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Some biological aspects of Mediterranean mud shrimp, *Upogebia pusilla* (Petagna, 1792) in the Boka Kotorska Bay, Southeastern Adriatic Sea

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Abstract: Biological aspects (length frequency distribution, length-weight ratio, sex ratio, allometric growth, Fulton's condition factor) of *Upogebia pusilla* were studied in Tivat Saline (Tivatska solila), Boka Kotorska Bay in 2019. A total of 1,415 individuals were collected. The total length of all studied individuals ranged from 27 to 55 mm (8 and 20 mm CL), while the total weights ranged from 0.29 to 2.56 g (the total length of males ranged from 27 to 51 mm, non-ovigerous and ovigerous females ranged from 27 to 51 mm and 30 to 55 mm, respectively; the weights ranged from 0.29 to 2.52 g, 0.34 to 2.52 g and 0.47 to 2.56 g for males, non-ovigerous and ovoid females, respectively). The overall female:male ratio was 1.2:1. Ovigerous females were recorded from April (74.3%) to June (23.8%), with a peak in May (91.4% of all females). Males dominated in July (66.4%). The relationship between CL and W was negatively allometric in both males and females and in all sampled individuals. Fulton's condition factor was higher only in females during the breeding season, while there were no significant differences between males and females during the rest of the year. Since this species is mainly used as live bait for commercial and sports fishermen, the results of this study would be useful for the sustainable management of this species.

Keywords: *Upogebia pusilla*, breeding period, condition factor, Boka Kotorska Bay

INTRODUCTION

The mud shrimp *Upogebia pusilla* (Petagna, 1792) is a small decapod species of the family Upogebiidae, which consists of benthic and burrowing species. According to [1-2], this species is the most common member of this family. It is widely distributed throughout the Mediterranean Sea and Eastern Atlantic from Mauritania in the south to Brittany in the north [2-5] and in the Black Sea [6-8]. It lives on muddy and sandy silty bottoms in intertidal and subtidal zones up to 45 m in depth, where it usually digs Y-shaped burrows. This species belongs to a special group of organisms called bioturbators and "ecosystem engineers" [9]. Through their bioturbation activities, Mediterranean mud shrimps configure benthic habitats and influence benthic communities [10-11]. Therefore, they are considered important ecosystem engineers [12]. *U. pusilla* occurs in high densities in lagoons [13].

In the Adriatic Sea, the Mediterranean mud shrimp has been described by several authors [14-16]. For the northern Adriatic, valuable information on some aspects of the biology and ecology of this species was provided by [17-19] and [20]. In other parts of the Mediterranean, a few studies on population dynamics, growth and reproduction have been carried out in the estuary of the Evros River in northern Greece [21], in the coastal area of Dioni Bay in western Greece [22] and in Izmir Bay, Turkey [23]. The existing information on *U. pusilla* in Montenegrin territorial waters is very limited, focusing mainly on the distribution of the species [24-27].

This species has economic importance for commercial fisheries because it is used as live bait. It is heavily exploited in some parts of the Mediterranean, for example in western Greece [22]. Mud shrimp are an important component of benthic food webs [28], and *U. pusilla* is a food source for a variety of fish and seabirds.

The aim of this article was to provide new information on the biological aspects of the Mediterranean

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mud shrimp, such as length frequency distribution, sex ratio, length-weight ratio, breeding season and Fulton's condition factor, and to compare the results with similar data from other regions of the Mediterranean and Adriatic seas.

MATERIALS AND METHODS

Study area

The study was conducted from January to December 2019 in the Tivat Saline (salt pans) area, which is situated in the wetland part of the coastal strip of Boka Kotorska Bay (Supplementary Fig. S1). In the last century, about 75 years ago, this area was designated as a salt pan, with basins, irrigation-drainage channel systems and communication dikes [29]; however, it was been put into operation. The Tivat Saline receives water from two rivers draining the Tivat fields – the Široka and the Koložun. The salt pan is divided into pools, each of which is approximately 3 ha (150×200 m) in size, and covers an area of 150 ha (Supplementary Fig. S1). The original marshlands were divided into artificial basins and separated from the southwest to the northeast by a 714-m-long dam into a smaller coastline and a much larger inland part [30]. The seaward section along the shoreline is dominated by shallow waters and temporarily flooded mudflats. In 2008, the Tivat Saline became a Special Nature Reserve in order to preserve rare, threatened and endangered plant and animal species, predominantly birds and plant communities.

