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Morphometric Relationships of the Global Invader *Callinectes sapidus* Rathbun, 1896 (Decapoda, Brachyura, Portunidae) from Papapouli Lagoon, NW Aegean Sea, Greece. with Notes on Its Ecological Preferences

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Abstract: *Callinectes sapidus* is native to the Atlantic coasts of the Americas. In the Mediterranean, it appeared around 1949 and though that it is established in East Mediterranean waters, relevant studies are limited. The aim of the present study is to report quantitative and qualitative data on the blue crab's biology and ecology in its non-native range, that are indispensable for management purposes. Papapouli Lagoon is in Thermaikos Gulf and is ecologically impacted by the blue crabs. Fyke nets with a 20 mm mesh opening were soaked for 12 to 14 h during each survey. Abiotic environmental parameters were obtained. The length–weight relationships were expressed by the equation $W = aL^b$. The species' ecological preferences at Papapouli Lagoon were assessed with PERMANOVA analysis, using the abiotic parameters as factors. Student's *t*-tests were used to assess the differences between sexes. The sex ratio of the blue crab's population was assessed by a Chi-square (χ^2) analysis. The sex ratio was found to be 1.28:1, in favour of males. The most dominant group size of male blue crabs was the 61–70 mm of CL and 130–139 mm of CW. Also, the dominant size group of female blue crabs was 60–69 mm of CL and 120–129 mm CW. The maximum abundance of blue crabs was recorded at a salinity range from 24‰ to 25‰ and the water temperature range was from 26 to 28 °C.

Keywords: alien species; blue crab; biodiversity; East Mediterranean Sea

1. Introduction

The blue crab *Callinectes sapidus* Rathbun, 1896, is distributed from Nova Scotia to Argentina including the Gulf of Mexico and the Caribbean Sea. During their complex life cycle, blue crabs inhabit marine and brackish environments and they can tolerate freshwater conditions [1,2]. They commonly occur at the continental shelf in estuaries, lagoons and near-shore waters from depths of 0 to 90 m on sandy and muddy substrates with or without vegetation [3,4]. In the Americas and in some countries of north-western Europe, blue crabs are commercially important [5–7]. In the Mediterranean Sea, the species is exploited in Turkey [8] and, to a lesser extent, in Italy [9], Egypt [10] and Greece [11–14]. Blue crabs are a well-studied species—at their native distribution. Relevant studies regard the species' life history and growth [1,3,15], diseases [16] and foraging behaviour [17,18]. There have also been studies related to the reproductive biology [19], population structure [20] and fisheries [6,21].

The first report of the blue crab in Europe was in 1900 from the Atlantic coast of France [5,22]. It has since been introduced to the European North East Atlantic Ocean [5,22], with no clear introduction vector [22]. However, introduction through ballast water and/or for aquaculture purposes seems the most probable [22]. In the Mediterranean Sea, the blue crab appeared around 1949 [12,23] and is widely distributed along the coasts. Also, blue crabs were reported in the Black Sea [12,23–25]. Furthermore, they have been recorded in Japan [26] and Hawaii [27].

Morphometrics and length–weight relationships are often used to study population characteristics and at stock assessment of commercially important species, for example, in [28,29]. These equations may reveal important biological aspects such as the size of sexual maturity and sexual dimorphism [30], or potential variability amongst populations of the same species [31] but see [32] for further details.

In Greek waters, the species is far from well-studied, however it is commercially exploited. Most of the studies are related to new occurrences of blue crab or review the blue crab current distribution. Information on blue crabs' fishery biology and population dynamics is very limited, with a few exceptions [11,13,14,33]. The blue crab's length–weight relationships have been studied in some cases along the East Mediterranean coasts: 1. Antalya, Turkey, [34,35], 2. Adana, Turkey [36], 3. North-eastern Mediterranean coasts, Turkey [37], 4. Greece [33] and 5. Sinai Peninsula, Egypt [10]—none of these studies assesses the species' ecological preferences.

The aim of the present study is to report quantitative data regarding the species' allometric growth and qualitative data on the blue crab's biology ecological preferences in its non-native range in the Eastern Mediterranean and Northwest Aegean Sea for the first time. Studies like the present are quite useful not only for scientists but for policymakers and at non-indigenous and invasive species management plans.

2. Results

The maximum, minimum and average values ± SD (standard deviation) are presented in Table 1.

Table 1. Mean, Standard Deviation, Minimum and Maximum values of the abiotic parameters during the study period.

