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ABUNDANCE AND POPULATION STRUCTURE OF THE BLUE CRAB *CALLINECTES SAPIDUS* (DECAPODA, PORTUNIDAE) IN THERMAIKOS GULF (METHONI BAY), NORTHERN AEGEAN SEA

BY

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ABSTRACT

The blue crab *Callinectes sapidus* is an alien decapod established in the Mediterranean Sea. Since 2007, increased abundance has been reported from the northern Aegean Sea sustaining local scale fishery. The present work aims to assess the abundance and population structure of *C. sapidus* in Thermaikos Gulf using fyke nets. Population abundance, estimated as CPUE, exhibited strong temporal variability with decreased values in the cold season; this pattern was correlated with seawater temperature. Females exhibited also spatial differences with increased abundance close to the Aliakmon estuary. In total, 543 individuals were measured for carapace width. Males prevailed in the population; however, mean size was similar between sexes. Larger individuals were caught from deeper waters and the estuarine areas, whereas mean size decreased temporally. The fyke nets used proved to be size-selective, thus preventing fisheries mortality for juveniles. However, recurrent monitoring is necessary for a sustainable management of blue crab fisheries in the gulf.

RÉSUMÉ

Le crabe bleu *Callinectes sapidus* est un décapode étranger établi en Mer Méditerranée. Depuis 2007, une augmentation de son abondance a été signalée par les petites pêcheries locales du nord de la Mer Egée. Ce travail à pour but, en utilisant des nasses, d'évaluer l'abondance et la structure de la population de *C. sapidus* dans le Golfe de Thermaïque. L'abondance de la population, estimée comme CPUE, montre une forte variabilité temporelle avec des valeurs plus faibles en saison froide; ce modèle a été corrélé avec la température de l'eau de mer. Les femelles ont aussi montré des différences spatiales avec une augmentation de l'abondance près de l'estuaire de l'Aliakmon. Au total, 543 individus ont été mesurés. Les mâles dominent dans la population; cependant la taille moyenne est similaire dans les deux sexes. Les individus les plus grands ont été capturés dans les eaux plus profondes et les zones estuariennes, tandis que la taille moyenne décroit en juillet. Les nasses utilisées sont sélectives pour la taille, donc évitent la mortalité des juvéniles liée à la pêche. Cependant, une surveillance récurrente est nécessaire pour une gestion durable de la pêcherie du crabe bleu dans le golfe.

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INTRODUCTION

The blue crab *Callinectes sapidus* Rathbun, 1896, of West Atlantic origin, is among the alien decapods found in the Mediterranean Sea (Galil et al., 2015). The species has probably been introduced multiple times through ballast waters and has successfully established, especially in the eastern basin (see Nehring, 2011 for a comprehensive review of *C. sapidus* introduction and distribution in European and adjacent waters). Its first record derives from Thermaikos Gulf (northern Aegean Sea), dating back to 1935, where local fishermen harvested the crab known there as "italos" (Serbetis, 1959). A few years later, the sporadic presence of *C. sapidus* individuals has been reported from Porto Lagos lagoon (Vistonikos Gulf, Greece) and from Italian, Israeli, Lebanese, Egyptian, and southeastern Turkish coasts (Galil et al., 2002; Nehring, 2011). In the past decade, the species expanded throughout the Mediterranean (e.g., Genaio et al., 2006; Florio et al., 2008; Onofri et al., 2008; Beqiraj & Kashta, 2010; Castriota et al., 2012; Castejón & Guerao, 2013; Thessalou-Legaki & Pafilis, 2014; Perdikaris et al., 2016; Daban et al., 2016; Mancinelli et al., 2017a).

Callinectes sapidus is distributed in the sublittoral zone, on sandy or muddy bottoms. It has a complex life history, demanding both oceanic and estuarine habitats (e.g., Van Engel, 1958; Millikin & Williams, 1984; Carr et al., 2004). It is a top predator of bivalves, including mussels, as well as of fishes and other crustaceans, and also a scavenger (e.g., Ropes, 1988; Hines et al., 1990; Belgrad & Griffen, 2016). The species is thought to be extremely fertile (e.g., Hines, 1982; Darnell et al., 2009), a critical biological feature for its relative success as an invasive species in the Mediterranean Sea (Streftaris & Zenetos, 2006; Galil et al., 2015).

