

RESEARCH ARTICLE

Environmental drivers of size-based population structure, sexual maturity and fecundity: A study of the invasive blue crab *Callinectes sapidus* (Rathbun, 1896) in the Mediterranean Sea

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Abstract

The blue crab *Callinectes sapidus* (Rathbun, 1896) is one of the most invasive species in the Mediterranean Sea. Understand how the populations are maintained and how the environment is driving the populations in the areas invaded is the key to an effective future management. This current study is presenting a monthly long-term monitoring of the blue crabs' population structure, body size, sexual maturity, reproduction periods and fecundity, and their relationships with environmental factors in a saltmarshes system in Italy. During winter, high densities (15 ± 8 ind m^{-2}) of early juveniles (< 2 cm) were observed, and their numbers decreased due the population growth until summer. The size-spectra showed that across different temperature ($18\text{--}26^\circ\text{C}$) and salinity ($24\text{--}40$ psu) gradients, the growth period for males is faster than for females. Based on abdominal analysis, sexual maturity was defined at ~ 12 cm for males and females but the population was in favor of males that were more than 66% of the time mature throughout the year. Copulations periods were identified between spring and autumn when more than 50% of females were matures, and ovigerous females' migrations were observed in late summer. Our study expand our understanding of how the environment interacts to effect physiological and biological processes of alien species and improve our ability to make predictions of how environmental change the distribution of the alien species in the future. Based on our results, we also discuss which population control strategy would be most effective based on the data available in the literature.

Introduction

An important point in ecology is to understand how environmental factors are affecting species distribution and population structure [1] which could be profoundly affected by disturbance

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(*sensu* Sousa [2]). This understanding involves study the population size and dynamics, reproduction strategy and species ability to adapt to environmental changes [3]. One of the main factor to consider is the species body size which is one of the main performance traits having a strong potential to determine the negative impact of environmental factors on the composition of local populations [4]. Studying the population size-spectra and how the environmental factors are able to shape the size based population structures, constitutes a powerful indicator of marine ecosystems status [5]. In the case of non-indigenous species (NIS) this approach proposes new monitoring descriptors such as (i) the quantification of ability of NIS to adapt in new habitats and (ii) the understanding of how environmental factors are able to address the population size structure, dynamics and biology.

The blue crab *Callinectes sapidus* Rathbun (1896), a portunid native from the Atlantic coasts of America (from Nova Scotia in the north to Argentina), was recorded in the Mediterranean Sea in 1949 and the Black Sea and the Sea of Azov in 1967 [6]. The species is particularly fertile [7, 8] and tolerates a large range of temperatures [9] which contribute to its invasion, expansion and maintenance success in the Mediterranean in the last decades [10]. *Callinectes sapidus* is widely distributed in the Mediterranean Sea along the Northern (from Spain to Turkey) and southern (Egypt, Tunisia, Algeria and Morocco) coasts [11]. This species is an opportunist predator [12, 13] with a particular aggressive behavior [14] inducing strong impacts on native biodiversity (competition with autochthonous species, local species extinctions) and artisanal fisheries (net destruction, fish mutilations) [15]. It's now accepted that the only way to control the species is to fish it and use it as a resource. In its native range, blue crab fisheries are recognized for their commercial value and catch volume, making the blue crab fishery one of the most important fisheries on the USA east coast [16]. Blue crab is of major fishing interest, consumed in large quantities, mainly in the USA and Mexico [17, 18]. In some Mediterranean regions (e.g. Adriatic Sea; Egypt; Turkey; Tunisia, Spain), the blue crab fishery is used as a population control measure, and its use and sale has made it possible to compensate for the economic losses suffered by the artisanal fishery [19, 20].

Due to its complex life cycle—comprising numerous larval (zoeae), post-larval (megalopae), and juvenile phases punctuated by an abrupt transition to adults [14]—*C. sapidus* occurs in various habitats and salinity gradients (brackish and sea waters) [21]. Reproduction between males and females takes place in low salinity waters, and female crabs migrate to polyhaline zones to produce and incubate eggs [22]. The adult males remain in the low salinity waters [23]. Once the eggs mature, the females migrate to the sea (salinity up to 30) to release the planktonic zoeae larvae [24]. After a long transport in the sea, the zoeae larvae differentiate into megalops that gradually return to the desalinated areas (e.g. lagoons, estuaries, saltmarsh, etc.) which play a role of nursery [24, 25]. Juvenile crabs use a diverse range of nurseries throughout ontogeny, mainly in structurally complex habitats (dense aquatic vegetation) for individuals smaller than 25 mm carapace width [26–30], then non-vegetated secondary nurseries with a sandy-muddy substrate and low salinity for individuals > 25 mm [25, 31]. The juvenile blue crabs stay in these sandy-muddy substrate until they reach sexual maturity at about 100–200 mm body weight (age at maturity and longevity ~ 1–2 years and 3 years, respectively, depending on phenology and geographic location) [23, 32]. The complex blue crabs life cycle present currently a barrier to the possible control measures and some answers are urgent about when and how to implement an effective control measure. Thus, studying the blue crabs life cycle, especially the population growth could be the key to define the sensitives periods where control measures could be implemented.

In the Mediterranean Sea, *C. sapidus* is found in various habitats (river mouths, brackish lagoons, on the coast) but patterns of blue crab population size structure and maturity stages in these diverse regions are still poorly understand. The land structure geography of Sicily does

not offer favorable desalinated habitats (e.g. estuaries, lagoons) preferred for the blue crabs' life-cycle. However, the high frequency of sightings and occurrences of *C. sapidus* in Sicily [33] suggests that they find favorable conditions for maintaining sustainable populations. Following the numerous reports of *C. sapidus* by fishermen on the western Sicilian coasts, an investigation was conducted to identify viable populations. In July 2021, a population of blue crabs was found in the heart of the natural reserve of Trapani ("Saline di Trapani e Paceco") which includes a large complex of human-made ponds hosting important aggregations of saltmarshes [34, 35] with some currently exploited for salt and others in restauration.

Shallow subtidal areas of saltmarsh are known to be inhabited by the blue crab *C. sapidus* in their native areas [36–39]. Although blue crabs have been reported to move into the intertidal zone of polyhaline [40, 41] and oligohaline waters [42], there are few data on the extent of intertidal salt marshes used by this large decapod crustacean in the Mediterranean Sea. These microhabitats are known to be refuge areas for *C. sapidus* where it found protection and high food quantities to sustain growth and reproduction [27, 31, 43, 44]. Even if *C. sapidus* is widespread in the Mediterranean Sea, little information exists on population structure, body size, sexual maturity, reproduction periods and growth. Few studies show the blue crab populations dynamics in the Mediterranean. Even if some efforts are performed to quantify the population size structure, data are missing on the males and females size at maturity, population growth and juveniles (< 2 cm) abundance, and, more importantly, the effect of environmental drivers conditioning the blue crab populations.

In the case of the blue crab, which is a generalist species with an important capacity of adaptation and larval and adult stages dispersal, using such descriptors allows to quantify the environmental key-factors inducing blue crab's population size structure well-being in order to implement adaptative and effective management plans (e.g. population control). Salinity and temperature gradients are the major environmental factors that affect the metabolism [9] and the life cycle of *C. sapidus* [18]. In Sicily, the pronounced temperature and salinity oscillations in the Trapani saltmarshes (Italy) may have favored the establishment of *C. sapidus*. However, to understand population dynamics, in addition to allometric relationships, it becomes necessary to follow seasonal size spectra, sexual maturity and reproductive events, establishing migration routes and recruitment periods in saltmarshes.

Considering the above information, here we show a study case on how monitoring and inspecting blue crabs *C. sapidus* performance traits (population structure, body size, sexual maturity, copulation periods and fecundity) in relationships with environmental factors can assist practitioners in addressing effective NIS management measures in in the Mediterranean Sea.