Parameter	Mean	SD	Minimum	Maximum
Humidity (%)	68.22	±9.71	63.56	81.52
Air temperature (°C)	24.61	±4.02	19.3	29.6
Wind velocity (km/h)	9.83	±5.92	0	21
pH	8.68	±0.37	7.39	9.25
Dissolved oxygen (mg/L)	5.8	±1.76	3.9	9.5
Salinity (‰)	28.27	±2.35	23.5	32.1
Water temperature (°C)	27.07	±1.41	24.7	30.3

During this study, a total of 516 blue crabs were sampled, of which 226 were females and 290 were males. The sex ratio was 1.28:1, in favour of males, which was significantly different; the Chi-square test yielded $\chi^2 = 7.9$, $p < 0.05$. Regarding the somatic dimensions, there were no significant differences in CL (carapace length) and W (weight) between sexes. During the sampling period, the mean carapace length (CL) was 56.07 ± 10.15 mm with a maximum and minimum value of 95.89 mm and 12.57 mm, respectively, for females. The mean CL for male crabs was 59.83 ± 10.77 mm with maximum and minimum values of 92.97 mm and 25.27 mm, respectively. The carapace width (CW) for female blue crabs ranged from 52.64 mm (min) to 183.33 mm (max). The mean CW of females was 126.92 ± 25.7 mm. Also, males had a mean CW of 127.01 ± 26.38 mm, with maximum and minimum values of 209.01 mm and 50.87 mm, respectively. Moreover, the mean weight (W) of females was 112.92 ± 49.65 g with maximum and minimum values of 292 g and 8 g, respectively. In male crabs, the mean (W) was 148 ± 84.88 g, with maximum and minimum values of 540 g and 12 g, respectively (Table 2). Merged sexes data regarding CL, CW and W mean, maximum and minimum values are presented in Table 3.

Table 2. Mean and Standard Deviation of CL, CW and W regarding blue crab measurements.

Blue Crabs' Morphometric Measurements, Papapouli Lagoon													
Sex	**n**	**Carapace Length**				**Carapace Width**				**Weight**			
		Mean	**SD**	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**
Female	226	56.07	10.15	12.57	95.89	126.92	25.70	52.64	183.33	112.92	49.65	8	292
Male	290	59.83	10.77	25.27	92.97	127.01	26.38	50.87	209.01	148.00	84.88	12	540

Table 3. CL–W and CW–W relationships' parameters during the sampling period.

Sex	**CL–W Relationship Parameters**				**CW–W Relationship Parameters**			
	a	**b**	**SE(b)**	$\mathbf{r^2}$	**A**	**b**	**SE(b)**	$\mathbf{r^2}$
Female	2.287	1.985	0.1793	0.6879	2.310	2.840	0.1266	0.8671
Male	2.990	3.197	0.1525	0.8072	2.568	3.284	0.1486	0.8199
Merged	2.664	2.629	0.1183	0.7532	2.451	3.082	0.1092	0.8114

The size-frequency distributions of male and female blue crabs from the study area are presented in Figure 1. Two group sizes of male blue crabs were dominant, these were the 61–70 mm of CL and 130–139 mm of CW. Also, the dominant size group of female blue crabs was 60–69 mm of CL and 120–129 mm CW. The CL–W and CW–W relationships for males, females and merged sexes are presented in Figure 2.

