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Abstract

Population structure, age, growth, mortality, and reproduction patterns of the Mediterranean green crab *Carcinus aestuarii* were determined for the native population in Parila Lagoon (Neretva Estuary, Middle Adriatic, Croatia). The population size structure showed two distinct cohorts: (1) specimens with a carapace width of 20–34 mm and dominated by females and (2) large-sized specimens with a carapace width > 34 mm with males significantly dominating and no females found above 46 mm. Males appeared to grow faster than females in the first and second year of the life cycle. Most of the natural mortality (70.4%) occurred during the first year of life. This indicates high predation pressure from fish and other crab species on small-sized (less than 25 mm) *C. aestuarii* cohorts. The peak of ovigerous female occurrence occurred in January 2015. A very small percentage of ovigerous females appeared in June 2015. The 50% ovigerous size for the population was estimated at a carapace width of 29.65 mm and weight of 10.39 g. The average fecundity was 61,017 eggs/female, with an average fecundity per gram of female wet weight of 4,804 eggs. The biological characteristics and

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population dynamics of *C. aestuarii* show that life history traits of this species (such as the smallest and average ovigerous female size, the mortality rate during the first year of life or the establishment of a second year, and a late-spring reproduction period) should be used to monitor potential changes in ecosystem properties of Mediterranean transitional waters and to manage potential fisheries.

Estuaries and other transitional habitats are impacted worldwide by an increasing number of pressures, including physical and chemical transformation, habitat destruction, changes in biodiversity, and recent climatic warming (Halpern et al. 2008; Robins et al. 2016). Given this general scenario, the Parila Lagoon (Neretva Estuary, Adriatic Sea, Croatia), representing the largest transitional system and the most important nursery ground along the eastern Adriatic coast (Bartulović et al. 2007, 2009; Dulčić et al. 2007; B. Glamuzina et al. 2014), makes no exception. The good ecological status and function of the lagoon is of crucial importance for many species targeted by artisanal fishery (Bartulović et al. 2006; L. Glamuzina et al. 2014) and to the socioeconomy of the area (Glamuzina 2010). However, the lagoon currently suffers from several impacts, including biological invasions (e.g., the Atlantic blue crab *Callinectes sapidus* [Onofri et al. 2008; Dulčić et al. 2011; Mancinelli et al. 2016]) and anomalous water temperature rises in summer (Sarà et al. 2014). Management and monitoring actions of the Parila Lagoon, as well as of other transitional ecosystems, are complex and costly, requiring the development of biological indicators effective in assessing the current ecological status, indicative of the underlying causes of environmental change and suggestive of future environmental changes (Van Den Broeck et al. 2015).

One of the species with a recognized potential to be used as a biological indicator is the Mediterranean green crab *Carcinus aestuarii*. It is a member of the Portunidae family, shares great similarities with the congeneric European green crab *C. maenas*, and was considered a subspecies of *C. maenas* until a molecular biological study based on the cytochrome *c* oxidase gene from samples originating from the North Atlantic and the Mediterranean showed that the difference was substantial enough to separate them into two different species (Roman and Palumbi 2004). The basic differences between the two species are that (1) the proportion between the width and the length of the carapace is typically less than 1.27 for *C. aestuarii* and more than 1.29 for *C. maenas* and that (2) the form of the frontal spines and the pleopods in males are straight and parallel for *C. aestuarii* while those of *C. maenas* are curved (Behrens Yamada and Hauck 2001).

Commonly, *C. aestuarii* is an inhabitant of coastal areas, estuaries, and lagoons, with a rather small depth distribution down to 26 m (Abelló et al. 1988). Until the end of the 20th century, it was recorded only in the Mediterranean and Black seas. However, it was recently introduced in the Canary Islands region, Tokyo Bay in Japan, and South Africa through ballast waters, and therefore it can be considered as much of a global invader as the related *C. maenas* (Sakai 1986; Furota et al. 1999; Behrens Yamada and Hauck 2001; Carlton and Cohen 2003; Chen et al. 2004). *Carcinus aestuarii* is currently used as a biomarker species due to its high adaptability and resilience in coastal eutrophic waters (Losso and Ghirardini 2010; Ricciardi et al. 2010; Leignel et al. 2014). The species is known to play an important role in native coastal ecosystems due to its value as a food item for several high-market-value fish species, such as Gilthead Seabream *Sparus aurata*, European Sea Bass *Dicentrarchus labrax*, and European Eel *Anguilla anguilla* (Mori et al. 1990; Özbek et al. 2012). Based on its diet, the species exhibits a rather high trophic level value in invaded ecosystems of 3.43 ± 0.35 (adults; Chen et al. 2004), similar to native ecosystems, such as 3.63 ± 0.11 in Parila Lagoon (Mancinelli et al. 2016).

Less attention has been given to the analysis of population characteristics as ecological indicators in *C. aestuarii* and, in general, in other bioindicator crab species. For fish, analysis of length–weight population structure and other growth parameters has been proven useful in providing insight into the status of the estuarine ecosystem (Whitfield and Elliott 2002). In addition, body condition, fecundity, and other metrics of marine vertebrate and invertebrate species have been recently acknowledged as "surveillance indicators" to be necessarily implemented within the Marine Strategy Framework Directive (Shephard et al. 2015).

Several biological characteristics and population dynamics parameters of *C. aestuarii* have been recently described for coastal lagoons in Turkey (Özcan et al. 2009; Koçak et al. 2011; Özbek et al. 2012), Tunisia (Baklouti et al. 2013), and Italy (Lumare et al. 2009; Cilenti et al. 2014), making possible comparison of crab properties in different Mediterranean ecosystems. *Carcinus aestuarii* has no commercial value along the Croatian coastline, but in some areas of the Mediterranean Sea (for example in Venice Lagoon, Italy), it is collected from the wild and sold as a delicacy in the phase of molting during spring and autumn (product locally named "moleche"; Cilenti et al. 2014).

This study evaluated population dynamics and reproduction characteristics of *C. aestuarii* in Parila Lagoon to enhance species fishery management and to select robust early warning species life patterns to be used as indicators in a holistic ecosystem management framework.

METHODS

Study area description.—The study was carried out in the Parila Lagoon in the southeastern Adriatic Sea (Figure 1). The lagoon is located north of the Neretva River mouth, between the river estuary and the city of Ploče (34°1′49.1″N, 17°26′59.4″E). The lagoon is characterized by a muddy bottom with patches of seagrass *Cymodocea nodosa* and a maximum depth of 1 m. Water temperature in the sampling area varied from a low of 9.7°C in February to a high of 26.4°C in August. Average annual water temperature was 17.61°C. Salinity varied from 14.5‰ in February to 17.3‰ in May, 26.3‰ in August, and 12.2‰ in November.

Sampling and measurements.—Sampling was carried out on a monthly basis between January 2015 and January 2016 using a static traditional trap used for the eel fishery, deployed during a 24-h period and at a depth between 0.5 and 1 m. A total number of 1,844 male and 2,112 female individuals of *C. aestuarii* were collected. Crabs were analyzed in vivo immediately after sampling, while gravid females were preserved in 4% formalin for reproduction analysis. The presence of crabs in the stomachs of fish species was recorded when fish specimens were available.