Materials and methods

Study site and sampling strategy

The study site (named "Salina Grande"; 37°57'13.7"N, 12°29'51.3"E) is located in the Nature Reserve "Saline di Trapani e Paceco" (WWF Italy; SIC: ITA01007), Sicily (Italy) [34]. The reserve (area of 960 ha) consists of a plain characterized by a sandy coast and a large wetland (80% of the area). The remainder of the area is divided between areas of intensive human activity (10%), wooded and shrubby areas (5%), and agricultural areas (5%). Wetlands are represented by the following categories: reed thickets, ponds (30 ha) and salt marshes (750 ha). It is an important wetland used as a resting place by thousands of migratory birds and characterized by a purely halophilic environment of great naturalistic value. The vast wetland has different habitats such as natural mudflats, salt marshes, canals that are home to over 200 species of birds.

The study site includes a complex of 9 saltmarshes (max depth: ~ 40 cm) with 2 connections to the sea though a channel (Fig 1). We identified 3 substrate types from the field observations:

sandy-muddy, *Cymodocea nodosa*, and *Ruppia maritima* [45, 46] (Fig 1 and S1 Table): three with only sandy-muddy substrate, six with sandy-muddy and *Ruppia*; and only one with sandy-muddy and *Cymodocea* substrates. The channel connecting the saltmarsh system and the sea was only composed by a sandy-muddy substrate. The first blue crab, *C. sapidus*, was observed on 20th July 2021. Since, a monitoring was performed for 18 months. A total of 49 sampling campaigns were carried out between July 2021 and December 2022. This study was performed in collaboration with the WWF Italy, manager of the "Saline di Trapani e Paceco" natural reserve, they give to us the permission to perform sampling and environmental study in this site.

Four traps (100 cm x 80 cm; 4 apertures of 30 cm) specifically designed for this study (S1 Fig) were used to sample blue crabs. The duration of the traps immersion was 5 days using chicken pieces as a bait. A minimum of 30 blue crab organisms were sampled in the 4 saltmarshes, and placed in a cooler. Due to the small size of the study site, the total specimen number across all 4 saltmarshes was considered for the study. At the beginning of our study, the northern salt marshes (S5 to S9, Fig 1) were disconnected from the others by wooden gates and no blue crabs were observed in. However, after a storm during autumn 2022, they are currently connected to the rest of the system. In order to maintain consistency in our study, we have therefore chosen not to include these northern salt marshes.

During the survey blue crab early stages (< 2 cm) and juveniles (2–5 cm) were observed in samples of sand-mud performed in the saltmarsh S3. To sample them and to quantify their local density, 10 replicates of 40 x 40 cm quadrats were performed in S3 at 1 m from the edge of the basin. The content of the quadrat was collected with a hand net and was gently rinsed with saltmarsh water on a 500 µm mesh. Juveniles of blue crabs were gently picked up by hand and conserved in a 1 L plastic bottle with saltmarsh water for biometry measurements. The density of juveniles was reported per square meter and monthly averaged (\pm standard deviation).

To determine the natural behavior of the organisms, the monitoring operators walked along the edges of all the saltmarshes making 10 round trips to observe the organisms. The shallow depth (< 40 cm) allowed for easy observation of the individuals. During this visual monitoring, the operators reported the presence of couples of blue crabs in reproduction (male and female abdomen against abdomen). This information made it possible to determine the reproduction periods in the study site.

Temperature was recorded continuously in each pond every hour using a HOBO Pendant® loggers (mod. MX2201, $\pm 0.5^\circ\text{C}$ accuracy), while salinity was measured for each sampling date using a multiparameter probe (HANNA® HI 98194, ± 0.1 unity accuracy).

Size structure, sexual maturity and females fecundity

In laboratory each blue crab individual was sexed and the maturity determined (immature or mature) based on the abdomen morphological analysis (Fig 2). The maturity of blue crab female was determined by the shape of the abdomen: a triangular abdomen was considered immature if it was sealed and the vulvas were indistinct. The mature adult female has the characteristic enlarged, heavily pigmented and fully expandable abdomen and pleopods [14, 47]. For males, organisms were immature if the abdomen was closed and/or partially locked to the sternum with the penis inserted into the gonopods [18, 48–50]. A mature adult male showed a free and fully expandable abdomen (Fig 2).

For all organisms with size up to 5 cm, the carapace width (CW, distance between the two dorsal spines) and the abdomen width (ABD) were measured using a graduate ruler (± 1 mm accuracy). The wet weight (WW) of each individual was measured using a digital balance (± 0.1 g accuracy). For the smaller organisms (< 5 cm), CW and ABD were measured using the

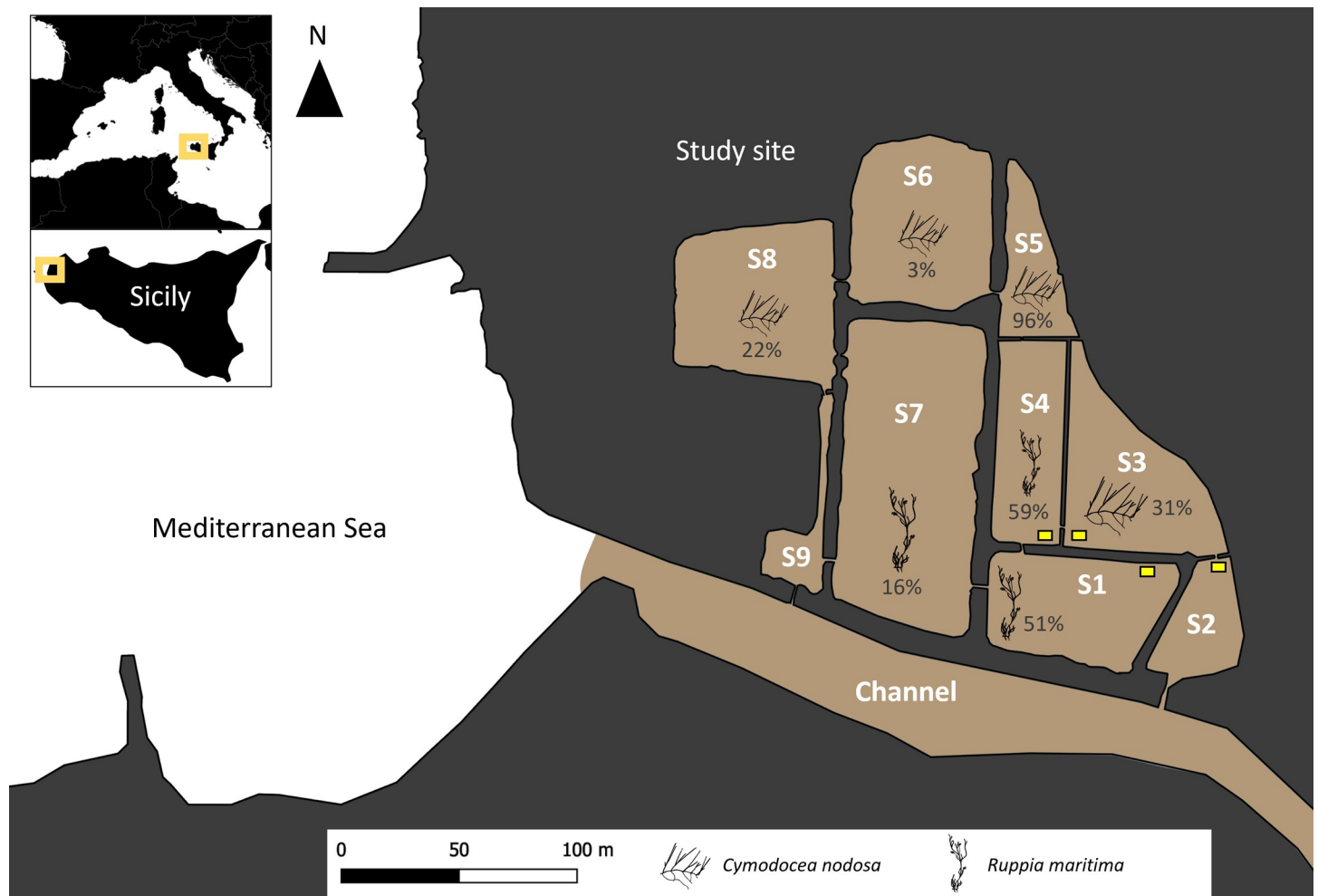


Fig 1. Map of the study site in the natural reserve of Trapani (Sicily). The yellow squares represent the position of blue crab traps. The white numbers correspond to the ID of each saltmarsh, the percentages represent the percentage of coverage by seagrasses, and the brown color represents the sandy-muddy substrate. Copyright: @Marchessaux, CC BY 4.0 license.

