# Population structure of the invasive Atlantic blue crab, *Callinectes sapidus* on the Eastern Adriatic coast (Croatia, Montenegro)

# Struktura populacije atlantskog plavog raka, Callinectes sapidus na istočnoj obali Jadrana (Hrvatska, Crna Gora)

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DOI 10.17818/NM/2023/SI3 UDK 595.3 (261)\*595.3 (262.3) Original scientific paper / Izvorni znanstveni rad Paper received / *Rukopis primljen*: 22. 2. 2023. Paper accepted / *Rukopis prihvaćen*: 23. 5. 2023



#### **KEY WORDS**

blue crab *Callinectes sapidus* Eastern Adriatic Sea sex ratio length-weight relationships

## Abstract

The Atlantic blue crab, Callinectes sapidus, is a highly invasive species that poses a significant threat to Mediterranean ecosystems. In the last two decades, it has become established in several marine and estuarine areas of the eastern Adriatic Sea, resulting in a decline in commercial catches and damage to fishing gear. This article reviews the current status of blue crab invasion in Montenegro and Croatia and analyses its abundance and population structure. Overall 619 crabs were sampled (male:female ratio, 1:1.91). Both carapace width and weight differed significantly between males and females, with males having a wider carapace and more weight. There was a significant difference in carapace width and weight among sites. For the total population, the mean male and female carapace width was 130.3  $\pm$  30.8 and 108.8  $\pm$  41.4 mm, respectively. In addition, the mean male and female weight was 187.2  $\pm$  85.6 and 132.5  $\pm$  39.1 g, respectively. Coefficient b between the weight and carapace width of blue crabs was significant at all locations, although it varied between males and females. This work will also document the impacts of the blue crab invasion on local ecosystems and provide comprehensive overview of populations structures and shed light on this important aspect of blue crab ecology.

#### Sažetak

Atlantski plavi rak, Callinectes sapidus, invazivna je vrsta koja predstavlja značajnu prijetnju mediteranskim ekosustavima. U posljednja dva desetljeća udomaćio se u nekoliko morskih i estuarijskih područja istočnog Jadrana, što je rezultiralo padom komercijalnog ulova i štetama na ribolovnim alatima. Cilj ovog rada je dati pregled trenutnog stanja širenja populacije plavog raka u Crnoj Gori i Hrvatskoj te analizirati njegovu brojnost i strukturu populacije. Ukupno je uzorkovano 619 rakova (omjer mužjaci:ženke, 1:1,91). Širina oklopa i ukupna masa značajno se razlikuju između mužjaka i ženki, pri čemu mužjaci imaju širi oklop i veću masu. Utvrđena je značajna razlika u širini i težini oklopa među lokacijama uzorkovanja. Prosječna širina oklopa mužjaka i ženki mjeri 130,3 ± 30,8 odnosno 108,8 ± 41,4 mm. Osim toga, srednja težina mužjaka i ženki mjeri 187,2 ± 85,6 odnosno 132,5 ± 39,1 g. Koeficijent b između mužjaka i ženki. Ovim radom zabilježit će se utjecaj invazije plavog raka na lokalne ekosustave te dati sveobuhvatan pregled strukture populacije istraživanih područja.