The carapace width (CW) was measured using a digital caliper (in mm), and the total body wet weight (TW) was
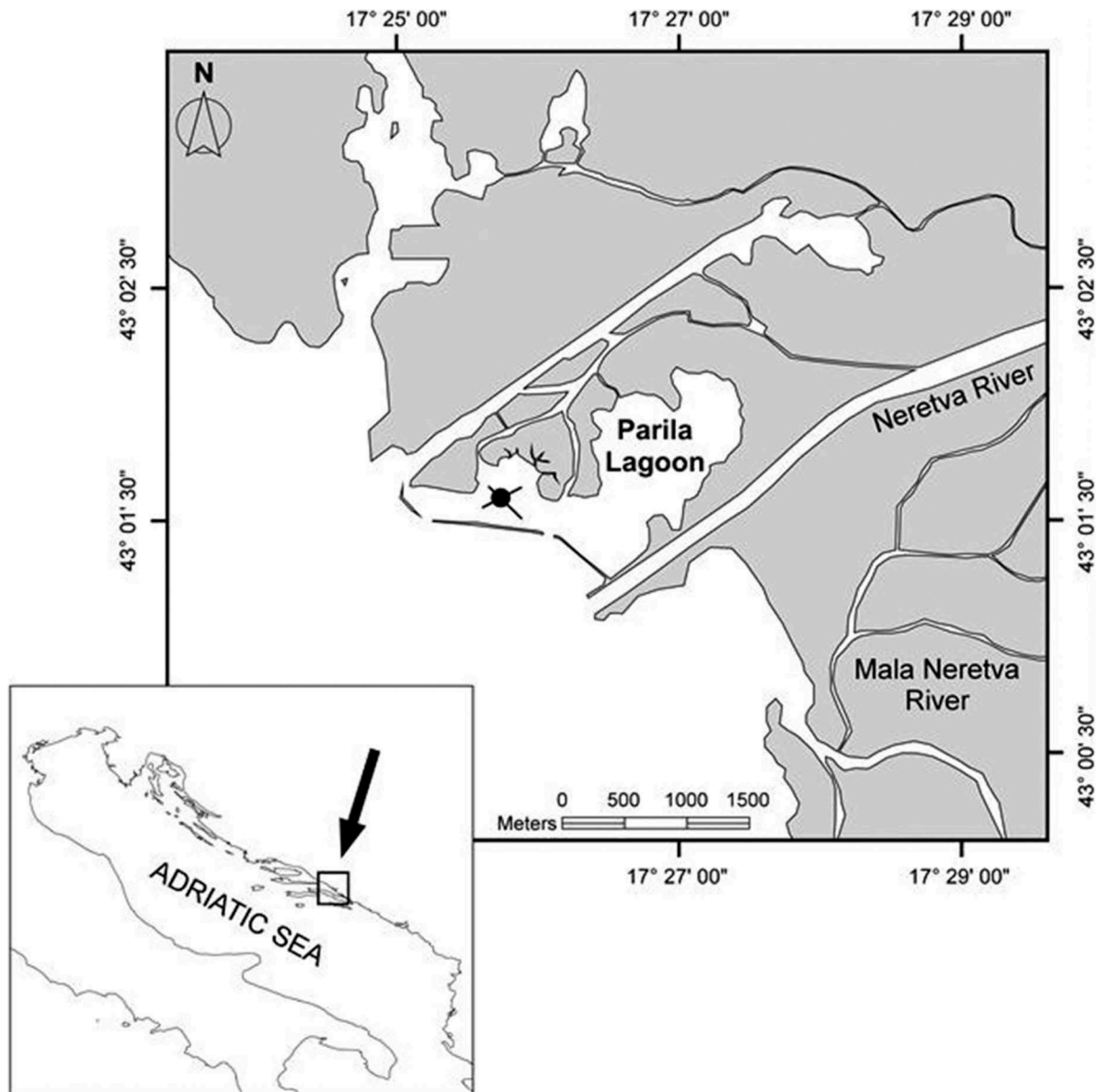

FIGURE 1. Map of the study area, the Parila Lagoon (Neretva Estuary, Adriatic Sea, Croatia), showing the sampling station (black dot).

measured using a digital scale. The sampled individuals were sexed and the sex ratio was estimated as female : male equations on a monthly and yearly basis. During the spawning period, fecundity was estimated as the total number of eggs per female. Eggs were counted using the gravimetric approach (measure the number of the eggs in a weighed portion of the egg mass and calculate the total number of eggs based on the total weight of the egg mass). The egg mass was separated from the body of the female using a soft paintbrush to avoid damage to the pleopods and bias to TW estimations.

Analysis of data.—The CW measurements for males and females separately were analyzed using the Bhattacharya method (Bhattacharya 1967) in order to separate age-classes in the samples and construct the length-frequency distribution of the sexes separately and for the total population. The Bhattacharya method is one of the modal progression analysis methods that infer growth by analyzing the shift of the modes of a time series of the length-frequency data based on the length-frequency histogram (Gayanilo et al. 2005). This analysis provided the essential information to estimate the von Bertalanffy (1938) growth curve parameters (CW∞, K, and t_o; Gayanilo et al. 2005). In addition, the growth data were used to estimate the parameters of the Gompertz and three-parameter logistic curves. The best model to explain growth for the species was selected based on the Akaike criterion (Akaike 1974). This criterion offers a relative estimate of the information lost when a given model is used to represent the process that generates the data. In doing so, it deals with the trade-off between the goodness of fit of the model and the complexity of the model.

Fulton's condition index (Fulton 1904) was used and calculated as $K = 100 \times W/L^3$, where W is the wet weight of the crab in grams and L is the length of the crab in millimeters.

Natural mortality (M; per year) was estimated using the age-dependent equations of Chen and Watanabe (1989), while the overall natural mortality was estimated using the empirical equations of Pauly (1980; which is based on $L\infty$, K, and the average ecosystem temperature [T]), Rikhter–Efanov (Rikhter and Efanov 1976), and Jensen (2001). The formulas for the above-mentioned equations to estimate M are as follows:

$$\text{Pauly}: \ln(M) = -0.0152 - 0.279\ln(L\infty)$$
$$+0.6543\ln(K) + 0.463\ln(T)$$

$$\text{Rikhter–Evanov}: M = \frac{1.52}{t_{\text{mass}}^{0.72}} - 0.16$$

$$\text{Jensen}: M = 0.21 + 1.47K$$

where t_{mass} = age at massive maturation.

Total mortality (Z; per year) was estimated using the length-converted catch curve based on the monthly samples of the length-frequency distribution (Jones 1981; Pauly 1983).

The method of length-converted catch curve is based on the estimation of the slope of a linear regression between the number of the organisms (n) in various age-groups and their corresponding age in years. The disadvantage of several organisms that cannot be aged individually (decapods for example) is overcome by using length-frequency data without the need for age–length keys (Pauly 1983). Since there is no fishing mortality in the target area, it was considered that $Z = M$ and, therefore, the overall natural and total mortality were calculated as the total average of all the empirical approaches.