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software ImageJ (± 0.1 mm accuracy) after taking a picture of the dorsal and ventral sides with a stereomicroscope Zeiss Axio Zoom V16. WW was measured using a digital microbalance Metro Toledo EL104 (± 0.0001 g accuracy).

For ovigerous females, the egg masses were carefully removed from pleopods and their wet weight (± 0.1 g accuracy) was measured using the digital microbalance. The embryonic development was determined on the basis of the eggs' color [51–53]: I-initial (yellow), II-intermediate (orange), and III-pre-hatching (dark brown). In a second time, eggs were separated in a 5% sodium hypochlorite solution [52]. The total eggs samples were separated in two homogeneous partitions and one was counted in a Dollfuss cuvette under the stereomicroscope. 1 000 eggs were measured using the software ImageJ (± 0.1 μm accuracy) after taking pictures with a stereomicroscope.

Data analysis

The length-weight (CW-WW) allometric relationship was determined by an exponential equation for males and females respectively, and the males/females abdomen-CW relationship was

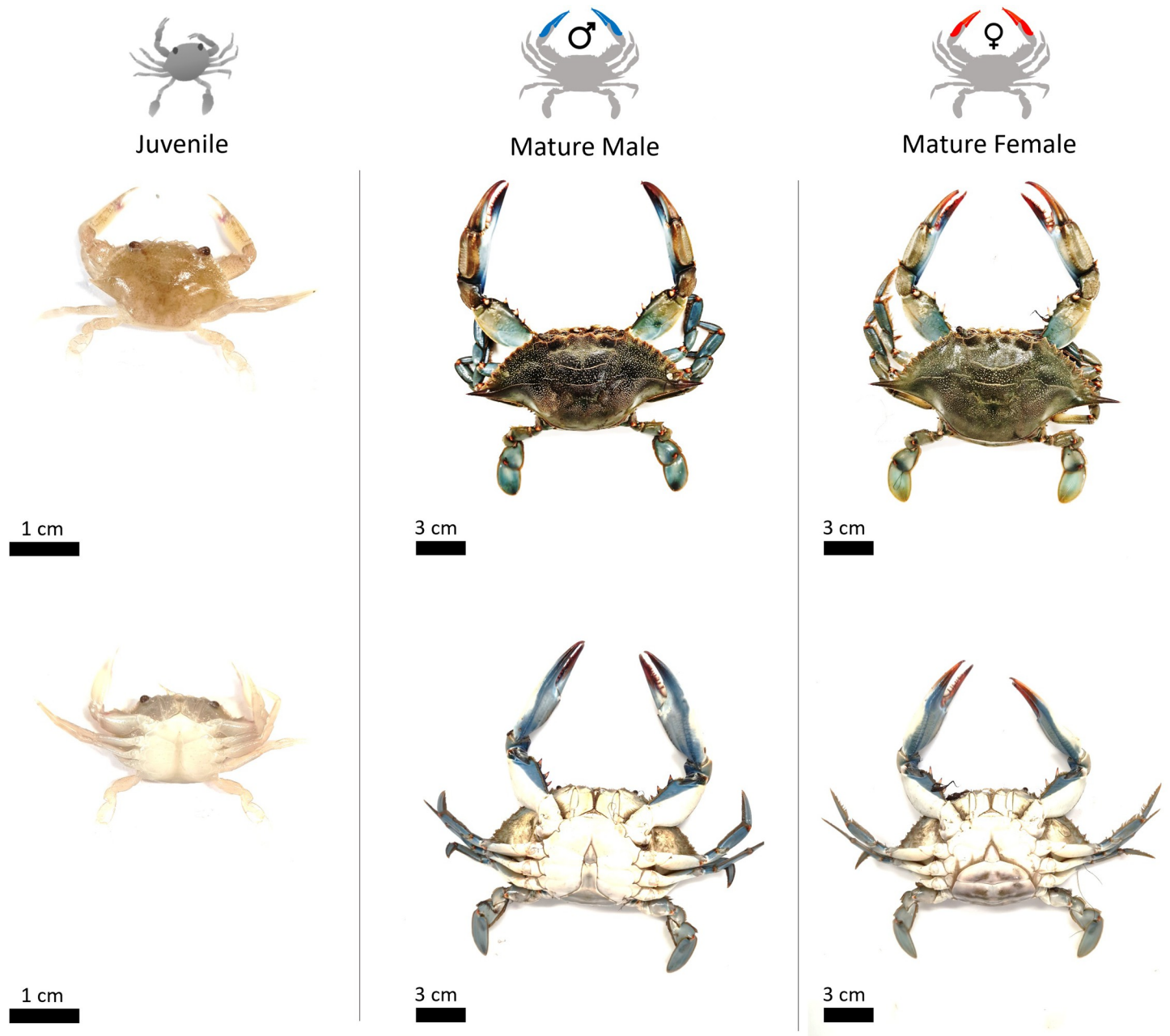


Fig 2. Pictures of the juveniles, matures males and females *Callinectes sapidus* sampled in the saltmarshes of Trapani (Italy); dorsal and ventral side. Photos: G. Marchessaux. Copyright: @Marchessaux, CC BY 4.0 license.

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determined by a linear regression. Size distribution (frequency for every 1 cm) for males and females was seasonally calculated and the Gaussian regressions determined. The seasons were determined as: winter: January-Mars; spring: April-June; summer: July-September; autumn: October-December. For each month, the allometric CW-WW regressions were performed for both sexes and the slopes, after linearization, were extracted. Using a 3-dimensional plot, slopes were represented as a function of temperature and salinity to determine the males and females growth.

To follow the population goodness condition, the Fulton's condition factor (K) was monthly calculated following the equation (1): $K = (W/L^3) \cdot 100$ where W and L are respectively the total weight and carapace width [54]. 3-dimensional plots were performed to correlate the Fulton's condition factor with temperature and salinity for both sexes and as a function of the maturity stage (immature; mature).

The monthly percentage of immature and mature males and females was calculated in order to quantify the sexual population structure. The monthly sex ratio (Males/Females) was estimated. The length at first maturity of females and males (the length at which 50% of the organisms had become mature) was determined from the relationship between the percentages of mature crab (males and females separately) and the CW classes of 1 cm. The proportion (P) of sexually mature of CW (males and females) was fitted to the logistic equation (3): $P =$

$\frac{1}{(1 + \exp[-r(L - L_m)])}$ which in straight line form is: $\ln\left[\frac{1-P}{P}\right] = rL_m - rL$ where r (-b) is the slope of the curve and L_m is the mean length at sexual maturity on the CW which corresponds to a proportion of 50%. Size at sexual maturity (L_m) was calculated from $-(a/b)$. A logistic function was fitted to the proportion of mature males/females. All plots and regressions were performed using the software SigmaPlot 12.5.

To test the differences between the eggs' diameter at the different embryonic development stages identified, an ANOVA and a Bonferroni post-hoc test (significant difference = $p < 0.05$) were performed using R Studio (version 2021.09.0). ANOVA was also used to identify the differences between matures males and females periods.