# KLJUČNE RIJEČI

plavi rak Callinectes sapidus istočni Jadran omjer spolova odnos duljina-masa

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# **1. INTRODUCTION**

The Atlantic blue crab, Callinectes sapidus, Rathbun, 1896, is a portunid brachyuran native to the western Atlantic Ocean from Nova Scotia to Uruguay and Argentina [1]. The first appearance of the blue crab in European waters was reported in 1901 in Rochefort, France [2], where it was probably introduced with ballast water [3]. Since then, the species has spread to the Mediterranean Sea, where it was first described in 1947 and has spread widely in the northwestern areas of the Mediterranean, including the Adriatic Sea [4,5]. The blue crab's excellent swimming abilities, opportunistic feeding behaviour, broad habitat preferences, high fecundity, and wide larval distribution are all biological characteristics that contribute to its invasive nature and allow it to spread successfully in different regions [6]. These characteristics have led to its recognition as one of the 100 worst invasive alien species in the Mediterranean [7]. The first detection in the eastern Adriatic was in 2004 in the Neretva estuary and in the salt pans of Ston [8], and a strong population was established in the Neretva estuary after six years [9]. The first information about the occurrence of blue crab in Montenegro, specifically in Port Milena, comes from a personal observation in 2006 [10]. The first verified record dates back to 2013, when two males were caught with a gill net in Boka Kotorska bay, while the first female was discovered in 2017 in the lagoon of the Tivat Solila nature reserve [11]. This study addresses the population characteristics of blue crab using common tools such as morphometrics, length to weight ratio and sex ratio. In addition, the study provides guantitative data on allometric growth and qualitative data on the biology and ecological preferences of the blue crab in its non-native range in the eastern Adriatic Sea. Samples from Montenegro and Croatia were analysed to provide new insights into population structure that will be valuable to scientists, policy makers, and those involved in the management of non-native and invasive species, and will contribute to the understanding and management of the blue crab population in the affected regions.

#### 2. MATERIALS AND METHODS 2.1. Sampling area

Samples of blue crab were collected between 2018 and 2021 at six different sites, three of them in Croatia and the remaining three in Montenegro. The study area in Croatia was located in Dubrovnik-Neretva County and included the rivers Neretva (Parila lagoon), Ston and Mlini, which differ significantly in terms of water temperature and salinity. The Neretva and Mlini areas are characterized by the inflow of fresh water into the sea, but in different ways. The Neretva River (43°01'55.3 "N 17°27'05.2 "E) has a main channel with lagoons on both sides, which are not affected by strong river currents and have a higher temperature and salinity than the surrounding areas. The temperature in the delta of the Neretva River ranges from 9.2°C to 26.7°C and the salinity from 8.0 to 34.4 ppt (in the Parila lagoon from 7.2°C to 33.4°C and the salinity from 11.4 to 34.6 psu. Mlini (42°37'18.0 "N 18°12'19.0 "E), on the other hand, is a small town that owes its name to the old mills still located in the old part of the town. These mills were located on the freshwater rivers of the area, and in 1952 a small hydroelectric power plant with a generator called "Zavrelje" was built, using water from the same river. The water is discharged to the sea through an outflow channel, and to reduce the impact of sea waves on the Zavrelje, a breakwater was built. The site in Ston (42°49'44.4 "N 17°41'53.4 "E) is characterized by shallow water with low circulation, walled with stones, which serves as a basin for the company Saline Ston. The term "saline" refers to a facility or site where salt is produced by the evaporation of salt water. The water for the salt works is transported to the basins through a series of canals that remain unused for most of the year. As part of the study, these canals and the adjacent lagoon were used as sampling sites to monitor the presence of blue crab.

The Bojana River (41°52'30.0 "N 19°22'24.3 "E), together with the Port Milena wetland and Šasko Lake, is the largest body of water on the entire Montenegrin coast [12]. The Bojana River is 43 km long, starting at Lake Skadar and flowing through Albania for 18 km before marking the border between Montenegro and Albania for the remaining 25 km. At its mouth into the Adriatic Sea, the Bojana River forms a small delta with two side channels, the left of which forms the border with Albania and the right of which, together with the island between the channels, belongs to Montenegro. Island of Ada Bojana today represents a unique ecosystem in Europe [13] and covers an area of 6 km2. The water temperature in Bojana varies between 4.8°C and 25°C throughout the year. Salinity ranges from 0.1 to 2.6 psu, but can reach up to 20 psu in the lower water layers near the delta during strong southerly winds. Port Milena Channel (41°54'56.3 "N 19°15'11.8 "E) has been transformed from a natural lagoon into a channel whose cleaning is obstructed by the Bojana River, dammed by a salt processing plant built in 1934. Hladna Uvala (41°59'38.4 "N 19°08'41.4 "E) is a fishing cove between towns Bar and Ulcinj, where there are several freshwater springs.