The Tivat Saline represents one of the last habitats of halophytic vegetation on the east coast of the Adriatic, and one of the key points on the Adriatic migratory corridor for birds (the Adriatic Flyway) [31]. It is an important wintering place for birds in the country, and a resting place during autumn and spring migrations. In 2013, this reserve was put on the List of Wetlands of International Importance of the Ramsar Convention [32]. The Tivat Saline is also an Emerald habitat of the Bern Convention and the Area of International Importance of Birds in Montenegro – IBA.

Sampling procedure

Individuals of *U. pusilla* were obtained from local fishermen on a monthly basis. Samplings of mud shrimp were carried out on days with the lowest monthly tides, at depths of about 50 cm. It is important to note that fishers mainly collect large individuals because they sell mud shrimps as live bait to professional and sport-recreational anglers. As a result, there were only a few (5) juveniles in the entire sample. Samples were collected by a traditional method, using a metal square metal frame 2 m×2 m, with a surface of 4 m^2 and a height of 50-100 cm. The fisherman stands in the square and kicks the muddy bottom in the fenced area. The disturbance causes *U. pusilla* individuals to come out of their holes and the fishermen then collect them by hand or with a hand net of 5-mm mesh size. This traditional way of gathering individuals of mud shrimps is less aggressive than using a large suction pump known as a yabby pump or a high-pressure gasoline engine-operated water pump. The main disadvantage of the yabby pump is that the specimens are often damaged; chelipeds or other legs can become detached, or the body ruptured [33], which makes proper examination difficult or impossible.

Measurements

Samples were stored on ice immediately after capture and transferred to the laboratory for further analysis. In the laboratory, the following parameters were measured for each individual: carapace length (CL) in mm, from the tip of the rostrum to the dorsal posterior end of the carapace; total length (TL) in mm, from the tip of the rostrum to the end of the telson, and body weight. The length was measured using calipers with an accuracy of 0.1 mm, while the body weights were measured using a digital balance to the nearest 0.01 g. Sex was determined by macroscopic analysis of the sexual dimorphism of the first pereiopod and based on the presence (in females) or absence (in males) of the first pair of pleopods [19]. Females with eggs were recorded and weighed with eggs. As the maturation stage of the gonads was not examined, only females with a TL equal to or larger than the TL of the smallest ovigerous female were considered adults. Available bibliographical data [5] uses a 12-mm CL as the division between juveniles and adults. However, as the smallest ovigerous female recorded in this study had a 10-mm CL, this length has been used as a division length instead.

Statistical analysis

The sex ratio was expressed as the female-to-male ratio (i.e. the number of females to number of males), on a monthly and yearly basis. The χ^2 goodness of fit test was used to check whether the number of females and males deviated from the expected 1:1 ratio for each month and for the sampling period. One-way ANOVA was performed to analyze differences in the mean carapace and total lengths of sexes in the total sample.

Length-weight relationships were determined separately for males, non-ovigerous and ovigerous females, and for the total sample using the general formula:

$$W=aL^b,$$

where W is weight in g, L is the total length in mm and *a* and *b* are the coefficients of the functional regression between W and TL. The hypothesis of isometric growth was tested using the Student's *t*-test.

Fulton's condition factor (K) is often used to quantify an animal's physical wellbeing and is considered to be a useful complement for growth estimation in crustaceans [34]. Fulton's condition factor was determined by using the formula:

$$K=(W/L^3)*100,$$

where K is the condition factor, W is weight in g (ovigerous females were weighed with eggs) and L is carapace length in mm [35]. One-way ANOVA was used to compare the differences in mean Fulton's condition factors between sexes (P<0.05).

Descriptive statistics as well as all statistical analyses were performed in MS Excel 2010.

RESULTS

Length distributions and sex ratio

During this study, a total of 1,415 *U. pusilla* specimens were analyzed, 771 (54.5%) females and 644 (45.5%) males. Of the 1,415 sampled individuals, 1,410 were adults and only five were juveniles (0.4%), of which three individuals were female and two were male. The total length of all sampled individuals ranged between 27 and 55 mm with a mean value (TL±SD) of 37.68±4.72 mm. The total length of females ranged between 27 and 55 mm (37.47±4.58 mm) while the males had a smaller range of lengths, between 27 and 51 mm (37.93±4.88 mm).