The blue crab supports an important fishery in its native range (i.e., the western North and South Atlantic Ocean), reaching 75 000 t for 2014 (FAO, 2018), which is marked, however, with severe interannual fluctuations (e.g., Hines et al., 1987; Lipcius & Stockhausen, 2002; Zohar et al., 2008). Within its introduced range, a small-scale fishery of local importance has been reported from the eastern Mediterranean (Nehring, 2011). Blue crab catches have also fluctuated therein, and the species disappeared or was only sporadically recorded for several years, which phenomena were attributed to the combined effects of overharvesting, pollution and epidemic diseases (Georgiadis & Georgiadis, 1974; Nehring, 2011). In recent years, *C. sapidus*' abundance seems to be increasing in several Mediterranean areas (Enzenroß et al., 1997; Dulcic et al., 2011; Mancinelli et al., 2013, 2016, 2017c; Carozzo et al., 2014; Abdel-Razek et al., 2016; Milori et al., 2017); the Aegean Sea being a major spot in its distribution pattern (Kevrekidis, 2010; Nehring, 2011; Mancinelli et al., 2017a, b).

In the Aegean Sea, the crab's key fishing grounds are located in the north, in Thermaikos Gulf and Vistonikos Gulf. In Thermaikos, the annual production ranged from 7 t (2007) to 84 t (2010) (mean 37 t) during the 2007-2017 period (source: Auction Agency of Greece, 2018); relevant data from Vistonikos are available for the 2010-2017 period reporting 1 t (2007) up to 34 t (2015) (mean 13 t) (source: Fisheries Cooperation of Vistonis Lake and Vistonikos Gulf). In the nearby area of SW Turkey, the annual production quadrupled in just one year, from 17 t in 2008 to 77 t in 2009 (Ayas & Ozogul, 2011), but drastically declined thereafter to only 1 t in 2013 (Tureli et al., 2016). Historically, the species supported a notably commercial fishery in Thermaikos during the 1950s, but its population collapsed after the mid 1960s. Thereafter, blue crab specimens were rarely reported from fisheries and annual landings were very low (<0.5 t) until 2007 (Auction Agency of Greece, 2018), when the population boomed, especially in Methoni Bay, an estuarine area of Thermaikos Gulf (Kevrekidis, 2010; Kevrekidis et al., 2013). Despite the crab's low sale price, of 3-5 €/kg, there is an ongoing increase in demand due to its high nutritional value and quality of its taste (Celik et al., 2004; Kucukgulmez et al., 2006), leading to the development of a targeted artisanal fishery utilizing fyke nets and exporting the species mainly to Europe and Asia. In Methoni Bay, the number of fishery-boats increased from 2-3 in the period 2007-2009 to 20-22 in 2017. The fishing period ranges from May till November, with a summer peak (June-September), despite legislative restrictions of not allowing the use of fyke nets in July and August and at depths <2 m.

The biology of *C. sapidus* has been extensively studied in the Atlantic (e.g., Hines et al., 1987; Lipcius & Van Engel, 1990; Carr et al., 2004; Graham et al., 2012). In the Mediterranean Sea, little information exists on population structure, body size, reproduction, growth and trophic position from SE Turkish (Enzenroß et al., 1997; Atar & Secer, 2003; Ozcan & Akyurt, 2006; Sumer et al., 2013, Tureli-Bilen & Yesilyurt, 2014; Tureli et al., 2016, 2018), Egyptian (Abdel-Razek et al., 2016), Adriatic and Ionian lagoons (Mancinelli et al., 2013, 2016, 2017c). Relevant data, however, are lacking for the Aegean Sea populations.

Considering all the above, the present work aims at studying the population dynamics of *C. sapidus*, a species of ecological and economic importance, in Methoni Bay (Thermaikos Gulf, northern Aegean Sea), where a fully established population exists, focusing on the assessment of abundance and population structure of its natural stocks.

MATERIAL AND METHODS

The study has been conducted in Methoni Bay, a relatively small and shallow (up to 15 m depth) embayment, SW of the Aliakmon estuary (fig. 1). The sea