#### 2.2. Sampling and gear description

Sampling was conducted with two types of fishing gear. The first was a locally remodelled version of American wire crab traps with a rectangular shape, 60 cm long, 60 cm wide, and 40 cm high, a mesh size of 40 mm, two entrances, and a compartment for storing bait (Figure 2A). The second type of gear used in the study was gillnets. Gillnets are vertical fishing nets suspended in the water and anchored to the bottom. The net material consists of a mesh large enough for the fish to fit their head through, but not their body. The fish's gills then become entangled in the mesh when the fish tries to free itself from the net, hence the name "gill net."

Fish waste from non-commercial or low-value species such as thin-lipped mullet (*Chelon ramada*) was used as bait for the wire traps. In addition, Gibel carp (*Carassius gibelio*) and pumpkinseed (*Lepomis gibbosus*) were also used as bait for the traps [14]. Both types of gear were deployed in the afternoon. The traps were soaked for 24 hours while the gillnets were hauled in the next morning. Catches from the crab traps were collected (Figure 2B), the traps were rebaited and returned to the sea, while the gillnets were brought ashore and cleaned manually.

After collection, specimens were identified to species level based on Galil et al. (2002). Weight (W) and carapace width (CW, maximum distance between posterior anterolateral spines) and sex were measured for all 619 individuals. Measurements were made using a digital caliper (Mitutoyo) for carapace width and a digital scale for weight (Mettler Toledo JL602-G/L).2.3.

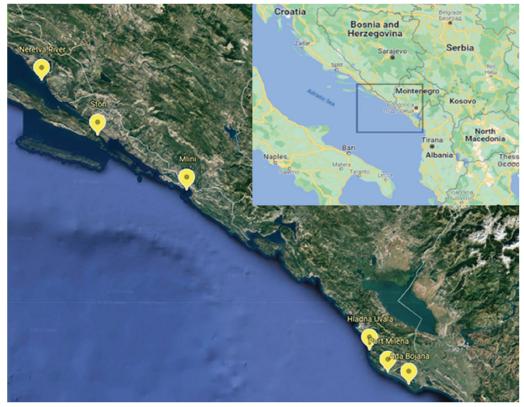


Figure 1 Sampling locations of the Atlantic blue crab Callinectes sapidus (Rathbun, 1869) in Croatia and Montenegro



Figure 2 A - American design wire crab trap, B - collection of the Atlantic blue crab Callinectes sapidus specimens from the trap

# 2.3. Statistical analysis

Statistical analyses were performed using SPSS Statistics v.26. Mean differences in carapace width (CW) and total weight (W) between males and females were tested with an independent T-test. Differences in sex ratio between sites were tested with the nonparametric chi-square ( $\chi$ 2) test. Mean differences in carapace width (CW) and total weight (W) between sites were tested using the 1-way test ANOVA. If a statistically significant

difference was found, a Tukey HSD post hoc test was performed for multiple comparisons. Differences in *b* coefficients between males and females were tested with an independent T test and between sites with a 1-way test ANOVA. Before analysis, data were visually checked for normality with Q-Q plots and tested for homogeneity of variance with Levene's test. All assumptions were met. For all tests, the significance level was set at 0.05 ( $\alpha = 0.05$ ).

# 3. RESULTS 3.1. Distribution

A total of 619 crabs were sampled (212 males and 407 females), (male:female ratio 1:1.91). Sex ratios differed significantly among sites ( $\chi$ 2 (5) = 33.49270, p = .001); ratios at each site are shown in Figure 3. In Montenegro, in the Ada Bojana site the most crabs were captured, 149 females and 48 males (197 total). In Croatia, the most crabs were caught in the Neretva River delta, including 118 males and 44 females (162 total) (Table 1).