The carapace length (CL) of the sampled individuals was between 8 and 20 mm, with a mean value of 13.74±1.98 mm CL. The mean size (CL±SD) of females was 13.38±1.83 mm (8 to 19 mm), with a mode at 12-14 mm, while the mean size of males was 14.16±2.07 mm (9 to 20 mm), with a mode at 13-15 mm (Fig. 1).

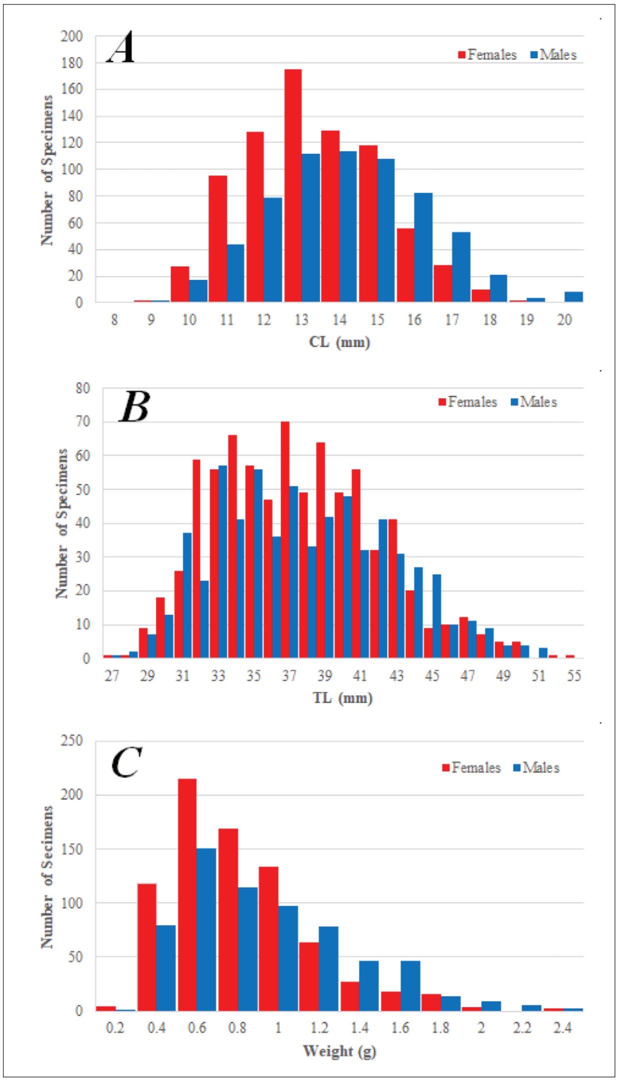

Fig. 1. Length and weight frequency distribution of *U. pusilla* for females and males from Tivat Saline, Boka Kotorska Bay. **A** – carapace length (CL) frequency distribution; **B** – total length (TL) frequency distribution; **C** – weight frequency distribution.

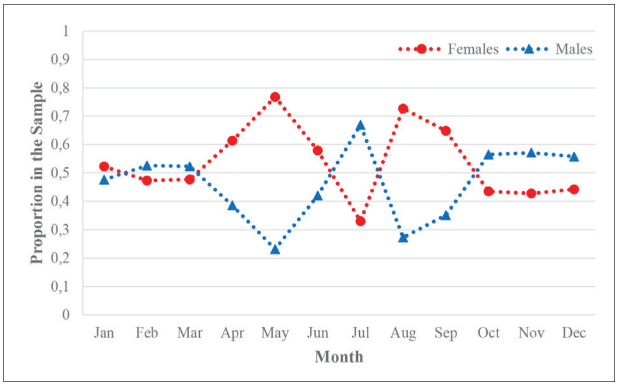

Fig. 2. Monthly sex ratio of *U. pusilla* from Tivat Saline, Boka Kotorska Bay.

The mean CL between the sexes was statistically significantly different (F=56.72, P<0.05), while the mean total length between the sexes showed no statistically significant difference (F=3.31, P=0.07).

The smallest observed individual was collected in November with a total length of 27 mm (8 mm CL) and a weight of 0.34 g while the largest observed individual was collected in May with a total length of 55 mm (19 mm CL) and weight of 2.56 g. The overall sex ratio (females/males) was found to be 1.2:1. There was a statistically significant difference between the number of females and males (χ^2=11.399, P<0.001). The monthly sex ratio varied from 0.9:1 to 3.3:1. The lowest value was in February (χ^2=0.316, P=0.574) while the highest value was in May when the sex ratio was found to be significantly different from the expected value of 1:1 (χ^2=51.983, P<0.005). A prevalence of females was found from April to June. Males are predominant in July. After that, the sex ratio again favored females until October (Fig. 2).