Fecundity (number of eggs) and egg mass (g) were correlated with CW (mm) based on a standard regression model of the following form: Fecundity = $a \times \text{CW}^b$, where a is the $\log_{10}$ coefficient of the slope and b is the power coefficient (Pitcher and Hart 1982). Unlike other organisms, the number of eggs produced per individual for marine species increases with age as a consequence of indeterminate growth patterns. In general, fecundity is related to some power of length although considerable variability is usually observed (Bagenal 1973; Pitcher and Hart 1982). Size-at-maturity analysis was based on the proportion of ovigerous females in the total of sampled females during the spawning period (December–February). Size at maturity was correlated with CW based on a standard three-coefficient logistic equation model:

$$\text{Ovigerous females}(\%) = \frac{a}{1 + e^{\frac{\text{CW}-b}{c}}},$$

where a, b, and c are coefficients. The CW and TW were correlated based on the power model ($y = ax^b$). Changes in the monthly population structure were studied based on an analysis of covariance test (ANCOVA) of monthly CW/TW log-linear regression relationships. The ANCOVA is a combination of ANOVA tests and regression analysis and aims to examine the influence of TW on CW using the month number (dummy variable = 1–12) as a separation factor (explanatory variable) in order to examine the results on a per month basis. The grouping of the month equations based on similarity was based on Fisher's least-significant-difference multiple-range test. One-way ANOVA was used to compare multiple sample means instead of a t-test (an ANOVA and t-test give the same results in the case of two samples).

Statistical analysis was carried out using XLSTAT for PC, while stock assessment was carried out using FiSAT II FAO/ICLARM Stock Assessment Tool (http://www.fao.org/fishery/topic/16072/en).

RESULTS

Population structure is presented in Figure 2. Females dominated in the cohort with a carapace width between 20 and 34 mm. In the cohort of specimens above 34 mm, the males significantly dominated, and no females were found above 46 mm.

Length–weight relationships and the equations for females and males are presented in Figure 3. The ANCOVA showed
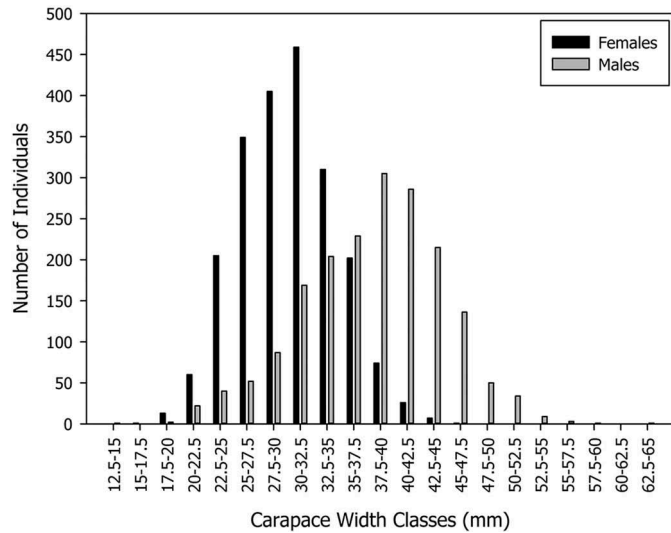
FIGURE 2. Population structure for males and females of *C. aestuarii* in Parila Lagoon (Neretva Estuary, Croatia).

Females
$$CW = 0.00083TW^{2.742},$$
$$r^2 = 0.938, SE = \pm1.49mm$$

Males
$$CW = 0.00043TW^{2.939},$$
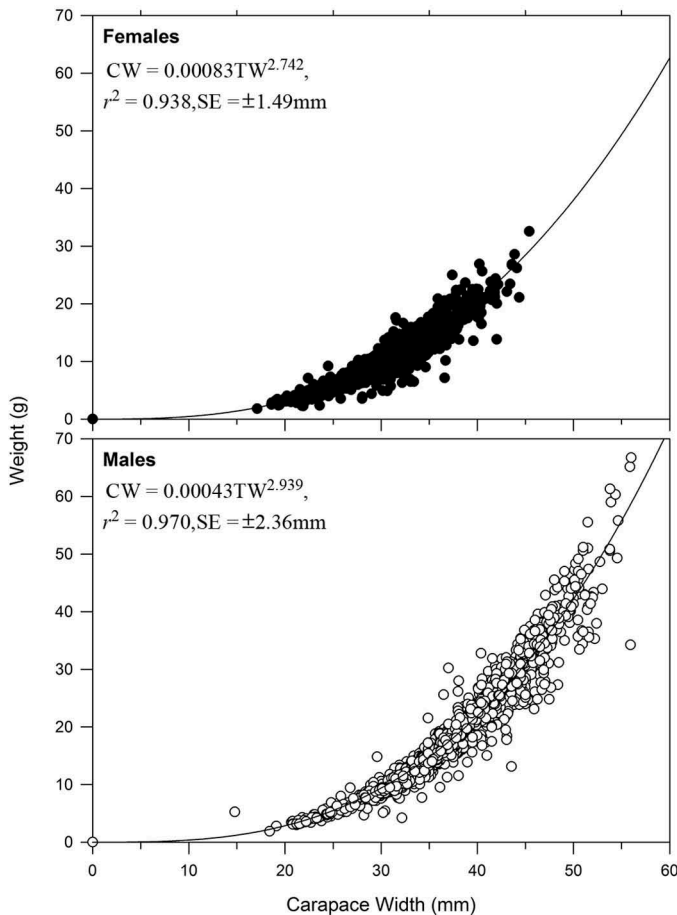$$r^2 = 0.970, SE = \pm2.36mm$$

FIGURE 3. Length–weight relationships for *C. aestuarii* in Parila Lagoon (Neretva Estuary, Croatia).

no significant differences between the monthly CW–TW relationships, indicating that the population structure does not change throughout the year (Table 1).

The condition index is significantly different ($P < 0.05$) between males and females during most of the year ($t = 7.15$, $P = 0.000$). This is only not the case in February and March after egg releasing ($t = 0.24$, $P = 0.810$) and in December ($t = 1.88$, $P = 0.062$). In the April–November period, the condition index is significantly higher for males and is highest in August ($t = 11.28$, $P = 0.000$) (Figure 4).

The growth and equation formulas of females and males are presented in Figure 5. The CW∞ and K parameters of the male and female growth equations were statistically different using a two-sided t-test (df = 3,954; level $a = 0.05$). Males grew faster than females in the first and second year of life. The difference was statistically significant in the second year of the life.

Since there is no fishing practice on *C. aestuarii* in the Parila Lagoon, there is no fishing mortality. The natural mortality (0.704) is a consequence of the high mortality occurred during the first year of the life (Figure 6).