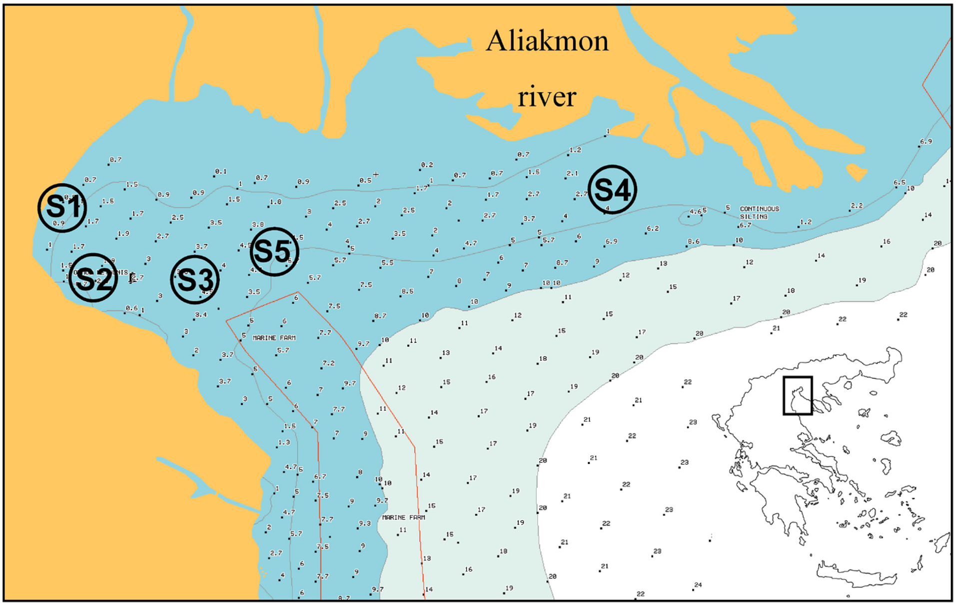

Fig. 1. Map of the study area indicating the location of sampling stations (S1-S5) in Methoni Bay (northern Aegean Sea). Stations depth: S1 = 0.5 m, S2 = 1.0 m, S3 = 3.0 m, S4 = 3.5 m, S5 = 5.0 m.

bottom consists of soft substratum: unvegetated sediments composed mainly of mud or muddy sands. After preliminary surveys, five stations were set that differed in depth and distance from the mouth of the Aliakmon river: S1 = 0.5 m, S2 = 1 m, S3 = 3 m, S4 = 3.5 m, S5 = 5 m. The distance of the shallower stations (S1, S2) from the Aliakmon mouth was approximately 4.5 km; S3 and S5 were placed in intermediate distance, whereas S4 was the closest (approximately 350 m) to the Aliakmon mouth. Sampling took place from March 2011 to January 2012, at bimonthly intervals (26 Mar. 2011, 14 May 2011, 23 Jul. 2011, 24 Sep. 2011, 26 Nov. 2011, 23 Jan. 2012) using 5 pairs of fyke nets (mesh size 40 mm) per station, following local fishery practice. Fyke nets were deployed in the previous afternoon and collected the next morning, i.e., after about 15 hours. In March 2011 no samples were taken from stations S3, S4 and S5. Surface water temperature and salinity were also recorded with a Hang-Lange automatic recorder at each sampling station and period.

Abundance was estimated through catch per unit effort (CPUE), as the number of individuals collected by the 5 pairs of fyke nets (indiv./10 fyke nets). All the collected *Callinectes sapidus* specimens were transferred to the laboratory and sexed according to the shape of their abdomen. The presence of ovigerous females, and the stage of egg development based on the coloration of the egg mass were also recorded (early stages: bright orange, late stages: dark brown, see Van Engel, 1958). For each crab, the carapace width (CW), including lateral spines, was measured with a digital Vernier calliper to the nearest 0.01 mm.

Analysis of variance (two-way balanced ANOVA) was used to test for significant spatial (among sampling stations, 5-level fixed factor) and temporal (among sampling periods, 6-level fixed factor) differences, and of their interactions, in the abundance and the biometry (CW) of the blue crab's population, through a general linear model (Underwood, 1997). Prior to the analyses, the homogeneity of variances was tested by Cohran's test. The Fisher Least Significant Difference test was used for post hoc comparisons. ANOVAs were repeated for male and female crabs, separately. Crabs of CW <60 mm were considered as juveniles (Olmi & Bishop, 1983; Cadman & Weinstein, 1985; Rugolo et al., 1998).

Size-frequency distributions were calculated per 10 mm CW classes to study population structure. Size-frequency distributions were constructed for each sex separately and significant differences between female and male size distributions and medians were tested with two-sample Kolmogorov-Smirnov (z) and Mann-Whitney (U) tests, respectively.

The sex ratio (♂/♀) was estimated per sampling period and deviations from 1 were estimated applying a chi-square (χ^2) test; the test was applied only for samples of >50 individuals.

All statistical analyses were performed using the SPSS software package.

RESULTS

The sea surface temperature followed the seasonal pattern of atmospheric warming, ranging from as low as 3.6°C (S2) in January to 28.4°C (S1 and S2) in July. In March, July and September slight temperature differences were recorded between stations (less than 1°C) in contrast to the large spatial fluctuations (4-5°C) observed in January (from 3.6°C in S2 to 8.8°C in S4), May (from 19.1°C in S4 to 23°C in S2) and November (from 10.5°C in S2 to 14.7°C in S4). Salinity ranged from 26.2 psu in S1 and S2 (May 2011) to 37 psu (S1) in March. In general, decreased values were recorded in May and July (<30 psu) and increased in January and March (>34 psu), with intermediate values in-between. Only slight spatial differences (2-3 psu) were recorded, mostly referring to S4, the station closest to the Aliakmon estuary.