For the total population, the mean carapace width (CW) of males was  $130.3 \pm 30.8$  mm with maximum and minimum values of 182 mm and 51.5 mm, respectively. The mean carapace width of females was  $108.8 \pm 41.4$  mm with a maximum and minimum value of 182 mm and 45.1 mm, respectively. In addition, the

mean weight (W) of males was  $187.2 \pm 85.6$  g with a maximum and minimum value of 449.2 g and 17.2 g, respectively. The mean weight of females was  $132.5 \pm 39.1$  g, with maximum and minimum values of 256 g and 13.7 g. A summary of data for the Croatian and Montenegrin catches at each site is presented, including mean carapace width (CW) and weight for males and females (Table 1).

Both carapace width (CW) and weight differed significantly between males and females (t (597) = - 2.558, p <.011 and t(597) = -10.913, p = .001, respectively), with males having a wider carapace and higher weight (MD = 7.092, SE = 2.772, MD = 56.925, SE = 5.21). There was a significant difference in carapace width between sites (F(5, 598) = 211.809, p=.001). Significant pairwise comparisons are shown in Table 2.

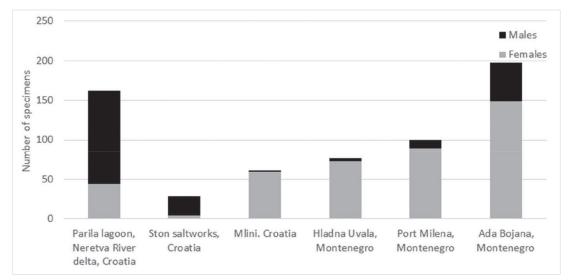


Figure 3 Sex ratio of the Atlantic blue crab Callinectes sapidus (Rathbun, 1869) at each sampling site on the Eastern Adriatic Sea coast

Sampling location	Total number	Males	Females	Male: Female ratio	Mean CW, males (mm ± st.dev.)	Mean CW, females (mm ± st.dev.)	Mean weight, males (g $\pm$ st.dev.)	Mean weight, females (g ± st.dev.)
Parila lagoon, Neretva River delta, Croatia	162	118	44	1:0.37	128.7 ± 28.7	120.5 ± 34.4	196.1 ± 99.2	115.1 ± 60.2
Mlini, Croatia	61	2	59	1:29.5	118.3 ± 2.3	137.9 ± 25.4	104.1 ± 3.2	129.4 ± 27.2
Ston saltworks, Croatia	29	25	4	1:0.16	133.9 ± 20.1	149.8 ± 10.5	$205.4 \pm 92.8$	194.2 ± 27.5
Ada Bojana, Montenegro	190	44	144	1:3.27	145.5 ± 13.3	147.2 ± 13.1	166.8 ± 34.3	156.1 ± 29.6
Hladna Uvala, Montenegro	77	4	73	1:18.25	85.7 ± 35.3	$61.71\pm5.8$	$169.8\pm42.6$	118.9 ± 33.1
Port Milena, Montenegro	100	11	89	1:8.09	134.1 ± 11.5	134.9 ± 11.9	153.2 ± 57.7	115.3 ± 28.1

Table 1 A summary of catch data for the Atlantic blue crab Callinectes sapidus (Rathbun, 1869) at each site (CW = carapace width)
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Table 2 Significant pairwise comparisons (Tukey HSD,  $\alpha$  < .05) of mean carapace width (CW) between sites.

(I) Location	(J) Location	Mean Difference (I-J)	SE	p	95% Confidence Interval	
(I) LOCATION					Lower Bound	Upper Bound
Ada Bojana	Hladna Uvala	83.69643	2.65273	.000	76.1121	91.2808
	Neretva	19.06394	2.10641	.000	13.0416	25.0863
	Port Milena	11.00816	2.44472	.000	4.0185	17.9978
	Mlini	10.43323	2.87078	.004	2.2255	18.6410
Hladna Uvala	Neretva	-64.63249	2.68243	.000	-72.3017	-56.9632
	Port Milena	-72.68827	2.95553	.000	-81.1383	-64.2382
	Ston	-74.19528	4.21108	.000	-86.2350	-62.1555
	Mlini	-73.26320	3.31660	.000	-82.7456	-63.7808
Neretva	Port Milena	-8.05579	2.47692	.015	-15.1374	9741
	Mlini	-8.63071	2.89824	.036	-16.9170	3445

There was a significant difference in the mean weight between sites (F(5, 598) = 20.598, p=.001). Significant pairwise comparisons are presented in Table 3.