The overall sex ratio for females to males was 0.53:0.47. The sex ratio was in favor of females during the spawning period from December to February. It was in favor of males in the period from April to September. In March and October, the sex ratio was marginally in favor of males (almost within the confidence interval of the chi-square test for deviation from a 1:1 proportion), while in November it was marginally in favor of females (Figure 7).

Based on the presence of ovigerous females in the population, spawning occurs during the period of mid-December to early February. The peak of ovigerous female appearance (100% of the females in the samples) occurs at the end of December and in January. A very small percentage of ovigerous females appeared in June (4%), when three individuals were also found in European Sea Bass stomach contents (Figure 7).

The smallest ovigerous female was found on January 21, 2016, and exhibited a CW of 19.3 mm, a TW of 3.2 g, and carried an egg mass weighing 0.67 g. The egg mass weight per female ranged between 0.21 and 6.04 g (average = 2.07 ± 1.06 g). The average percentage of egg mass in fresh weight of females was 18.06% and ranged from 8.47% to 35.26%. The analysis of the egg mass weight frequency among the ovigerous female cohort showed that most of the ovigerous females in the samples carried eggs with a weight between 1 and 3 g and belonged to the 24–29 mm CW class (Figure 8).

The relationship and equation between the percentage of ovigerous females to the total female population is presented in Figure 9. The analysis showed that the 50% gravid size for the population exhibits a size of 29.65 mm and weight of 10.39 g (based on the CW–TW relationship for females).

The relationship and equation showing fecundity in relation to CW is included in Figure 10. The average fecundity was 61,017 eggs/female and ranged from 6,482 to 169,622 eggs/female. The average fecundity per 1 g of female wet

TABLE 1. Analysis of variance in covariance models of the relationship between carapace width and total body wet weight for *C. aestuarii* in Parila Lagoon (Neretva Estuary, Croatia).

Source	df	Sum of squares	Mean squares	F	Pr > F
Females					
Model	12	147,787.33	12,315.61	252.43	< 0.0001
Error	2,100	102,454.75	48.78		
Corrected total	2,112	250,242.09			
Males					
Model	12	159,356.93	13,279.74	1,546.05	< 0.0001
Error	1,832	15,727.30	8.5895		
Corrected total	1,844	175,084.24			

weight was 4,804 eggs. The average egg diameter was 332 µm, with a range among females from 311 to 363 µm. The color of the eggs varied from orange to yellow, gray, and finally dark, corresponding to the domination of yolk in orange eggs and developed larva in the dark eggs.

DISCUSSION

Classified as one of the world's 100 worst invaders by the International Union for Conservation of Nature, green crabs (*C. aestuarii* and *C. maenas*) are the most widely distributed intertidal crabs in the world. Although, data on the life cycle, diversity, physiological tolerance, genomic investigations, historical invasion, control programs, and putative economical valorisation of green crabs exist (Leignel et al. 2014), information on the biological traits of *C. aestuarii* and *C. maenas* in different geographical areas is still deficient.

The population structure of *C. aestuarii* from Parila Lagoon showed that their size distribution is consistent with those reported for Tunisian populations (Baklouti et al. 2013), in Turkish Aegean Sea ecosystems such as Homa Lagoon in İzmir Bay (Koçak et al. 2011), and along the Italian Adriatic coast (i.e., Cilenti et al. 2014). However, the Parila Lagoon population was considerably different from the population analyzed in the eastern Black Sea, Turkey, where specimens showed a CW that was significantly higher than that in all Mediterranean populations, ranging from 12.5 to 85.5 mm in males and from 21 to 92 mm in females (Aydin 2013). A possible reason for this, as demonstrated by Matozzo et al. (2013), is that *C. aestuarii* morphometric features, as well as haemolymph cellular and biochemical parameters, varied markedly as a function of both season and gender. Thus, it is expected that morphometric differences will be expressed in different geographical areas based on population density, feeding efficiency, food availability, and local environmental conditions.

The weight–size relationship is a useful tool for population status analysis and can provide information about the increase
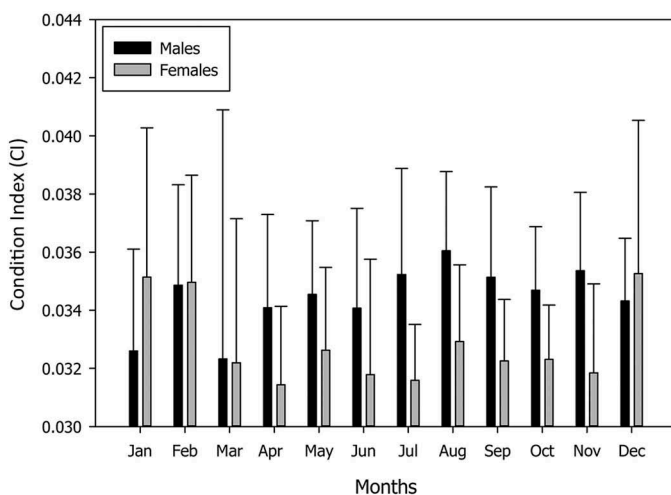

FIGURE 4. Mean condition index of males (black bars) and females (gray bars) of *C. aestuarii* from Parila Lagoon (Neretva Estuary, Croatia). Error bars represent SDs of the monthly mean condition index.

females : $CW_t = 50.49(1 - e^{-0.795(t+0.0018)})$

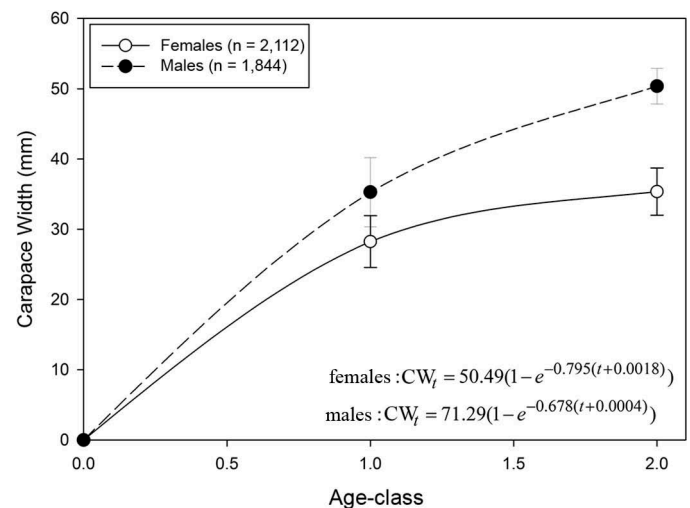males : $CW_t = 71.29(1 - e^{-0.678(t+0.0004)})$

FIGURE 5. Growth of *C. aestuarii* from Parila Lagoon (Neretva Estuary, Croatia). Error bars represent SD.
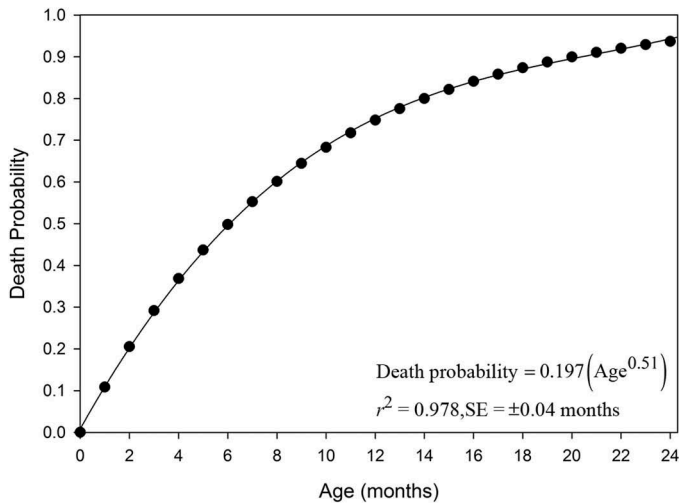
FIGURE 6. Death probability curve for *C. aestuarii* in Parila Lagoon (Neretva Estuary, Croatia).

