of males. The crayfish were irregularly distributed and this can be explained by the character of bottom h abitats as well as pressure from predators. The biggest concentration of the crayfish was fixed in the habitats with slow flow and presented of the debris and plant detritus, where small crayfish can hide from predators. Mass concentrations of the crayfish were fixed in the mouths of tributaries wherein highly heterogeneous habitats juvenile crayfish can hide in shallow waters. The obligatory precondition for the population reproduction

is the presence of habitats with a high amount of CPOM at the bottom. This fact should be taken into account during hydropower construction when downstream of the dike because of the phenomenon of sedimentation hunger and absence of the small fraction juvenile crayfish could lose an opportunity to hide.

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Received 23 February 2021
Accepted 1 July 2021