# 3.2. Length-weight relationship and length-weight population structure

The relationship between carapace width and weight was calculated for both sexes of blue crab using power regression:  $W=a\times Lb$ , where W is weight in grams, L is carapace width in

centimeters, a is the intercept of the regression, and b is the slope [15]. A strong correlation was found between the weight and carapace width of crabs, as shown by the high correlation coefficients for the sites in both countries (Figure 4). However, the slope b for males and females varied considerably between sites, with the lowest value being 1.1125 for Hladna Uvala (where only four males were captured) and the highest being 3.1607 for males from Port Milena (Table 1).

(I) Location	(J) Location	Mean Difference (I-J)	SE	p	95% Confidence Interval	
					Lower Bound	Upper Bound
Ada Bojana	Hladna Uvala	42.16242	8.37566	.000	18.2159	66.1090
	Port Milena	41.98553	7.71889	.000	19.9168	64.0543
	Ston	-41.65655	12.21814	.009	-76.5890	-6.7241
	Mlini	33.05851	9.06410	.004	7.1437	58.9733
Hladna Uvala	Neretva	-53.98573	8.46944	.000	-78.2004	-29.7711
	Ston	-83.81897	13.29595	.000	-121.8329	-45.8050
Neretva	Port Milena	53.80884	7.82055	.000	31.4494	76.1683
	Mlini	44.88182	9.15083	.000	18.7190	71.0446
Port Milena	Ston	-83.64208	12.89232	.000	-120.5020	-46.7822
Ston	Mlini	74.71506	13.74004	.000	35.4314	113.9987

Table 3 Significant pairwise comparisons (Tukey HSD,  $\alpha < .05$ ) of mean weight (W) between sites

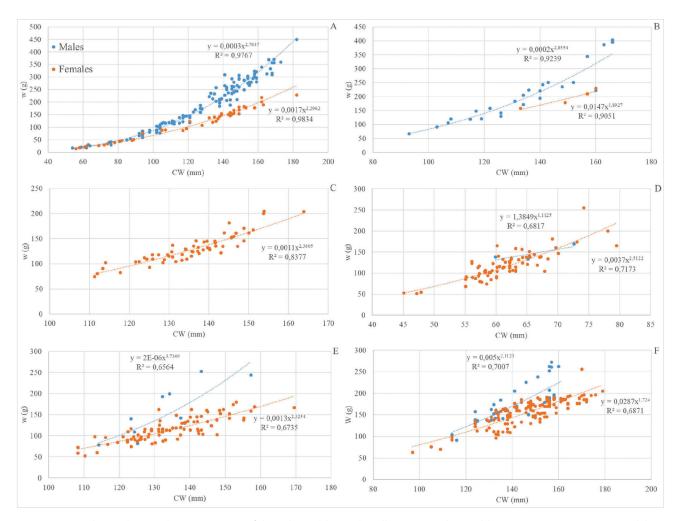


Figure 4 Length-weight population structure of the Atlantic blue crab *Callinectes sapidus* (Rathbun, 1869) (A - Neretva River delta, B - Ston, C - Mlini, D - Hladna Uvala, E - Port Milena, F - Ada Bojana)