FIGURE 8. Frequency of ovigerous females by carapace width and egg mass weight for *C. aestuarii* during spawning season.

or decrease in weight of populations. This parameter could also be important for comparative studies between populations. In Parila Lagoon, the power coefficient *b* (of the CW–TW power regression model) values are 2.742 for females and 2.939 for males, showing a negative allometric growth pattern both in males and females. Other populations exhibit similar growth patterns, such as 2.91 for males and 2.89 for females in Homa Lagoon (Koçak et al. 2011) or 2.30–2.92 for males and 2.66–2.81 for females in Çakalburnu Lagoon, İzmir Bay, Turkey (Can et al. 2004). However, positive allometric growth was reported for the Black Sea population, with a *b*-value of 3.117 (Aydin 2013), making this population specific. It seems that ecological conditions in the Black Sea were optimally set
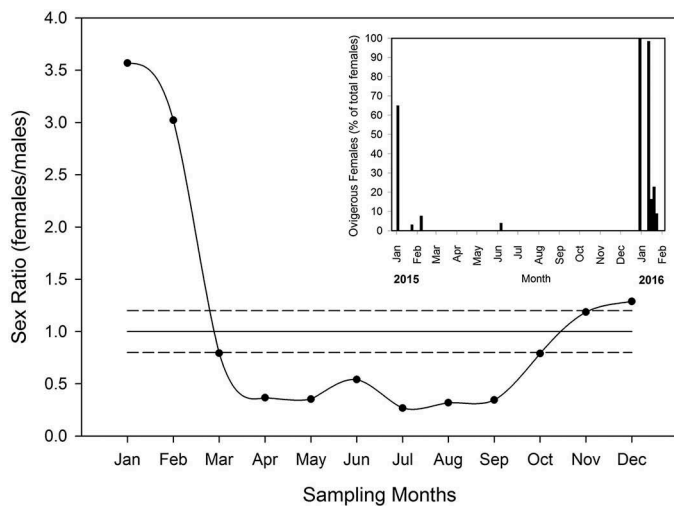
for *C. aestuarii* growth (both in mass and length), as mentioned earlier. The condition index of both males and females from Parila Lagoon is higher than that in Tunisian waters (Baklouti et al. 2013), where condition index variations between females and males were not observed as they were in our study. The condition index values for other investigated Mediterranean populations (i.e., Aegean Sea, western Adriatic coast, and eastern Black Sea) were not reported.

The high natural mortality (0.704) that occurred during the first year of life indicates, among other causes, a high potential predation pressure from fish and other crab species on smaller (less than 25 mm) crab cohorts, supported with crab records in

FIGURE 7. Monthly sex ratio and the percent of females that are ovigerous for *C. aestuarii* in Parila Lagoon (Neretva Estuary, Croatia).
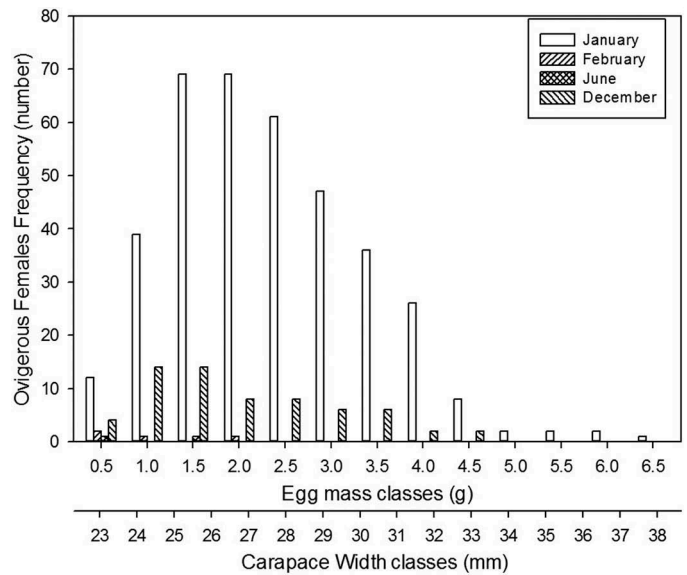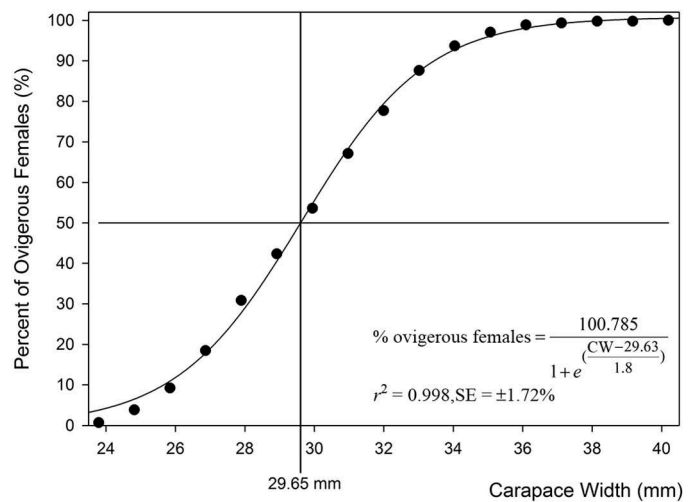

FIGURE 9. Percentage of ovigerous females in the total female population by carapace width for *C. aestuarii* in Parila Lagoon.
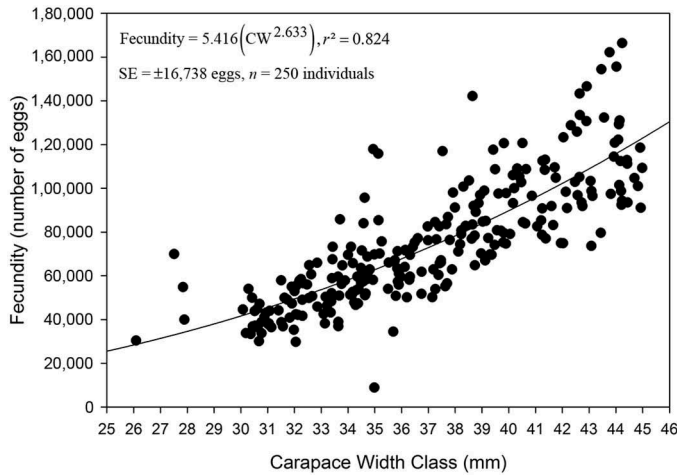
FIGURE 10. Relationship between the number of eggs and the carapace width of *C. aestuarii* in Parila Lagoon.