A rather dense *Callinectes sapidus* population was found in Methoni Bay. CPUE ranged from 2 to 46 indiv./10 fyke nets, with an overall mean (±sd) of 21.72 ± 13.64 indiv./10 fyke nets. Mean CPUE showed significant variation among temporal samplings (ANOVA, $F = 6.95$, $p<0.001$); the relevant spatial differences were non-significant (table I, ANOVA results). The highest and lowest

TABLE I

ANOVA results for spatial (sampling station) and temporal (sampling period) effects on the abundance (as CPUE) of *Callinectes sapidus* Rathbun, 1896 population and of male and female blue crabs, separately

Factor		Abundance as CPUE (N/10-fyke nets)					
		Blue crab population		Female blue crabs		Male blue crabs	
	df	F	p	F	p	F	p
Sampling period	5	6.95	0.001*	2.71	0.058	14.73	0.000*
Sampling station	4	1.45	0.264	3.64	0.027*	1.03	0.421

The population abundance of the blue crab and of male crabs only showed significant spatial differences, whereas female crabs displayed temporal differences. The relevant patterns are presented in fig. 2.
*Significant differences in abundance.

values were recorded in July and January, respectively (fig. 2), a pattern correlated with seawater temperature (Spearman, $\rho = 0.67$). CPUE also differed according to sex ($F = 7.26$, $p<0.05$) in favour of males; therefore, spatiotemporal trends were examined separately for males and females. Male crabs followed exactly the same

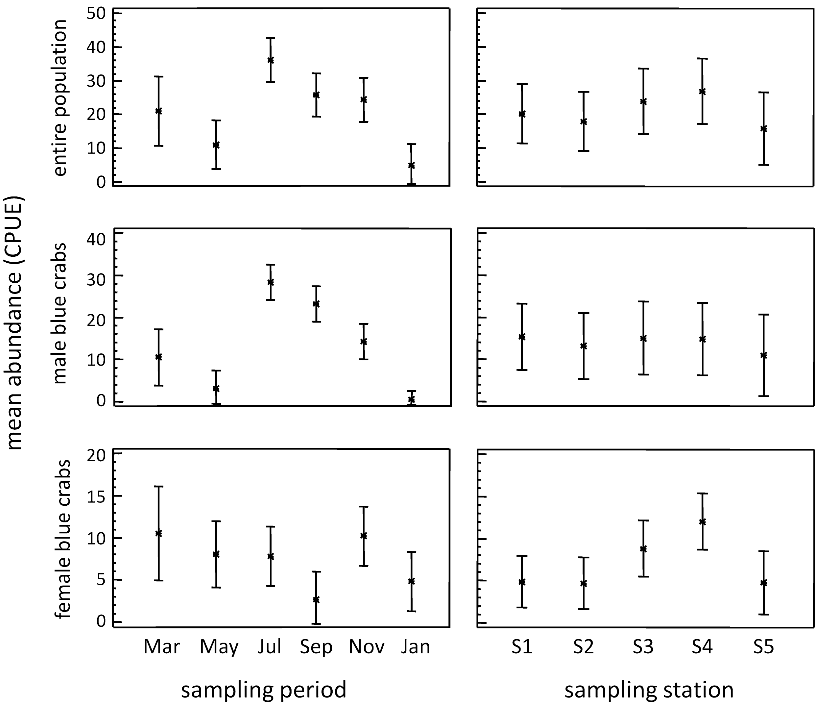

Fig. 2. Mean CPUE (± LSD value) of *Callinectes sapidus* Rathbun, 1896 population, and of male and female blue crabs, separately, in Methoni Bay per sampling period and station (S1-S5).

pattern as the *C. sapidus* population as a whole, in contrast to female crabs, that showed significant spatial differences, with increased values in S4 (table I, fig. 2). The temporal trends of male and female crab abundance were highly correlated with seawater temperature ($\rho = 0.76$ and $\rho = 0.96$, respectively).