W=0.00004TL2.93 represents the TL-W relationship for adult males, for adult non-ovigerous females it is $W=0.00007TL^{2.59}$, for adult ovigerous females $W=0.00004TL^{2.76}$, while the overall length-weight relationship was $W=0.00003TL^{2.84}$. The length-weight relationships and the equations for females, males and the whole population are presented in Fig. 3. The b values indicate that individuals of *U. pusilla* showed negative allometric growth in both sexes (t-test, P<0.05). The length and weight for males showed a strong relationship (r^2=0.91), while the r^2 values for adult females were lower, both for non-ovigerous (r^2=0.84) and ovigerous (r^2=0.89).

Breeding period

Ovigerous females were observed from April to June. The percentage of ovigerous females in the total number of adult females was found to be 74% in April, 91% in May and 24% in June. The smallest ovigerous female had a total length of 30 mm (10 mm CL), while the largest had a total length of 55 mm (19 mm CL). The mean total length of all ovigerous females was 40.89±4.68 mm. Adult females had a carapace length >10 mm.

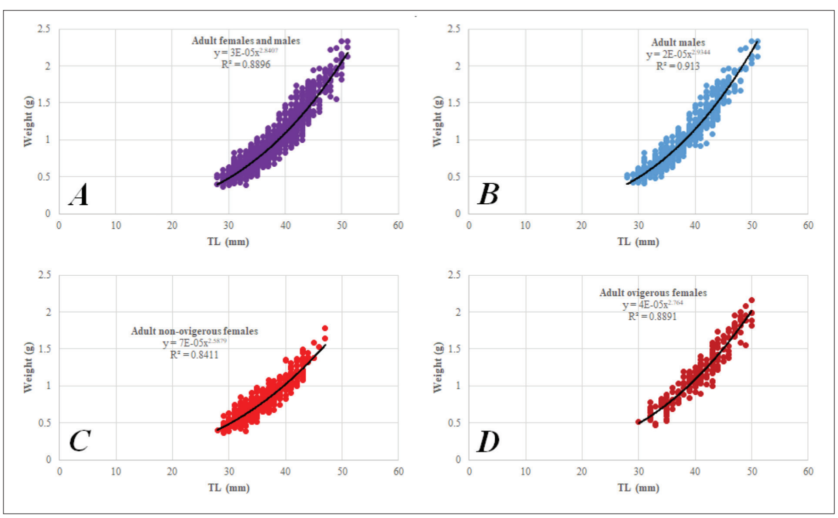

Fig. 3. Total length-weight relationship of adult *U. pusilla* specimens from Tivat Saline, Boka Kotorska Bay. **A** – LWR of both sexes combined; **B** – LWR of adult males; **C** – LWR of both adult non-ovigerous females; **D** – LWR of adult ovigerous females. TL – total length.

Fulton's condition factor

The values of Fulton's condition factor for both sexes showed very similar trends through almost the whole year (Fig. 4), except for the April-June period. In males, the highest mean condition factor was recorded in November (K=0.048±0.01) while the lowest result was recorded in February (K=0.03±0.004). Females follow the same pattern, with the highest mean condition factor value in November (K=0.051±0.01), and the lowest in (K=0.03±0.004) in February. During the April-June period, the condition factor of all (non-ovigerous and ovigerous) females was significantly

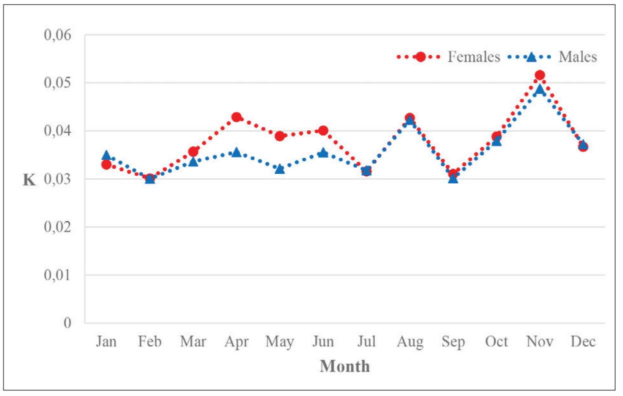

Fig. 4. Mean Fulton's condition factor (K) of *U. pusilla* females and males from Tivat Saline, Boka Kotorska Bay.

higher than that of males, and peaked in April when the breeding period starts (F=21.17, P<0.05). Separately, the highest mean condition factor of ovigerous females was also recorded in April (0.045±0.008), while the lowest K values were recorded in May (0.039±0.008).