European Sea Bass stomachs. Among other factors, the high natural mortality of *C. aestuarii* in Parila Lagoon could be result of the recent Atlantic blue crab population establishment (Dulčić et al. 2011) or the recent increase of the Gilthead Seabream population in Neretva Estuary (B. Glamuzina et al. 2014). Predation by Atlantic blue crab limited the abundance and geographic range of invasive *C. maenas* along the eastern USA coast and green crabs were significantly less abundant at sites and depths with Atlantic blue crab than areas lacking Atlantic blue crab (deRivera et al. 2005). As these two species are in opposite roles in the Parila Lagoon, the invader is a potential danger to the native *C. aestuarii*. Further monitoring will provide more information on the results of this combat.

The *C. aestuarii* species spawns mainly during the winter season, with some reports for late-spring spawning. The ovigerous females in Parila Lagoon first appeared in November, peaked in January, and disappeared in February. Mori et al. (1990) found ovigerous females in the lagoon of San Teodoro (Sardinia, Italy) in late November to early May, with a maximum in February. Similarly, Veillet (1945) and Démeusy (1958) reported that ovigerous females in the lagoon of Thau and in Séte (France) were present from November to March. In Tunisian waters the major peak was found earlier, with a peak in October and a minor peak in January (Baklouti et al. 2013). The results obtained in the Parila Lagoon were consistent also with data from Homa Lagoon (Aegean Sea, Turkey), where ovigerous females were caught only from November to February, with a peak in January (Özbek et al. 2012). However, a small number of ovigerous females were also found in Parila Lagoon in June, an event not reported in the above-mentioned literature sources. However, both Amanieu et al. (1978) and Crivelli (1982) reported two spawning periods in lagoons along the western Mediterranean coast. This finding potentially provides support for the hypothesis that the progressive warming of the Parila Lagoon waters observed in

the last years (Sarà et al. 2014) may promote the establishment of a second reproductive cycle. The findings from shallow French and Spanish lagoons should also speak in favor of a temperature-dependent reproductive event. However, this was not observed in Tunisian or Turkish lagoons. The observed second spawning season should be attributed to delayed reproduction of a relatively secondary aliquot of females. Further monitoring of this life history trait should be used as indicator of potential reproductive changes caused by warming and their impact on transitional ecosystems if the hypothesis of warming impact and second spawning season is correctly set.

However, histological examinations revealed that ovarian development of *C. maenas* in Irish and British waters occurred biannually, with a primary winter cycle when the larger crabs reproduced and a secondary summer cycle when smaller crabs reproduced (Crothers 1967; Lyons et al. 2012). Broekhuysen (1936) also recorded that this crab has two well-marked breeding seasons. He reported a secondary summer cycle, with spawning of smaller younger crabs that are physiologically unable to produce eggs in the primary breeding cycle. Further research on this subject should clarify this interesting finding in *C. maenas* and *C. aestuarii*.

The size at sexual maturity is an important part of biological and reproductive cycles in decapods and is a basic parameter allowing definition of the parental stock. This parameter should be defined as the smallest recorded ovigerous female or the size when 50% of females are ovigerous. Older research reported the smallest and average sizes of ovigerous females as follows: 21-mm smallest size in the Lagoon of Thau (Veillet 1945), 25-mm average size of ovigerous females (Démeusy 1958), and 28 mm in the Lagoon of Tampan (Crivelli 1982). Mori et al. (1990) observed a CW of 29.1 mm at puberty in the Lagoon of San Teodoro, Sardinia. In Homa Lagoon (Aegean Sea, Turkey), females reached puberty at a CW of 26.84 mm (Özbek et al. 2012). Our analysis shows that the 50% gravid size for the Parila Lagoon population exhibits a size of 29.65 mm and a weight of 10.39 g, similar to above-mentioned values. However, the results from Tunisian waters differ from most of those discussed above; there 50% of the population reached maturity at 34.56 mm CW for females and 43.93 mm CW for males (Baklouti et al. 2013). This result is difficult to discuss in the light of most other reports, as earlier maturation in Tunisian populations is expected due to higher water temperatures and a faster growth rate there.

The average fecundity of *C. aestuarii* from Parila Lagoon was estimated at 61,017 eggs/female, with an average fecundity per 1 g of female weight of 4,804 eggs and average egg diameter of 332 μm. In Tunisian waters, the average fecundity was 30,750 eggs/female, ranging from 3,620 to 55,400 eggs/female. The egg number per gram of weight was 7,857 (Baklouti et al. 2013). The average number and diameter of eggs from the Black Sea population were 272, 162.8, and 420 μm, respectively (Aydin 2013). The data from

the Black Sea are substantially higher than from other recorded populations in the Mediterranean, and this should be ascribed to the size of females, showing a CW almost three times larger than in the Parila Lagoon.

Carcinus aestuarii is one of the most important species in the ecology of transitional waters, serving as an energy carrier from common estuary shellfish (mussels and clams) to important fish species, such as Gilthead Seabream and European Sea Bass. The observed high natural mortality during the first year of life and the consumed crabs found in European Sea Bass stomachs indicate the importance of the species as a prey animal. Beside this ecological footprint, the species is also of significant fishery potential, although this is primarily exploited in Venice Lagoon and is not yet a regular activity in other parts of the Mediterranean. These peculiar ecological characteristics advocate for a better management of this species, including protection measures issued during the gravid stage (closed season) and the protection of minimal sizes, as well as protection of habitats. As the species is presently not the object of a fishery in Neretva Estuary, these analyses should serve as zero-status for future exploitation management.

In conclusion, the results obtained from research on *C. aestuarii* in Neretva Estuary confirm recent results on the species in different parts of the Mediterranean but enhance the knowledge of population dynamics for the species, providing deeper and richer data compilation and analysis. The overall results overlap with other recent reports in the Mediterranean. However, the comparison of these data with that of the Black Sea population point to significant differences between these two areas. This could be a result of different ecosystem properties but could also be a potential genetic distinction of the Black Sea population and deserves to be better investigated. As a species, *C. aestuarii* is specialized and restricted to estuaries, lagoons, and shallow embayments of the Mediterranean Sea, with overall patchy distribution and the absence of a continuous habitat, which may promote divergence among populations (Deli et al. 2014), as observed here.

The biological characteristics and population dynamics of *C. aestuarii* show that several parameters should be used to indicate potential changes in ecosystem properties of Mediterranean transitional waters. Among them, the most suitable include changes in the smallest and average size of ovigerous females, general changes in population structure, mortality rate during the first year of life, and intensification of a second late-spring reproduction season. The focus on a lower number of indicators will significantly contribute to better monitoring of transitional water ecology with less effort and lower financial needs.

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REFERENCES

Abelló, P., F. J. Valladares, and A. Castellón. 1988. Analysis of the structure of decapod crustacean assemblages off the Catalan coast (north-west Mediterranean). Marine Biology 98:39–49.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723.