Overall, 543 individuals, 364 males and 179 females, were collected and measured to describe the size structure of the population (fig. 3); the smallest crab (♀) measured 50.00 mm in CW and the largest 177.29 mm (♂). Five juveniles (three ♀♀ and two ♂♂) with CW ranging from 50 to 58.98 mm were only caught, but excluded from the further analyses. The mean CW (±sd) of the adult population was 134.75 ± 19.71 mm and 133.57 ± 20.14 mm for males and females, respectively. Mean CW showed non-significant differences between sexes ($F = 0.57$, $p = 0.45$), but exhibited significant spatiotemporal differences ($F = 4.57$, $p<0.001$ and $F = 16.84$, $p<0.001$, for spatial and temporal effects, respectively) with larger crabs caught in May and from the deeper station (S5) and

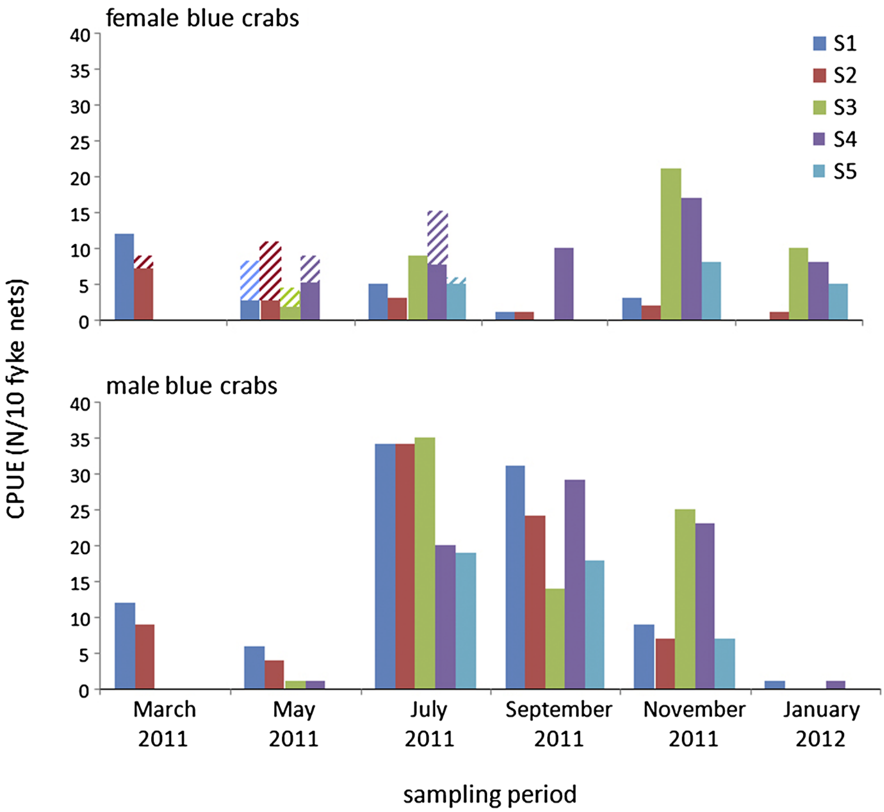

Fig. 3. Number of male and female specimens (including juveniles) of *Callinectes sapidus* Rathbun, 1896 collected using fyke nets in Methoni Bay per sampling period and station (S1-S5). Ovigerous females also noted with hatched bars.

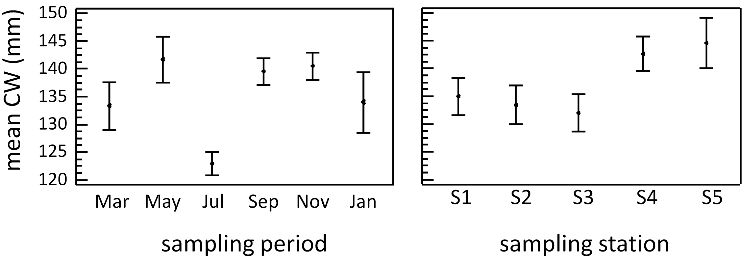

Fig. 4. Mean carapace width (CW) (± LSD value) of *Callinectes sapidus* Rathbun, 1896 population in Methoni Bay per sampling period and station (S1-S5).

the station closest to the Aliakmon estuary (S4) (fig. 4). However, a significant interaction was detected ($F = 8.96$, $p<0.001$), not allowing further comparisons: a result produced mainly by the presence of small crabs in January at S2. Size (CW) frequency distributions and medians showed non-significant differences between sexes ($z = 0.716$, $p = 0.68$; $U = 32\,119.5$, $p = 0.79$), and thus, they were constructed for the entire population by grouping stations in two sets, (A) by pooling data over S1, S2 and S3, and (B) by pooling data over S4 and S5 (fig. 5).