Results

A total of 750 blue crabs (445 males and 305 females) were evaluated for size-length characteristics and sexual maturity based on their morphology. The size distribution of immatures and matures males and females ranged for males from 0.75 to 10.8 cm (immatures) and from 8.2 to 19.8 cm (matures); for females from 0.74 to 10.4 (immatures) and from 9.6 to 16.2 (matures) (Fig 3A and 3B and Table 1A).

The length-weight relationships (CW-WW) followed an exponential curve for both sex (Fig 3A and 3B and Table 1B). The slope coefficient b values were similar for both sex maturity stage: immatures males: 0.21, immatures females: 0.17; matures males: 0.09, matures females: 0.07 (Table 1B). The male and female abdomen sizes showed different linear regressions depending of the sexual maturity (Fig 3C and 3D and Table 1C); with a marked separation between immatures and matures females in contrast to males where the slopes were almost identical. For males, immatures/matures abdomen size was not clearly separated and both immature and mature organisms were observed between 8 and 10 cm (Fig 3C). For females, the separation was more marked with only an overlap of the two stages of sexual maturity observed between 9.9 and 11.3 cm (Fig 3D).

Temperature and salinity showed a typical Mediterranean seasonal trend (S2A Fig). The monthly temperature ranged from $11.9 \pm 1.7^\circ\text{C}$ (Jan. 2022) to $30.6 \pm 2.1^\circ\text{C}$ in July; with lowest temperature value at 7.6°C recorded on 26th January 2022 and highest at 35.9°C on 20th July 2022. Salinity oscillated seasonally from 15 (17th Feb. 2021) to 42 (6th Aug. 2022) between winter and summer (S2B Fig).

The size distribution of males and females showed a seasonal pattern (Fig 4). During winter only the early stages (< 3 cm) were founded with a major contribution ($> 40\%$ of 0–1 cm size class for both sexes). Both sexes size distribution was overlapped (Fig 4). Both sexes grew rapidly during spring to reach a dominant size at 10.6 cm for males and 8.4 cm for females. On the other hand, males showed larger size ranged (from 1 to 19.8 cm) than females (1 to 16 cm)

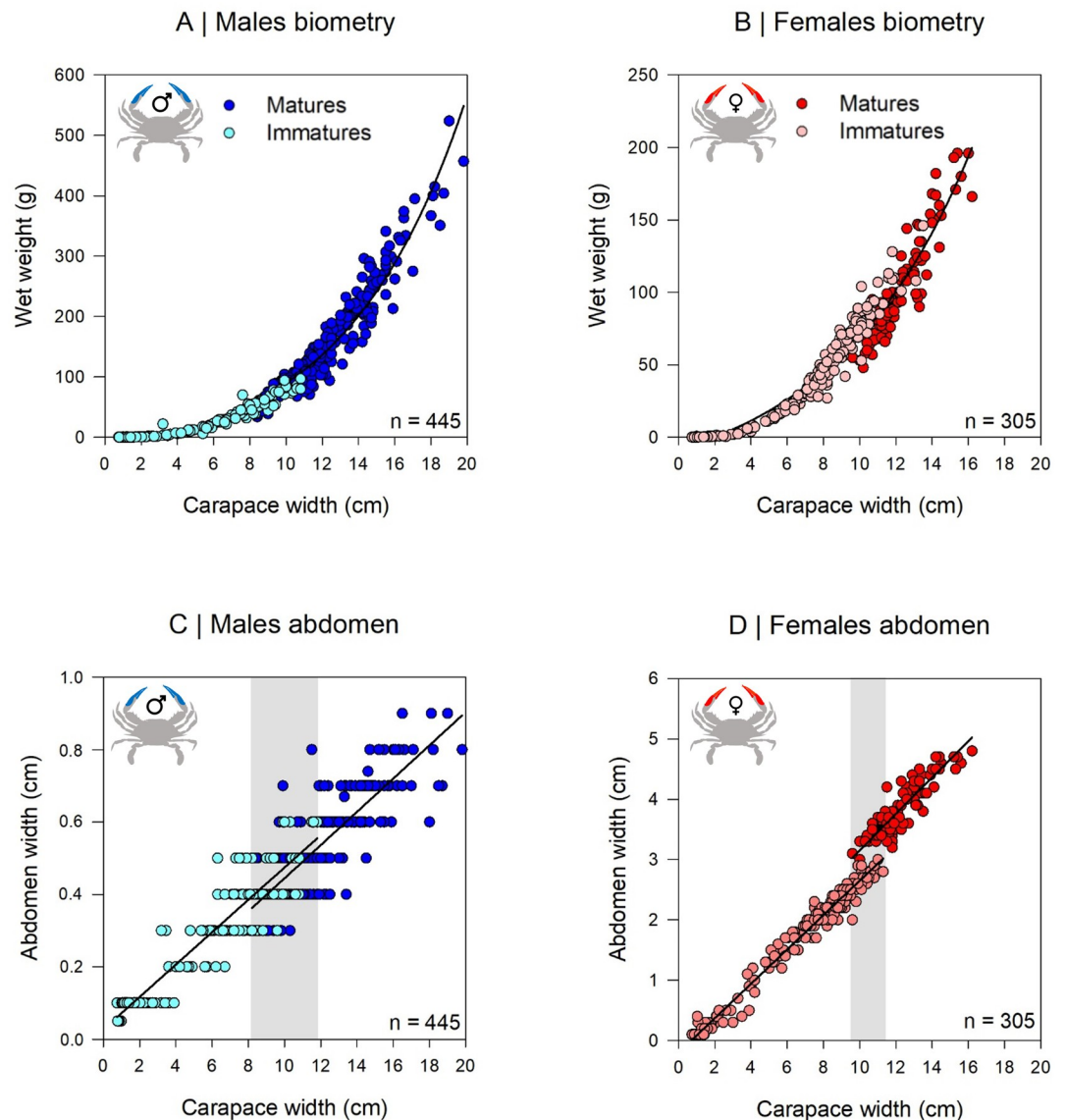


Fig 3. Relationship between carapace width (CW) and wet weight (WW) for (A) males and (B) females; relationship between CW and abdomen width for (C) males and (D) females. Copyright: @Marchessaux, CC BY 4.0 license.

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inducing a lag of size distributions which were 86% overlapped (Fig 4). During summer, the both sexes showed the same sizes ranges with, for both, the same dominant sizes (9.9 cm for males; 9.4 cm for females) with both size distributions overlapped (Fig 4). Autumn was the season regrouping two distinct cohorts: the adult one with dominant contribution of 11.3 cm and 10.8 cm size for males and females respectively; and the juvenile cohort mainly composed by 0.9 cm organisms for both sexes (Fig 4).

The early stage (< 2 cm) and juveniles (< 5 cm) densities showed a seasonal pattern (S3 Fig). They were observed in March 2022 and their density decreased from 6 ± 3 ind m^{-2} to 2 ± 3.0 ind m^{-2} in May. Early stage and juveniles were absent in the samples until September where low density was measured (1 ± 3 ind m^{-2}). Whereas a large arrival of early stage was recorded between November (15 ± 8 ind m^{-2}) and December (12 ± 5 ind m^{-2}).

Table 1. (A) Width (CW) and weight (WW) characteristics (mean, minimum (Min.), maximum (Max.)) for the blue crab caught in the saltmarshes of Trapani (Italy). (SE = standard error); (B) allometric parameters of the relationship ($WW = a \cdot \exp(b \cdot CW) + y_0$) between the carapace width (CW) and the weight (WW) and (C) allometric parameters of the relationship ($ABD = a \cdot CW + b$) between the abdomen width (ADB) and the carapace width (CW). *: significant difference.