Amanieu, M., T. Do Chi, R. Galzin, and G. Lasserre. 1978. Structure et importance des populations de crabe vert *Carcinus mediterraneus* Czerniavski, 1884 dans l'etang de Prévost (Languedoc, France). Bulletin d'Ecologie 9:329–341.

Aydin, M. 2013. Length–weight relationship and reproductive features of the Mediterranean green crab, *Carcinus aestuarii* Nardo, 1847 (Decapoda: brachyura) in the eastern Black Sea, Turkey. Pakistan Journal of Zoology 45:1615–1622.

Bagenal, T. B. 1973. Fish fecundity and its relations with stock and recruitment. Rapports et Proces-Verbaux des Reunions du Conseil International pour l'Exploration de la Mer 164:186–198.

Baklouti, S., A. Derbali, K. Dhieb, and O. Jarboui 2013. Reproductive biology of the Mediterranean green crab, *Carcinus aestuarii* Nardo, 1847 (Crustacea: portunidae), in the Gulf of Gabes (Tunisia, Central Mediterranean). Cahiers de Biologie Marine 54:411–417.

Bartulović, V., B. Glamuzina, A. Conides, A. Gavrilović, and J. Dulčić. 2006. Maturation, reproduction and recruitment of the Sand Smelt, *Atherina boyeri* Risso, 1810 (Pisces: atherinidae) in the estuary of Mala Neretva River (southeastern Adriatic, Croatia). Acta Adriatica 47:5–12.

Bartulović, V., B. Glamuzina, D. Lučić, A. Conides, N. Jasprica, and J. Dulčić. 2007. Recruitment and food competition of juvenile Thin-lipped Grey Mullet, *Liza ramada* (Risso, 1826), in the Neretva river estuary. Acta Adriatica 48:25–38.

Bartulović, V., S. Matić-Skoko, D. Lučić, A. Conides, N. Jasprica, A. Joksimović, J. Dulčić, and B. Glamuzina. 2009. Recruitment and feeding of juvenile Leaping Grey Mullet, *Liza saliens* (Risso, 1810) in the Neretva River estuary (south-eastern Adriatic, Croatia). Acta Adriatica 50:195–207.

Behrens Yamada, S., and L. Hauck. 2001. Field identification of the European green crab species *Carcinus maenas* and *Carcinus aestuarii*. Journal of Shellfish Research 20:905–912.

Bertalanffy, V. L. 1938. A quantitative theory of organic growth. Human Biology 10:181–213.

Bhattacharya, C. G. 1967. A simple method of resolution of a distribution into Gaussian components. Biometrics 23:115–135.

Broekhuysen, G. J. 1936. On development, growth and distribution of *Carcinides maenas*. Archives Neerlandaises Zoologie 2:257–399.

Can, E., E. M. Tirasin, and B. Cihangir. 2004. An Investigation of the crab *Carcinus aestuarii*, (Nardo, 1847) in the Çakalburnu Lagoon. Turkish Journal of Aquatic Life 2:277–284.

Carlton, J. T., and A. N. Cohen. 2003. Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. Journal of Biogeography 30:1809–1820.

Chen, R. B., S. Watanabe, and M. Yokota. 2004. Feeding habits of an exotic species, the Mediterranean green crab *Carcinus aestuarii*, in Tokyo Bay. Fisheries Science 70:430–435.

Chen, S., and S. Watanabe. 1989. Age dependence of natural mortality coefficient in fish population dynamics. Nippon Suisan Gakkaishi 55:205–208.

Cilenti, L., G. D'Errico, T. Scirocco, C. Manzo, and A. Fabbrocini. 2014. Spatial variability in the population structure of the *Carcinus aestuarii* in Varano lagoon. Transitional Waters Bulletin 8:24–31.

Crivelli, A. J. 1982. Biology of three Malacostraca (Decapoda) in a Mediterranean lagoon with particular emphasis on the effect of rapid environmental changes on the activity (catchability) of the species. Estuarine Coast Shelf Science 15:591–604.

Crothers, J. 1967. The biology of the shore crab *Carcinus maenas* (L.) 1. The background-anatomy, growth and life history. Field Studies 2:407–434.

Deli, T., K. Said, and N. Chatti. 2014. Morphological differentiation among geographically close populations of the green crab *Carcinus aestuarii* (Nardo, 1847) (Brachyura, Carcinidae) from the Tunisian coast. Crustaceana 87:257–283.

Démeusy, N. 1958. Réchèrches sur la mue de puberté du Decapode Brachyoure *Carcinus maenas* Linné. Archives de Zoologie Experimentale et Generale 95:258–490.

deRivera, C. E., G. M. Ruiz, A. H. Hines, and P. Jivoff. 2005. Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. Ecology 88:3364–3376.

Dulčić, J., P. Tutman, S. Matić-Skoko, and B. Glamuzina. 2011. Six years from first record to population establishment: the case of the blue crab, *Callinectes sapidus* Rathbun, 1896 (Brachyura, Portunidae) in the Neretva river delta (south-eastern Adriatic Sea, Croatia). Crustaceana 84:1211–1220.

Dulčić, J., P. Tutman, S. Matić-Skoko, M. Kraljević, N. Glavić, V. Kožul, B. Glamuzina, V. Bartulović, and B. Skaramuca. 2007. A list of Y-O-Y fish species found in the littoral shallows of the Neretva and Mala Neretva estuaries (eastern Adriatic, Croatian coast). Acta Adriatica 48:89–94.

Fulton, T. W. 1904. The rate of growth of fishes. Annual Report of the Fishery Board of Scotland 3:141–241.

Furota, T., S. Watanabe, T. Watanabe, S. Akiyama, and K. Kinoshita. 1999. Life history of the Mediterranean green crab, *Carcinus aestuarii* Nardo, in Tokyo Bay. Japan. Crustacean Research 28:5–15.

Gayanilo, F. C. Jr., P. Sparre, and D. Pauly. 2005. FAO-ICLARM Stock assessment tools II (FiSAT II), revised version, user's guide. Food and Agriculture Organization of the United Nations, Computerized Information Series (Fisheries) 8, Rome.

Glamuzina, B. 2010. Neretva River fishery-history and perspectives. Pages 20–30 in B. Glamuzina and J. Dulcic, editors. Proceedings of fish and fisheries of Neretva River: status and perspectives. University of Dubrovnik and Neretva-Dubrovnik County, Dubrovnik, Croatia.

Glamuzina, B., A. Pešić, A. Joksimović, L. Glamuzina, S. Matić-Skoko, A. Conides, D. Klaoudatos, and P. Zacharaki. 2014. Observations on the increase of wild Gilthead Seabream, *Sparus aurata* abundance, in the eastern Adriatic Sea: problems and opportunities. International Aquatic Research 7:1–8.