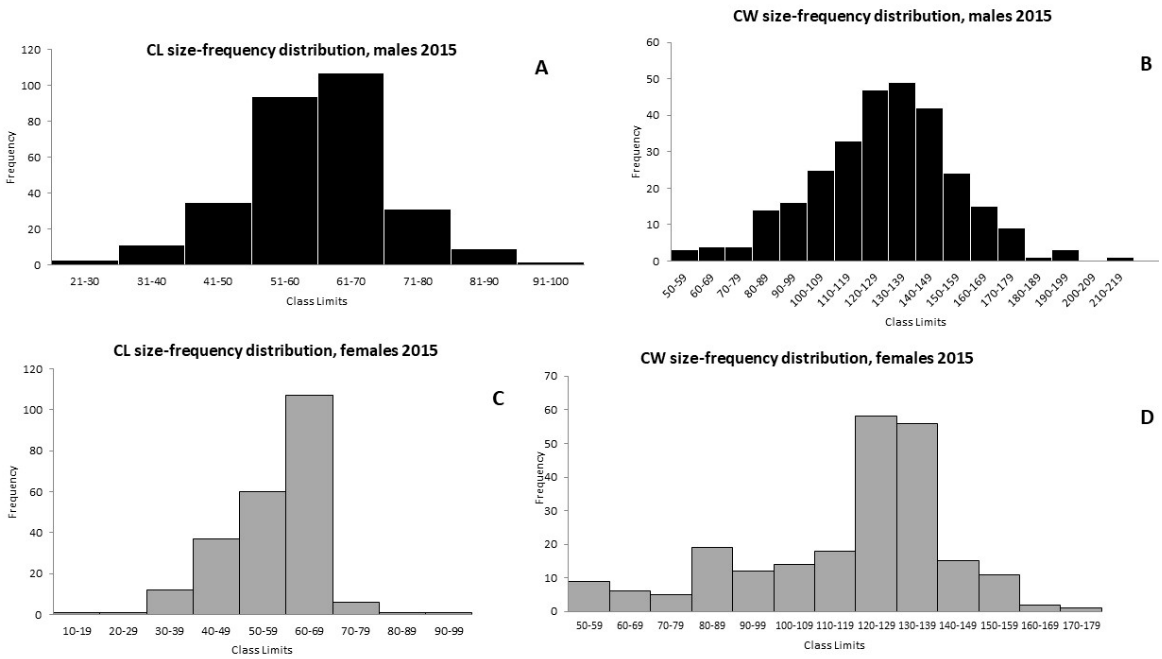

Figure 1. Size frequency distributions of Callinectes sapidus Rathbun, 1896. (**A**) carapace length (CL) frequency distribution in males, (**B**) carapace width (CW) frequency distribution in males, (**C**) carapace length (CL) frequency distribution in females and (**D**) carapace width (CW) frequency distribution in females.

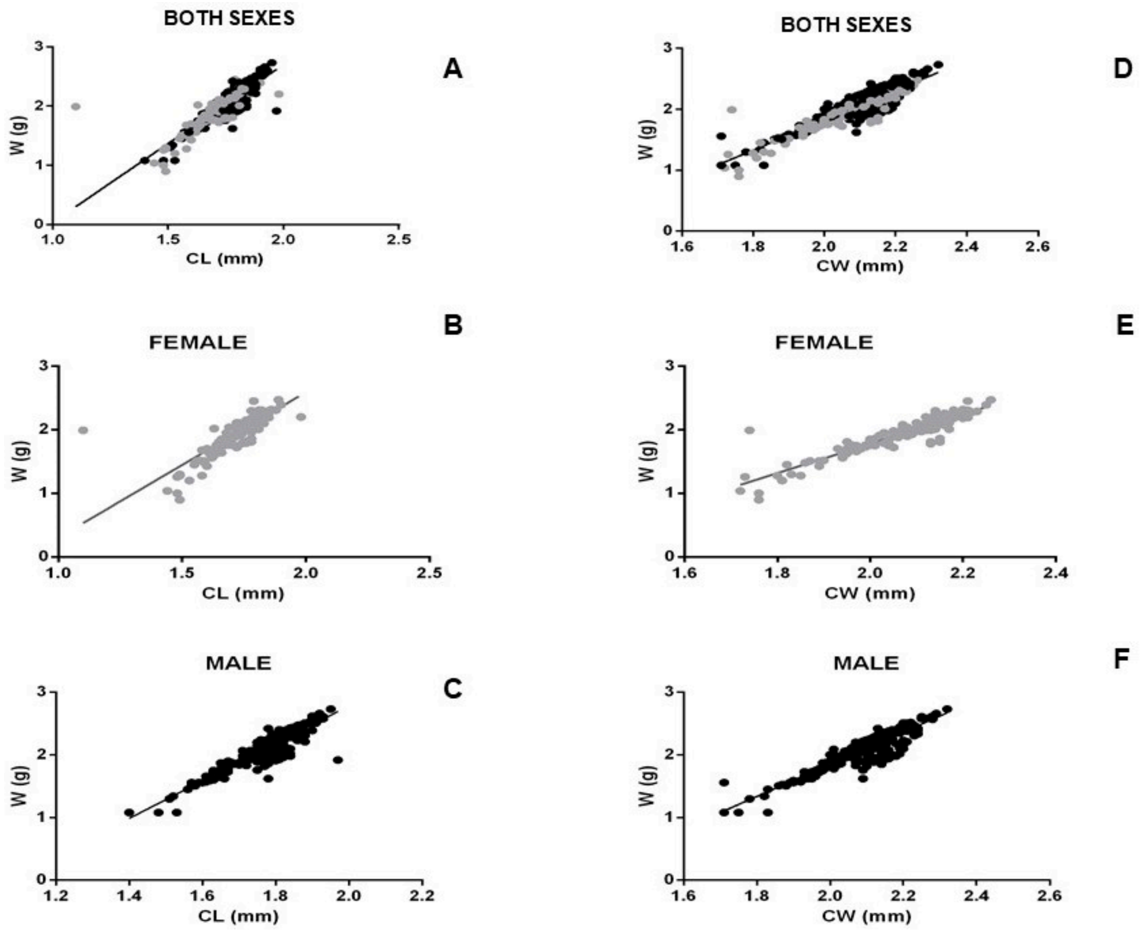

Figure 2. CL–W relationships (log transformed data), (**A**) both sexes, (**B**) female crabs, (**C**) male crabs. CW–W relationships (log transformed data). (**D**) both sexes, (**E**) female crabs, (**F**) male crabs.