A			CW characteristics (cm)				WW characteristics (g)			
Sex	Maturity	n	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.
Males	Immatures	168	5.02	± 3.26	0.75	10.80	25.16	± 27.18	0.02	97.00
	Matures	277	12.06	± 2.29	8.20	19.80	152.06	± 83.50	34.00	524.00
Females	Immatures	205	6.52	± 3.02	0.74	10.40	36.67	± 25.61	0.02	104.00
	Matures	100	12.34	± 1.48	9.60	16.20	105.30	± 35.93	48.00	196.00

B			Parameters of CW-WW relationship					
			$WW = a \cdot \exp(b \cdot CW) + y_0$					
Sex	Maturity	n	a	b	SE(b)	R ²	p value	y ₀
Males	Immatures	168	11.32	0.21	0.013	0.96	< 0.0001*	-15.63
	Matures	277	106.42	0.09	0.010	0.93	< 0.0001*	-188.33
	Both	445	29.56	0.15	0.004	0.95	< 0.0001*	-45.17
Females	Immatures	205	18.43	0.17	0.009	0.95	< 0.0001*	-25.99
	Matures	100	138.66	0.07	0.053	0.83	0.2082	-214.60
	Both	305	29.69	0.13	0.007	0.94	< 0.0001*	-38.20

C			Parameters of ABD-CW relationship				
Sex	Maturity	n	a	b	SE(a)	R ²	p value
Males	Immatures	168	0.045	0.027	0.001	0.84	< 0.0001*
	Matures	277	0.046	-0.014	0.002	0.66	< 0.0001*
	Both	445	0.043	0.028	0.001	0.88	< 0.0001*
Females	Immatures	205	0.285	-0.199	0.003	0.98	< 0.0001*
	Matures	100	0.301	0.139	0.017	0.76	< 0.0001*
	Both	305	0.333	-0.424	0.004	0.96	< 0.0001*

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The relationship between temperature, salinity and the CW-WW slope coefficient as a proxy of growth change showed opposite results between males and females (Fig 5). For both sex, temperature had a positive effect on growth: higher the temperature, the larger the growth. Salinity had an opposite effect for males and females: the growth was higher for male for a large salinity range (16 units; from 24 to 40) than females where the highest growth values were observed on a smaller salinity range (12 units; from 28 to 40) (Fig 5). In other words, the size spectra showed that across different temperature and salinity gradients, the growth period for males is faster than for females (Fig 5).

The size at first maturity showed same values (males: 11.75 cm, females: 12.0 cm; Fig 6A). The temporal evolution of mature/immature stage frequency showed that matures males dominated significantly (ANOVA, Bonferroni post-hoc test, $F = 84.6$, $p < 0.001$) the population throughout the 61% of the time (Fig 6B). In contrast, mature females were observed only 33% of the time (Fig 6B) with matures females mainly observed between late summer and early fall. In fact, it appears that the population studied in the saltmarshes of Trapani is substantially composed by a population of young individuals. The temporal evolution of sex ratio Males: Females (M:F) showed highest values in September and October for both years (Fig 6C) indicating a dominance of males during these periods and a decrease in the number of females. For the rest of the time, the M:F sex ratio was close to 1 suggesting a relative equilibrium between both sex. The reproduction events (i.e. field observations of breeding couples face to face abdomens) were observed in July and September/October in 2021; and between May-July and in October for the year 2022 (Fig 6C). In the saltmarshes of Trapani, large matures males were always paired with large matures females (S4 Fig) illustrating here the reproduction capacity of large specimens only.

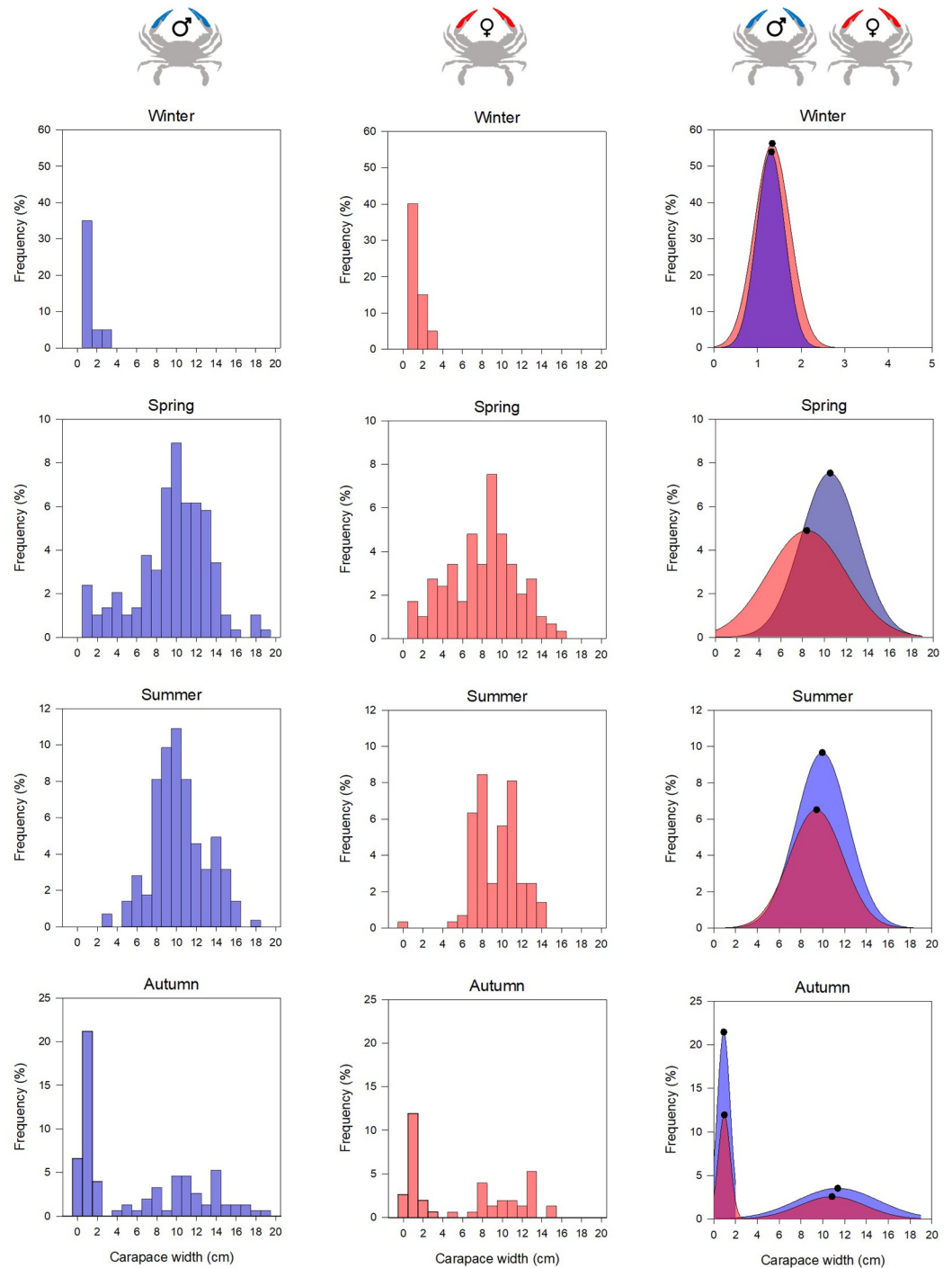


Fig 4. Seasonal blue crab size distribution of males (on the left, in blue), females (in the center, in red) and the estimated Kernel densities of each sex (on the right, the black dots represent the size with the highest frequency).
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The relation between the Fulton's condition factor (K) and temperature/salinity showed different patterns between sexes and maturity stage (Fig 7). For both sexes immatures stages, the condition factor was high for highest temperatures (30°C) but lower salinity ($S = 20$) (Fig 7A