Glamuzina, L., A. Conides, I. Prusina, M. Ćukteraš, D. Klaoudatos, P. Zacharaki, and B. Glamuzina. 2014. Population structure, growth, mortality and fecundity of *Palaemon adspersus* (Rathke 1837; Decapoda: palaemonidae) in the Parila Lagoon (Croatia, SE Adriatic Sea) with notes on the population management. Turkish Journal of Fisheries and Aquatic Sciences 14:677–687.

Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa, J. F. Bruno, K. S. Casey, C. Ebert, H. E. Fox, R. Fujita, D. Heinemann, H. S. Lenihan, E. M. Madin, M. T. Perry, E. R. Selig, M. Spalding, R. Steneck, and R. Watson. 2008. A global map of human impact on marine ecosystems. Science 319:948–952.

Jensen, A. L. 2001. Comparison of theoretical derivations, simple linear regressions, multiple linear regression and principal components for analysis of fish mortality, growth and environmental temperature data. Environnmetrics 12:591–598.

Jones, R. 1981. The use of length composition data in fish stock assessment (with notes on VPA and Cohort Analysis). FAO Fisheries Circular 734.

Koçak, C., D. Acarli, T. Katağan, and M. Özbek. 2011. Morphometric characters of the Mediterranean green crab (*Carcinus aestuarii* Nardo, 1847) (Decapoda, Brachyura), in Homa Lagoon. Turkish Journal of Zoology 35:551–557.

Leignel, V., J. H. Stillman, S. Baringou, R. Thabet, and I. Metais. 2014. Overview on the European green crab *Carcinus* spp. (Portunidae, Decapoda), one of the most famous marine invaders and ecotoxicological models. Environmental Science and Pollution Research 21:9129–9144.

Losso, C., and A. V. Ghirardini. 2010. Overview of ecotoxicological studies performed in the Venice Lagoon (Italy). Environment International 36:92–121.

Lumare, L., D. Lumare, M. Florio, T. Scirocco, and F. Lumare. 2009. I Crostacei Decapodi del Lago di Acquatina: struttura delle Popolazioni e Ciclo Riproduttivo. Thalassia Salentina 31:83–102.

Lyons, J. L., M. R. O'Riordan, F. T. Cross, and C. S. Culloty. 2012. Reproductive biology of the shore crab *Carcinus maenas* (Decapoda, Portunidae): a macroscopic and histological view. Invertebrate Reproduction and Development 56:144–156.

Mancinelli, G., B. Glamuzina, M. Petrić, L. Carrozzo, L. Glamuzina, M. Zotti, D. Raho, and S. Vizzini. 2016. The trophic position of the Atlantic blue crab *Callinectes sapidus* Rathbun 1896 in the food web of Parila Lagoon (south eastern Adriatic, Croatia): a first assessment using stable isotopes. Mediterranean Marine Science 17:634–643.

Matozzo, V., A. Boscolo, and M. G. Marin. 2013. Seasonal and gender-related differences in morphometric features and cellular and biochemical parameters of *Carcinus aestuarii* from the Lagoon of Venice. Marine Environmental Research 89:21–28.

Mori, M., R. Mancon, and G. Fanciulli. 1990. Notes on the reproductive biology of *Carcinus aestuarii* Nardo (Crustacea, Decapoda) from the lagoon of San Teodoro (Island of Sardinia, Italy). Rivista di Idrobiologia 29:763–774.

Onofri, V., J. Dulčić, A. Conides, S. Matić-Skoko, and B. Glamuzina. 2008. The occurrence of the blue crab, *Callinectes sapidus* Rathbun, 1896 (Decapoda, Brachyura, Portunidae) in the eastern Adriatic (Croatian coast). Crustaceana 81:403–409.

Özbek, M., C. Kocak, and D. Acarli. 2012. Reproductive biology of the Mediterranean green crab *Carcinus aestuarii* Nardo, 1847 (Crustacea, Brachyura, Portunidae) in Homa Lagoon, Aegean Sea, Turkey. Oceanological and Hydrobiological Studies International Journal of Oceanography and Hidrobiology 41:77–80.

Özcan, T., K. Bakır, and T. Katağan. 2009. Length/width–weight relationships of the Mediterranean green crab *Carcinus aestuarii* Nardo, 1847 in the Homa Lagoon, Aegean Sea Turkey. Journal of Fishery Science 3:1–4.

Pauly, D. 1980. On the interrelationships between natural mortality, growth parameters and mean temperature in 175 fish stocks. Journal du Conseil International pour l'Exploration de la Mer 39:175–192.

Pauly, D. 1983. Length converted catch curves: a powerful tool for fisheries research in the tropics (part I). Fishbyte 1:9–13.

Pitcher, T. J., and P. J. B. Hart. 1982. Fisheries ecology. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Ricciardi, F., V. Matozzo, A. Binelli, and M. G. Marin. 2010. Biomarker responses and contamination levels in crabs (*Carcinus aestuarii*) from the Lagoon of Venice: an integrated approach in biomonitoring estuarine environments. Water Research 44:1725–1736.

Rikhter, V. A., and V. N. Efanov. 1976. On one of the approaches to estimation of natural mortality of fish populations. ICNAF (International Commission for the Northwest Atlantic Fisheries) Research Document 79/VI/8.

Robins, P. E., M. W. Skov, M. J. Lewis, L. Giménez, A. G. Davies, S. K. Malham, S. P. Neill, J. E. McDonald, T. A. Whitton, S. E. Jackson, and C. F. Jago. 2016. Impact of climate change on UK estuaries: a review of past trends and potential projections. Estuarine. Coastal and Shelf Science 169:119–135.

Roman, J., and S. R. Palumbi. 2004. A global invader at home: population structure of the Mediterranean green crab, *Carcinus maenas*, in Europe. Molecular Ecology 13:2891–2898.

Sakai, T. 1986. Rare species and their genus of crabs in Japan. Research on Crustacea 15:1–4.

Sarà, G., M. Milanese, I. Prusina, A. Sarà, L. D. Angel, B. Glamuzina, T. Nitzan, S. Freeman, A. Rinaldi, V. Palmeri, V. Montalto, M. Lo Martire, P. Gianguzza, V. Arizza, S. Lo Brutto, M. De Pirro, B. Helmuth, J. Murray, S. De Cantis, and A. G. Williams. 2014. The impact of climate change on Mediterranean intertidal communities: losses in coastal ecosystem integrity and services. Regional Environmental Change 14:5–17.

Shephard, S., S. P. R. Greenstreet, G. J. Piet, A. Rindorf, and M. Dickey-Collas. 2015. Surveillance indicators and their use in implementation of the marine strategy framework directive. ICES Journal of Marine Science 72:2269–2277.

Van Den Broeck, M., A. Waterkeyn, L. Rhazi, P. Grillas, and L. Brendonck. 2015. Assessing the ecological integrity of endorheic wetlands, with focus on Mediterranean temporary ponds. Ecological Indicators 54:1–11.

Veillet, A. 1945. Recherches sur le parasitisme des crabes et des galathées par les rizocéphales et les épicarides. Annales de l'Institut Océanographie Paris 22:193–341.

Whitfield, A. K., and M. Elliott. 2002. Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. Journal of Fish Biology 61:229–250.