The results regarding the abundance of the blue crab showed significant differences among sampling stations (space), but not significant differences between sampling dates (time) (Table 4). Pair-wise analysis showed that station B has greater differences when compared with station D; the pair of stations C and D are similar; stations B and C demonstrated less significant differences (Table 5). The maximum abundance of blue crabs was recorded at a salinity range of 24‰ to 25‰ and at the water temperature range 26–28 °C.

Table 4. PERMANOVA analysis regarding blue crab abundances.

Source	dF	SS	MS	Pseudo-F	P	Unique Perms
Time	2	1738.7	347.74	0.45754	0.809	999
Stations	2	12.334	6167.1	8.1146	0.01	999

Table 5. Pair-wise comparisons between sampling stations.

Groups	t	P	Unique Perms
B-C	4.14	0.013	981
B-D	2.44	0.063	991
C-D	0.40	0.847	957

3. Discussion

The sampling size is sufficient, and the observed differences were expected, since male blue crabs are generally larger in size [14]. Also, the low abundance of female individuals during the summer can be explained by the species' complex life cycle that involves female migration in marine environments during the egg-release period. Regarding the morphometric relationships and the allometric growth of the blue crab at Papapouli Lagoon, the present results are partially in accordance with the earlier studies from the Eastern Mediterranean basin, even from Hellenic waters. When comparing CL–W relationships, the present results are in accordance with [34] only when comparing both sexes with a negative allometric profile. Also, female blue crabs demonstrated negative allometry that is in accordance with earlier studies from Turkey and Egypt [10,37]. Moreover, male blue crabs in the present study presented positive allometry and they exhibited the same patterns as blue crabs from Egypt and Greece [10,33]. When comparing CW–W relationships, females showed negative allometry and are in accordance with earlier studies from Turkish waters [34,37] and Greece [33].

It is known that decapods and other crustaceans demonstrate an important plasticity in their phenotypic morphology that can be associated with environmental and/or genetic factors [31]. Although data on blue crab's growth pattern (CL–W, CW–W, CL–CW) are available [33], their results are mostly in disagreement with the present study. The study of [33] presents some similarities with ours, such as the used gear (fyke nets), but there is no further information available. For instance, Kevrekidis (2019) [33] does not provide information such as the mesh opening, the net's length or the leader's net length. In addition, the sampling frequency is different—once every two months in [33] and four times per month in the present study. The potential differences could be vast, and they could reflect in differences in catch selectivity (male–female selectivity or size selectivity) or differences in maturity stages, which requires further research. Furthermore, our research was exclusively conducted within Papapouli Lagoon and an earlier study [33] regarding mainly marine environments, which could impact the observed differences. However, except for our study, there are no published data—to the best of our knowledge at least—that assess the species' ecological preferences.

The introduction of blue crab in Thermaikos Gulf and Thessaloniki Bay was around 1935, with a continuous decline in population numbers until 1959 [22]. This information appears to be correct and is in accordance with the data extracted by professional fishers and mussel farmers who are active in the area. Specifically, they stated: "the crab (they are calling it as Italians) was here before the war—meaning the 2nd World War—we caught them till the 60s, then it disappeared. Strangely, it repapered again around the 80s for a short period and now, they are everywhere, and they destroy everything ... " The negative ecological impact of the blue crab in Thermaikos Gulf was described by [14], reporting important ecological imbalances in the local food chain and associated economic impacts on the regional communities of the fishermen and mussel farmers.

Although Papapouli Lagoon is protected by national and European laws [38], illegal activities are conducted in the area. These included illegal hunting, trash landfill, illegal agriculture and grazing. This resulted in the degraded operation of the lagoon as a natural fish farm, which is an institutional obligation. In addition, all of the above may lead to the creation of poor environmental conditions. The above are reinforced by the data of this research, since the area of station A could be described as a "dead zone". Even though no sediment analysis was conducted, during the sampling period, black and muddy sediments were observed, indicating potential hypoxic or even anoxic conditions. Paradoxically, the highest values of dissolved oxygen were observed at station A. There were significant differences among the sampling stations. Station B was the one with the greatest difference. This variation can be explained by the increased presence of females. Summer is the spawning season of the species and the larger female blue crabs prefer to remain at sea, showing similarities with other estuarine crab species [39] (e.g., *Eriocheir japonica*). Moreover, the smaller females must avoid large males because the species gives a stark indication of cannibalism, as shown in earlier studies from the same region [14]. Blue crabs are known to inhabit brackish habitats and that can tolerate temperature and salinity changes. Nevertheless, based on the present data, the optimal areas within the lagoon, where blue crabs prefer to dwell, are the wide areas of stations B, C and D.