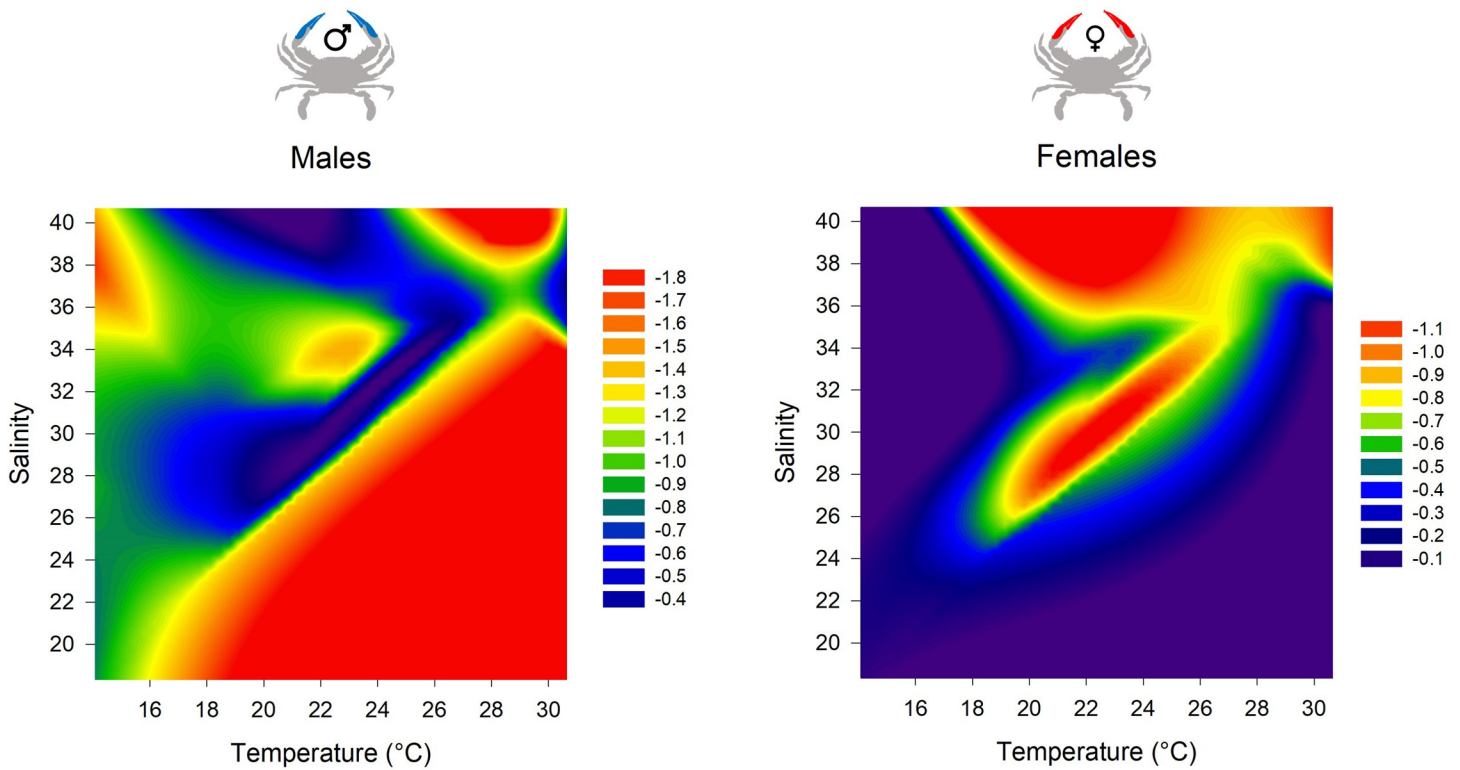


Fig 5. 3-dimensional relationships of the monthly males and females CW-WW slope coefficients (a) as a function of temperature and salinity. Copyright: @Marchessaux, CC BY 4.0 license.

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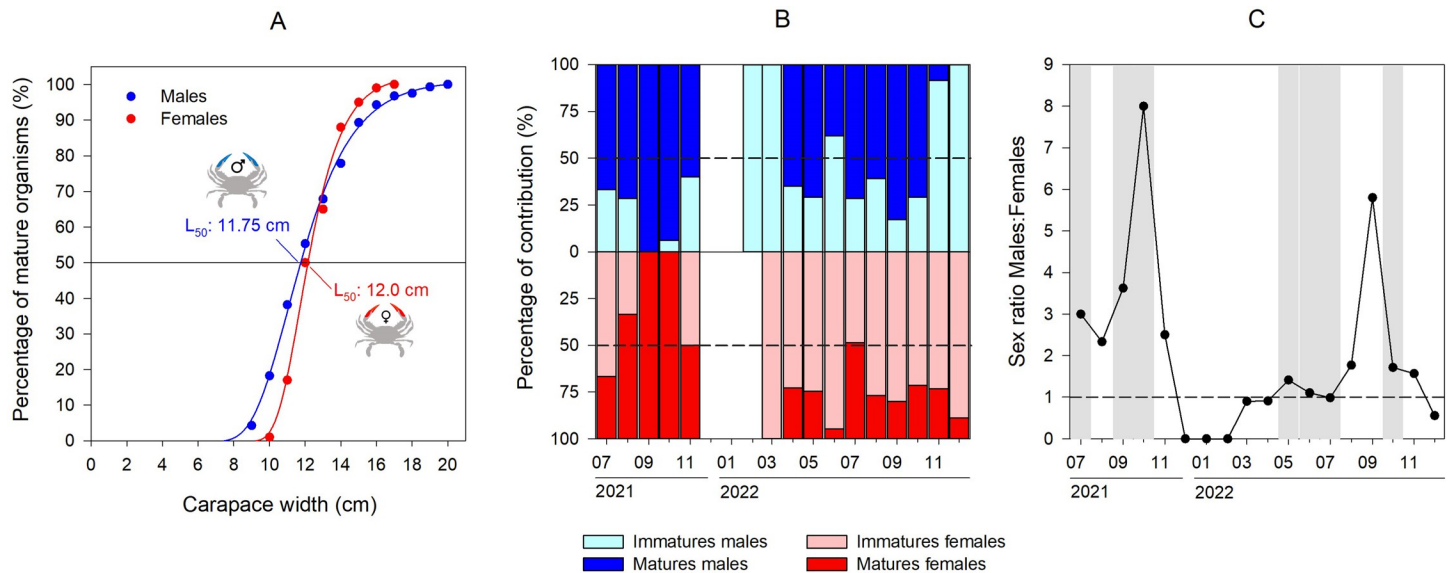


Fig 6. (A) Logistic regressions (solid lines) fitted to the percentage of mature males and females based on the carapace width (CW), L_{50} values represent the CW when 50% of the population was mature; (B) temporal evolution of the percentage of contribution of the immatures/matures males and females between July 2021 and December 2022; (C) temporal evolution of the sex ratio Males/Females between July 2021 and December 2022, the gray bars represent the reproduction periods observed and monitored on the field. Copyright: @Marchessaux, CC BY 4.0 license.

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and 7B). The same trends were observed for matures males where the condition factor was higher at high temperature (30°C) and at S = 25. In contrast, matures females showed an optimal condition factor for salinity S~32 and temperature at 22°C (Fig 7C and 7D). Ovigerous females appeared in early autumn (September and October) for both study years (Table 2), and were sampled in the traps in the channel connecting saltmarshes to the sea. The ovigerous females CW ranged from 10.1 to 15.6 cm and the egg mass weight wet from 17 to 56 g representing an average of $25 \pm 5\%$ of the total female body wet weight (Table 2). The number of eggs estimated ranged from 985 137 (Female CW = 10.1 cm) to 2 156 874 eggs (Females CW: 13.3 cm) (Table 2). Two different eggs embryotic development stages were observed: 7 females with stage II-intermediate (orange), and 5 females with a stage III-pre-hatching (dark brown) (Table 2). The mean eggs diameter was significantly different (ANOVA, Bonferroni post-hoc test, $F = 51.7$, $p < 0.002$) between the stage II ($209.4 \pm 30.6 \mu\text{m}$) and III ($303.0 \pm 4.3 \mu\text{m}$).