Only adult crabs were considered to describe the sex ratio, which was found biased in favour of males, ♂/♀ = 2.04 : 1. Male to female ratios deviated from unity in all tested samples ($p<0.001$), apart from March, where the population was equally represented by both sexes. However, in that time all the specimens of *C. sapidus* came from two out of the five stations (sampling was completed only in S1 and S2), and so, this result should be viewed with precaution. The proportion of females was higher in May and in January (fig. 6).

Thirty-three ovigerous females (18% of total females) were collected (fig. 3), with a CW ranging from 120.75 to 159.79 mm (mean CW ± sd = 138.2 ± 9.71). The highest number was caught in May ($N = 22$) followed by July ($N = 9$), and the remaining 2 specimens were fished in March. Ovigerous females were found at all stations. In May, 15 out of the 22 ovigerous females were caught from the shallower stations (S1, S2). In July, almost all of these (8 out of 9) came from S4, the station closest to the Aliakmon estuary, and the remaining one from the deeper station (S5). The highest number of ovigerous crabs with eggs in late developmental stages was recorded in May (13 individuals) at all stations except S5 (5 m); another two were caught in July from S4 and S5. In March, two ovigerous females were caught (S2) having eggs in early developmental stages.

DISCUSSION

Callinectes sapidus has a complex life cycle, involving specific seasonal and spatial migrations for copulation and reproduction (e.g., Van Engel, 1958; Hines,

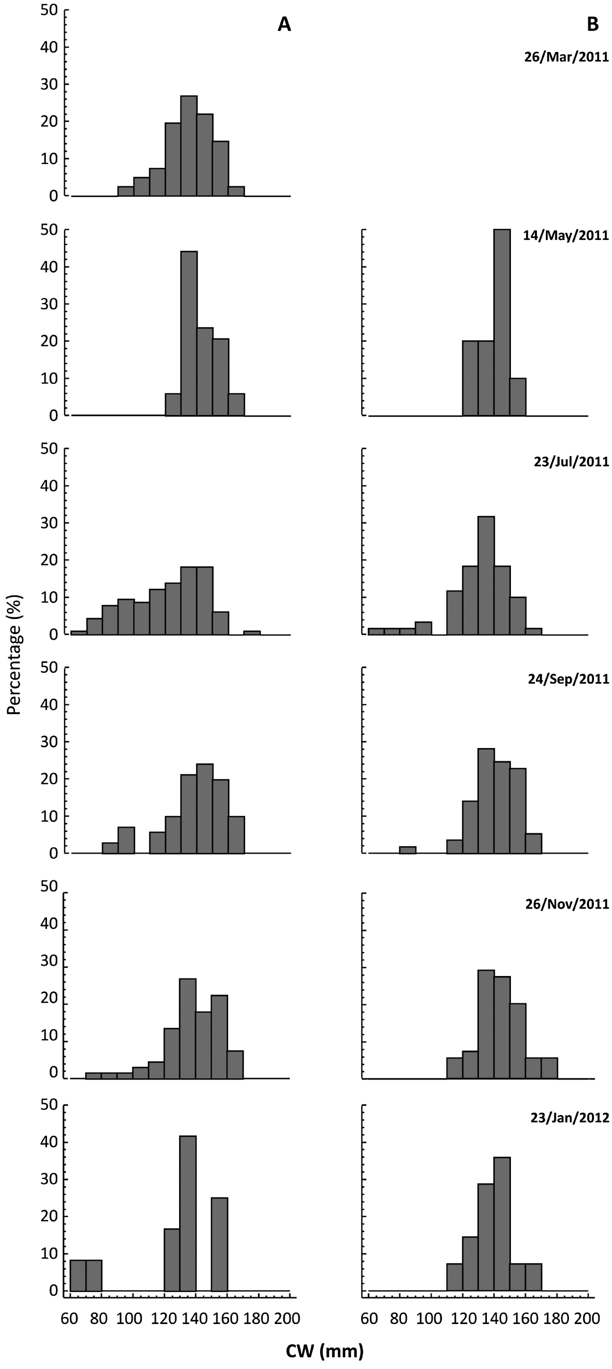

Fig. 5. Size (CW) frequency distribution analysis of *Callinectes sapidus* Rathbun, 1896 in Methoni Bay per sampling period. Stations are grouped as follows: Group A = S1, S2, S3 and Group B = S4, S5.