4. Materials and Methods

4.1. Study Area and Sampling Methodology

Papapouli Lagoon is in the Thermaikos Gulf in the North-west Aegean Sea (39°58′257″ N 22°40′204″ E; Figure 3). Its major component is the Papapouli River. Papapouli Lagoon is a chocked lagoon with two points of contact with the sea. The small inlet is almost always closed—it is open only under extreme conditions—and the large inlet is periodically open (June–December)—this is affected by the wind's direction and intensity [40]. The large central canal is 30 m long and 15–45 m wide, depending on weather conditions [41]. Fyke nets were deployed on a weekly basis at Papapouli Lagoon from May to December 2015 four times per month, at four sampling stations with different salinities and (B ≥ C ≥ D) with an isolated station (A) (Figure 3), following [14]. Fyke nets were soaked for 12 to 14 h during each survey. Fyke nets were equal in length (300 m), the mesh opening was 20 mm, and each was equipped with a leader net of 3.5 m in length. All nets had two inner chambers and a 22 mm mesh size. Both chambers were framed with plastic tubes of 0.5 cm in width. All tubes had a horseshoe shape. All frames were 0.6 m in height and 0.7 m in width. The ends of cod-end were fastened before deployment. Data on meteorological parameters were collected, these were: (i) humidity (%), (ii) air temperature (°C), (iii) wind velocity (km/h) and (iv) wind direction obtained with a meteorological station Vantage Pro2, Davis, while the physiochemical parameters were: (i) dissolved oxygen (mg/L), (ii) pH, (iii) salinity (‰) and water temperature (°C) obtained with a CTD complex of the DKK-TOA Japan. Anecdotal data on the blue crab's occurrence and fisheries were obtained by interviewing fishermen and mussel farmers that are active in the region.

Figure 3. Papapouli Lagoon and sampling stations in the Thermaikos Gulf in the Northwest Aegean Sea. Source: Google Earth, modified.

4.2. Morphometric Measurements

Measurements were made for the carapace width (CW), carapace length (CL) and for the weight (W) of blue crabs. Carapace width (CW), defined as the distance between the tips of the posteriormost lateral spines; carapace length (CL), defined as measured dorsally, as the distance of the posterior carapace's margin and between the frontal notch following the midline [29]. All dimensions (CW–CL) were measured using a digital calliper with an accuracy of 0.5 mm. The crabs' wet weight was measured using a digital scale and reported to the nearest gram (1 g) [42,43]. The assignment of the sex of blue crabs was based on differences in their abdominal morphology. Males have narrow and long inverted "T" shaped structures and females have round and wide "U" shaped structures [9].

The length–weight relationships were expressed by the equation $W = aL^b$ or in its logarithmic form $logW = loga + blogL$. Where W= wet weight, L = CW or CL, a = intercept and b = slope. The constants a and b were estimated by the linear regression from logarithmically transformed data. The degree of association between length–weight variables was determined by a coefficient (r^2) [32]. There are three types of allometric growth—negative allometry, isometry and positive allometry. When comparing length/weight, these types are determined by the values of constant b. When $b < 3$, the allometry is negative, when $b = 3$, there is isometry and when $b > 3$, there is positive allometry [39].

4.3. Statistical Analyses

The analysis of the ecological preferences of the blue crab in the Papapouli Lagoon was performed with PRIMER 6.0 [44] and the PERMANOVA+ add-on [45] by using the abiotic parameters as factors and with respect to blue crabs' biomass and abundance. Length/weight relationships and the relavant analyses were conducted with GraphPad Prism 6.0 software with log10 transformed data. Also, GraphPad Prism 6.0 was used to perform Student's t-test, which assessed differences between the sexes. Supplementary analyses were conducted with Microsoft Excel. The spatial and temporal differences and distribution were determined by the abundance and the biomass of the species. The sexes ratio of the blue crab's population was assessed by a Chi-square (χ^2) analysis and with the assumption of a theoretical ratio of 1:1, males to females. ANOVA tests were used to assess the significance of linear regression [37].

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