Discussion

Size spectra analysis is a powerful approach to describe the relationship between species size and abundance with environment [5]. Especially, in the case of invasive species, this approach is very useful to define the sensitive periods to implement management measures [5, 55]. By using this approach, our study demonstrates that the population of the invasive blue crab *C. sapidus* in the saltmarshes studied in Sicily is well-established consisting juveniles, subadults and adults. The analysis of the size spectra distribution showed integrative results on how environment was driving the blue crab's population in terms of growth, sexual maturity and copulation (Fig 8). In particular, we were able to provide more information on early stages (< 2 cm) that have been poorly studied because of the limited methodology to catch them (Fig 8).

Population size structure

Our results on the morphometric relationships and allometric growth of blue crab were partially consistent with previous studies carried out in the Mediterranean basin. Comparing size spectra, blue crabs (males and females) showed positive allometry which is in disagreement with published studies in Turkey [56] for example. This may be because our study included the early juveniles' stages (< 2 cm) in the allometric curves which is rarely presented in other studies. However, the positive allometry was in agreement with that presented for Egyptian [57] and Greek [58] blue crabs populations. The difference in the slopes of allometry equations was consistent with the fact that decapods and crustaceans exhibit significant plasticity in their phenotypic morphology that may be associated with environmental and/or genetic factors [59] such as time of year, temperature, food, stomach fullness, age differences, maturity stage, and sex [60, 61].

The largest crabs were caught in spring-summer and the smallest specimens in autumn-winter. While this pattern was not consistent with what observed in studies along the Atlantic coasts [62], but consistent with other populations in the Mediterranean Sea (e.g. Lesina Lagoon, Italy [63] and Monolimni Lagoon, Aegean Sea [64]). Local conditions are indeed crucial factors in addressing local presence of species as showed by our analysis of size spectra. Indeed, CW-WW allometric slopes estimated in Trapani saltmarshes showed opposite relationships to temperature and salinity between males and females and this corroborates the hypothesis that are local environmental conditions that are mostly able to shape local presence. In our study, males showed a faster growth under cooler temperatures (spring) and salinities higher than 24, while females had a slower growth in spring and reached a maximum size in summer (Fig 8). Our findings were able to show that our study site could be an important recruitment zone of early stages (< 2 cm) whose were coming here to growth as observed in

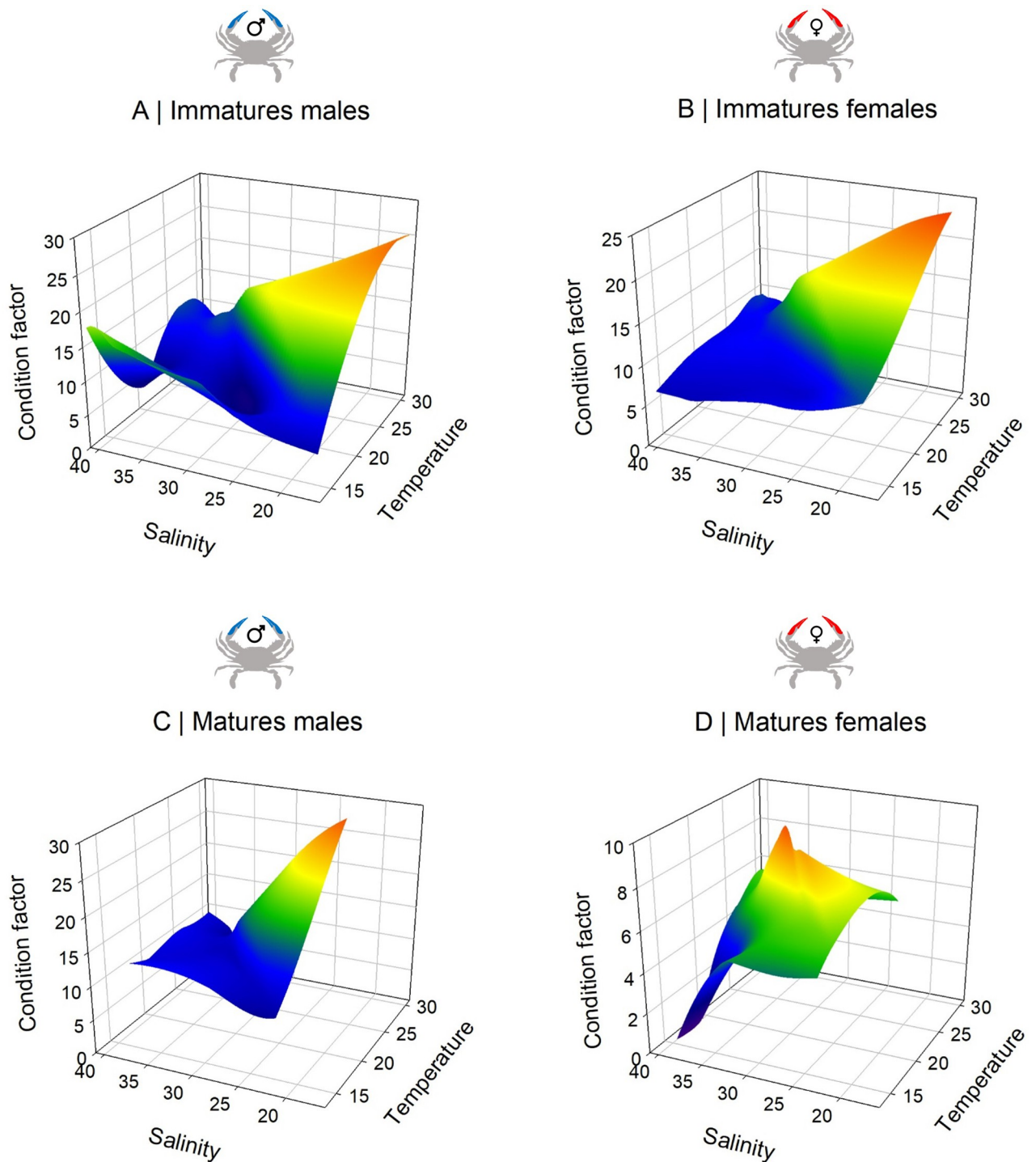


Fig 7. 3-dimensional relationships between the condition factor K, temperature and salinity for (A) immatures males, (B) immatures females, (C) matures males and (D) matures females. The color gradient represents the values of the Fulton's conditions factor (z axis). Copyright: @Marchessaux, CC BY 4.0 license.

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Table 2. Fecundity of females *C. sapidus*: Sampling dates, female carapace width and wet weight, egg mass wet, total number of eggs estimated, eggs embryotic development stage (II: Intermediate; III: Pre-hatching), and mean (\pm standard deviation) egg diameter.

Sampling date	Female Carapace width (cm)	Female wet weight (g)	Egg mass wet weight (g)	Total number of eggs	Eggs embryotic development stage	Eggs diameter ($\mu\text{m} \pm \text{SD}$)
2 Sept. 2021	11.0	168	42	1 769 297	II	241.1 \pm 10.5
2 Sept. 2021	10.7	142	56	1 814 390	III	310.0 \pm 14.5
2 Sept. 2021	13.2	147	36	1 278 276	II	197.9 \pm 9.4
2 Sept. 2021	12.6	144	38	1 464 727	III	301.6 \pm 16.1
6 Oct. 2021	10.9	91	21	1 621 248	II	231.2 \pm 11.4
6 Oct. 2021	15.6	180	44	1 924 586	II	178.6 \pm 8.5
6 Sept. 2022	11.9	87	19	1 675 254	III	298.5 \pm 6.4
6 Sept. 2022	10.1	79	17	985 137	II	176.4 \pm 8.7
6 Sept. 2022	10.4	90	22	1 053 546	III	301.7 \pm 10.6
7 Oct. 2022	13.0	112	27	2 023 587	II	249.6 \pm 4.6
7 Oct. 2022	13.3	90	20	2 156 874	III	303.2 \pm 8.1
7 Oct. 2022	12.6	108	31	2 057 964	II	190.8 \pm 4.5

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the native area in North Carolina (USA, native area) [65]. The recruitment period was identified in our study between September and November (Fig 8). The population's growth period was identifier from the end of winter to early summer (Fig 8).