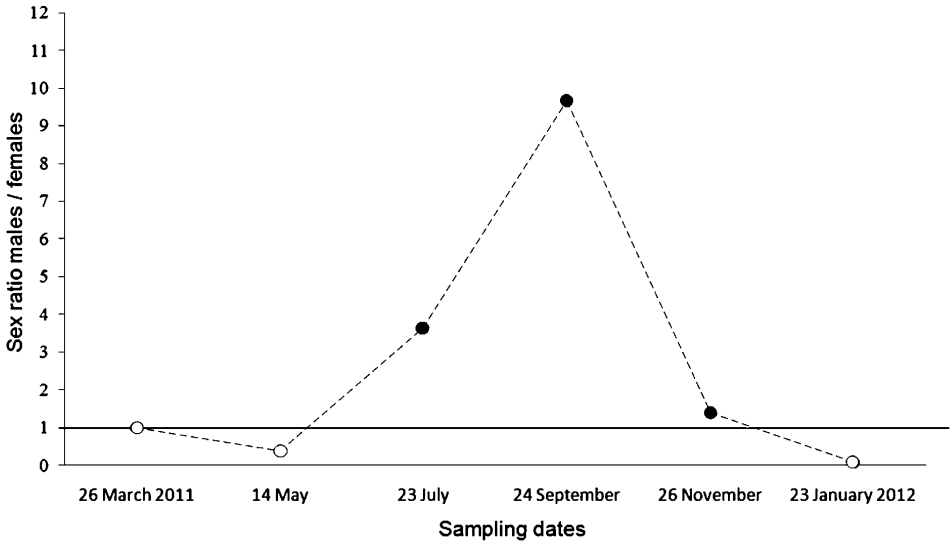

Fig. 6. Sex ratio (male/female) of the *Callinectes sapidus* Rathbun, 1896 population in Methoni Bay per sampling period (black spot denotes significant deviations from unity at $p<0.001$, white spot denotes non-tested samples).

2003; Turner et al., 2003; Carr et al., 2004; Aguilar et al., 2005; Forward et al., 2005; Jivoff et al., 2007) thus generating significant spatiotemporal variability in its population abundance on a local scale. In Methoni Bay, the population studied decreased in January, a pattern correlated with seawater temperature. Declining abundance during the cold season of the year has also been referred to from other Mediterranean and Atlantic regions (Hines et al., 1987; Lipcius & Van Engel, 1990; Pereira et al., 2009; Mancinelli et al., 2013; but see Sumer et al., 2013, reporting on an annually stable population in a warm Levantine lagoon). Migration towards greater depths of Methoni Bay for wintering may explain the significant reduction of the blue crab's abundance during the cold period, which in turn indicates a seasonal migration pattern of the species in the study area.

Abundance patterns differed between sexes; males followed the same pattern as the entire population, with non-significant spatial differences, in contrast to females, whose abundance was found increased closest to the Aliakmon estuary. The number of female crabs was increased at the brackish water station (S4) as well as at the station of moderate depth (S3), especially from May through November, suggesting a specific spatiotemporal migration pattern. This possibly reflects migratory trends for spawning, as the higher percentage of ovigerous females was found in May and in July, specifically at the shallow and at the estuarine stations, respectively. Female blue crabs generally extrude eggs during summer and the embryos develop for 2-3 weeks before hatching (Millikin &

Williams, 1984). As the first ovigerous females appeared in March, carrying eggs in early developmental stages, it seems possible that the spawning period in Methoni Bay begins in mid spring and lasts until mid summer. However, a more intensive sampling strategy is required for properly addressing the reproductive biology of the species in Methoni Bay.

Dulcic et al. (2011) reported that the blue crab population is partitioned with respect to sex in the Neretva River delta (Croatia) and Cilenti et al. (2015) mentioned that the higher proportion of females in the Varano and Lesina lagoons (Adriatic Sea, Italy) was area-dependent, as adult and ovigerous females were found near the mouth of the seaward channels, and they accordingly characterized these areas as spawning sites. Either males or females, or both, prevailed the studied population in different spatiotemporal samplings and accordingly, the fyke nets used for the blue crab fishery in Methoni Bay seem not to be sex-selective. Males predominated in the studied population, especially in July and September; in January females prevailed, as only two male crabs were caught. In May, the sex ratio approximated unity in the shallower station (0.5 m depth), while the majority of the catch of the blue crab originated from stations S1 and S2. As salinity had the lowest values in that period (26 psu), the blue crab population probably congregates in shallow, brackish waters for mating. At the deeper station (S3), including the estuarine station (S4), females outnumbered males; thus, a spatial divergence in sex ratio occurred, probably related to the spawning migration pattern of the species. Very different results have been reported earlier for the sex ratio of *C. sapidus* populations. A predominance of males has been reported in low-salinity areas of the Gulf of Mexico from March to November (Johnson & Perry, 1999), in contrast to results from SW Atlantic areas, where females predominated in most of the sampling months (Pereira et al., 2009). According to Fitz & Wiegert (1992), the sex ratio is heavily biased in favour of males when the size structure in the population shifts towards larger sizes. Within the Mediterranean Sea, our results conform to similar studies from Italian (Mancinelli et al., 2013; Carrozzo et al., 2014), Croatian (Dulcic et al., 2011) and Egyptian lagoons (Abdel Razek et al., 2016), but diverge with respect to sex ratio results from a southern Turkish lagoon, where the population of this crab was found to be biased in favour of females (Sumer et al., 2013). A seasonal transition of the sex ratio according to day-length and water temperature has been reported for *C. sapidus*, with a positive response in the case of the abundance of male crabs, and a negative one in the case of females (Harding & Mann, 2010).