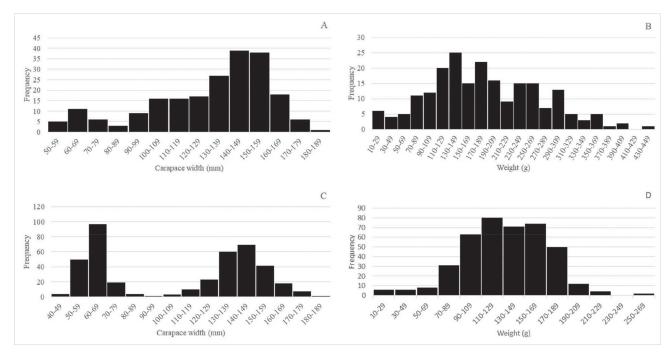


Figure 5 Carapace width and weight distribution for each sex (males = A - B, females = C - D) of the overall *Calinectes sapidus* population on the Eastern Adriatic coast

Blue crabs were divided into carapace width and weight classes and presented in diagrams (Figure 5). The greatest abundance of males is found in the 140-149 mm CW class and females in the 60-69 mm CW class. The majority of males fell into the 130-149 g weight class, while the majority of females fell into the 110-129 g weight class (Figure 5). There was no significant difference in b coefficients between males and females (t(10) = .144, p = .889) or between sites (F(5,11)= 2.666, p = .132).

#### 4. DISCUSSION

The study compared populations of blue crabs sampled at different locations along the southern east coast of the Adriatic Sea. The highest number of crabs was recorded at the mouth of the Bojana River (Montenegro), with a predominance of females in the total catch, while samples from the Neretva River estuary (Croatia) contained dominantly males. It should also be taken into account that in the area of Ston saltworks a majority of males were caught, and in Port Milena in Montenegro a majority of female crabs were caught, since in both cases they are lagoons located near saltworks with similar water conditions, but with opposite sex distribution. This indicates that sites with similar ecological conditions do not necessarily have similar population distributions. The village of Mlini in Croatia, closest to the border with Montenegro, is the only site with a similar sex distribution to all three sites in Montenegro. The similarity of catch data in Mlini and Hladna Uvala, both small bays with freshwater springs, suggests that females on the eastern Adriatic coast prefer marine areas with colder water in summer, as males were almost absent at these sites. This result is consistent with previous observations that males are more abundant in lower salinity environments as females migrate to higher salinity coastal waters to spawn [16]. This study has shown that the population structure of this species in these areas is characterized by a high proportion of females, a size distribution toward larger male individuals, and a high reproductive potential. In addition, as a strong swimmer, the blue crab can migrate between the sea and rivers throughout its life cycle, resulting in strong geographic dispersal and colonization [3]. Nevertheless, blue crab is an important target for commercial and recreational fisheries in its native range [17]. In the U.S., the commercial fishery for this species is estimated to be worth between \$141 million and \$205 million between 2000 and 2019, according to the National Marine Fisheries Service [18]. Because most stocks of commercially valuable crustacean species are currently in critical condition, new fishing grounds and species should be developed [19]. In some parts of Europe, such as Greece, this species has become a target of commercial fisheries [2]. It is expected that the future development of bottom and coastal fisheries in the Mediterranean will follow the changes already observed at the European level [19]. Creating a sustainable blue crab fishery that can transform invasive populations from a threat to an opportunity for valuable fishery resources with real market value and demand is crucial management step in control of this species. Involving tourists in the fishery and promoting blue crab as food could help regulate the crab population, which is important since both countries rely heavily on tourism.

Author Contributions: Conceptualization, L. G. and A. P.; Methodology, T. D.; Validation, L. G., A. P. and J. T.; Formal Analysis, A. C.; Investigation, O. M.; Resources, M. P.; Data Curation, S. G.; Writing – Original Draft Preparation, L. G. and A. P.; Writing – Review & Editing, L. G.; Visualization, M. B. S.; Supervision, T. D.

**Funding:** The research presented in the manuscript did not receive any external funding.

# Conflicts of Interest: None.

Acknowledgments: The authors would like to thank Slobodan Bedo Glamuzina and Stanko Glamuzina, fishermen from Mostina, Croatia, and to Ivo Knežević and Milan Milić, fisherman from Ulcinj region, Montenegro, for their help in organizing crab fishing, maintaining fishing gear, and providing their experience and skills for the project activities.

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