DISCUSSION

Size frequency distribution shows that both sexes were represented throughout the year with individuals having CLs between 8 and 20 mm (27- and 55-mm TL). The results of studies [19-22] report a length difference between the sexes, with males having total lengths greater than those of females. The largest individual recorded in this study was a female with a TL of 55 mm. However, the males were, on average, larger than females, both in TL (37.93±4.88mm TL in males vs. 37.47±4.58 mm TL in females) and CL (14.16±2.07 mm CL in males vs. 13.38±1.83 mm CL in females). In addition, the range of carapace lengths was higher in males (9-20 mm CL) than in females (8-19 mm CL).

According to the available data, the largest recorded individual was a male with a TL of 106 mm, sampled in the Evros delta [21]. The maximum lengths reported in other studies that determined the length distribution of this species were higher than the values reported in this study [20,22,23]. Only individuals from Rovinj [19] were smaller compared to individuals in this study (the largest mud shrimp measured had 47 mm TL). The size frequency distributions are generally dependent on the population dynamics and seem to be mainly influenced by individual growth rates [19].

We found that the overall sex ratio favored females, which is in accordance with studies in the area of Rovinj [19] and the eastern Ionian Sea [22]. Females were also predominant during April-June, especially in May, the breeding period. Males prevailed in July and the October-December period. The predominance of males in July has also been reported previously [19].

In this study, the b values indicate that specimens of *U. pusilla* show negative allometric growth in both sexes (t-test, P<0.05). Similar growth was observed in the Evros delta [21] as well as in western Greece [22]. Positive allometric growth was reported for the central Aegean Sea [23]. Various factors may be responsible for the differences in the b value of the length-weight relationships among seasons and years, such as temperature, salinity, food, time of year and stage of maturity [34]. In addition, differences in the b value could be also attributed to differences in sampling, sample size or length ranges.

Based on the presence of ovigerous females in the population, the breeding period occurs from April to June with a peak in May, when 91% of females were ovigerous. The smallest ovigerous female measured 30 mm TL, while in the mediolittoral zone in the north Adriatic [20] the smallest females measured 26.4 mm TL and 25 mm TL [19]. The smaller size could be explained by high oscillations in environmental factors such as temperature, salinity and oxygen availability [22]. The breeding season generally depends on the temperature cycle of the biotope [19]. In the north Adriatic, on the Slovenian coast [20], the reproduction period lasts from early April to late July, while in the lagoon of Grado, Italy [19], the presence of ovigerous females from March to late September has been reported, as in other regions of the Mediterranean [21-23]. In the Evros delta, ovigerous females can breed more than once in the same reproductive period [21].

In this study, we set a limit of CL=10 mm between juveniles and adults. Considering this limit and the fact that we received only large specimens of *U. pusilla* from fishermen, we conclude that our annual sample consisted of adult individuals, while juveniles were present in very small numbers.

Fulton's condition factor (K) provides information about the specific growing conditions of shrimps. The observed value of K of females and males showed that the condition of the studied shrimp population was almost uniform during the study period. The condition factor differs significantly between females and males only during the breeding season. The difference in condition can be attributed to the presence of ovigerous females in the period April-June. It could be due to the higher weight of female gonads. Many factors such as sex, the time of year, stage of maturity, stomach contents [35] influence the value of the condition factor. Since Fulton's condition factor of this species was not determined in other studies, a comparison between other regions could not be conducted.

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Author contributions: All authors performed the fieldwork, laboratory analysis, interpreted the data, analyzed the results and prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: Authors confirm no potential conflicts of interest.

Data availability: Data underlying the reported findings have been provided as part of the submitted article and are available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Markovic%20et%20al_8409-Data%20Set.pdf

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SUPPLEMENTARY MATERIAL

Supplementary Fig. S1. Map of the Boka Kotorska Bay showing its four small bays. Study area. **A** – Location of Tivat Salina in Boka Kotorska Bay (QGIS 3.10); **B** – Satellite image of Tivat Salina (modified from: www.maps.google.com).