The size spectra of the blue crab population were rather similar among temporal samplings; nevertheless, significant differences in mean size were detected seasonally. Larger crabs were caught in May and smaller specimens in July, which is in agreement with similar studies from the Atlantic (e.g., Graham et al., 2012). This may reflect the seasonal entry of new recruits in the fished population, together with the migratory trends of the species. It is worth noting that three out of the five collected juveniles were caught in July and the remaining two in March. Larger crabs were found at deeper stations and at the estuarine station, a pattern mostly produced by females. Mature, mated females, which are often $>$140 mm CW, are catadromous, migrating from hyposaline ($<$30 psu) to higher salinity waters, in the lower estuary and offshore, to spawn (Hines et al., 1987; Steele & Bert, 1994), and this may contributes to the increasing size of crabs in greater depths.

Mean size (CW) and size frequency distributions were similar between sexes, despite the visual predominance of males in size due to their larger propodus of the right claw. Larger males compared to females have been reported from Levantine populations, reaching larger CW_{∞} but following a lower k (Sumer et al., 2013; Turelli et al., 2016). Three size classes have been reported to constitute the age structure of the blue crab population as follows: 0^{+} ($<$60 mm CW), 1^{+} ($>$60 $<$120 mm CW) and 2^{+} ($>$120 mm CW) (Rugolo et al., 1998). Accordingly, as no relevant studies on growth and age structure are available from northern Aegean *C. sapidus* populations, the bulk of the fished blue crab population in Methoni Bay probably belongs to the 2^{+} age class.

The environmental characteristics of Thermaikos Gulf, such as the extended deltaic areas of the Axios-Loudias-Aliakmonas riverine system, with numerous small lagoons and channels, and the increased productivity (see Papathanassiou & Zenetos, 2005) seems to favour the *C. sapidus* population. However, the area is also one of the most anthropogenically impacted areas of Greece, with, since the 1990s, an extensive, industrialized mussel-culture zone that might have affected blue crab's abundance.

In the present study, the mesh size of the fyke nets used by fishermen seems to be adequately selective, as only five juveniles have occasionally been caught and most specimens were $>$120 mm CW. This highly contrasts the situation of the SW Atlantic fisheries, where about half of the catch concerns juveniles (Pereira et al., 2009). Therefore, the existing national (mesh size) fisheries legislation of fyke nets seems not to affect the species' recruitment. This, along with the high fertility of the species and the common good-practice of local fishermen targeting males and discarding ovigerous females, may prevent overfishing of *C. sapidus* natural stocks. However, a declining trend in landings after 2011 in Thermaikos may be inferred, according to the Auction Agency of Greece (2018), and this may cause concerns for future sustainability.

The *Callinectes sapidus* population seems to be well established in Thermaikos Gulf, consisting of large-sized individuals and completing the usual life cycle of the species, which involves complex spatiotemporal migratory patterns for both sexes, in Methoni Bay. Despite the fishing pressure applied by the local, artisanal fishery, the exploitation seems to be maintained at sustainable levels, so far. However, the increasing demand for food and the economic profit of exporting live and processed crabs, can not be ignored. This observation, in combination with the limited knowledge on key aspects of the species' biology, such as definition of reproductive and recruitment periods and areas, mapping of migratory patterns, and of spawning grounds, may well prove inadequate for a proper, knowledge-based management of this valuable natural resource. The above results, interesting as they may be, are insufficient to reveal the entire outline of this crab's biology.

Thus, in order to base measures for the management of the population of *C. sapidus* in this region on a firm scientific footing, more knowledge will have to be acquired, as a crucial prerequisite for such regulations. This obviously necessitates a more intensive study of the species in the area, including recurrent monitoring of its population: during more than one year cycle and in a larger area. Only then can scientists provide for the implementation of a sustainable management plan for the entire Thermaikos Gulf, under the auspices of seascape planning.

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