Our study in Trapani showed that *C. sapidus* has locally a complex life cycle, involving seasonal and spatially specific migrations for copulation and reproduction related to temperature and salinity [18, 22, 23, 26, 66, 67] thus generating spatio-temporal variability in population abundance at the small scale (Fig 8). In the saltmarshes, the population decreased in abundance and showed adult hibernation when water temperature was lower than 12°C (Fig 8) which is consistent both with what was observed by Marchessaux *et al.* [9] showing that this temperature represents the putative critical lower thermal threshold making local conditions unfavorable to the metabolism of *C. sapidus*, and with what is well known that specimens become inactive during col period [14], as reported in other Mediterranean and Atlantic regions [7, 68–70]. The relationship between Fulton's conditions factor and temperature/salinity for immature and mature males and females showed different environmental windows explaining the complex life cycle of *C. sapidus*. Immature stages were present at salinities < 25 corresponding to winter salinities and a significant effect of temperature was observed. On the other hand, mature stages showed marked differences between males and females: temperature had a marked effect on the condition factor of males for salinities between 15 and 30, corresponding to winter and spring periods. On the contrary, the condition factor of females presented a more restricted environmental window with a thermal optimum of 24°C and a salinity of 30, observations consistent with the thermal tolerance of *C. sapidus* recently studied [9] where an optimum of 24°C was defined for the species' metabolism. Our results showed a marked difference in environmental tolerance between males and females which could corroborated the idea of the complexity of the life cycle which occupies desalinated areas for reproduction and salty areas where the females mature their eggs. Thus, temperature and salinity play a key role in the maintenance of male and female populations throughout the year but also for sexual maturity, reproduction and the release of larvae at sea by the females. Indeed, mature females are catadromous, migrating from hyposaline waters (< 30 psu) to higher salinity waters to spawn [7, 71].

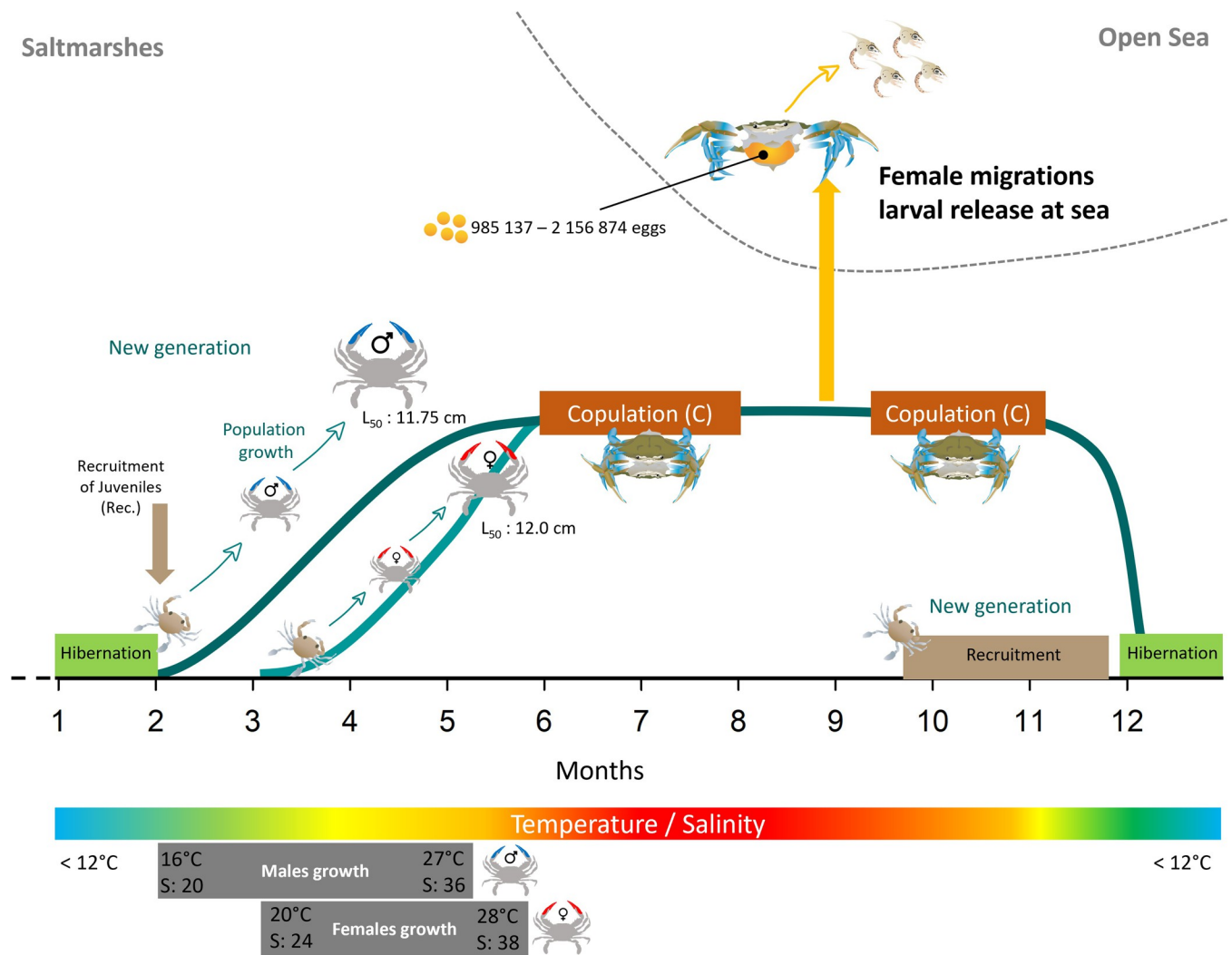


Fig 8. Schematic representation of the results obtained on the population size spectra dynamics in the saltmarshes of Trapani (Sicily, Italy). Copyright: @Marchessaux, CC BY 4.0 license.

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Sexual maturity and reproduction

The sizes at sexual maturity (L_{50}) estimated in our study at 11.75 cm for males and 12.0 cm for females were in the same range as those presented in the studies in native/introduced areas published (Table 3). However, it appeared that L_{50} differed locally. For example, in the native areas, females' L_{50} was estimated at 15–16 cm (Florida [72]), 12–14.7 cm in Chesapeake Bay [73, 74], and 10.33 cm in Brazil [52] (Table 3). For male, L_{50} obtained in our study was larger than those cited in the literature in Brazil [69], but consistent with data available in the USA by Van-Engel (1958) [18] (Table 3). Our study showed that mature males were observed 2/3 of the time in contrast to mature females which were observed only 1/3 of the time mainly in summer, corresponding to the periods of maximum size observed in females. These observations were in accordance with the copulation period of *C. sapidus* characterized in our study, divided into two periods: late spring-early summer and early autumn when the highest percentage of matures males and females was observed. There are few data on the precise copulation periods of *C. sapidus* available in the literature and our results were in the same trends in

the native areas in St. Johns River (Florida) and in the lower Chesapeake Bay [72, 75] with copulation observed during spring-summer. As determined by Jivoff [76], large matures males were always paired with large matures females, that is consistent with what we observed in our study. This strategy allows large males to be more competitive for access to females than small males, thus, large males win in aggressive interactions with rivals and transmit larger ejaculates to females [76]. The amount of time a male spends guarding influences female survival and access to her (this last phase is not clear). Male guarding time and ejaculate size increase when the male/female ratio is high, but ejaculate size decreases when males mate frequently, with short intervals between copulations [76]. In another hand, female blue crabs store sperm to fertilize the eggs they produce throughout their lives, where the reduced amounts of stored sperm may limit the total number of eggs they produce [76].

The variation in sex ratio could be explained by the different total mortality rates between sexes, the different migration patterns, and these parameters seemed to affect the males/females relative occurrence [77]. Especially, the sexual proportion of *Callinectes* species is related to reproductive behavior, female migrations, seasonal temperature variation, and salinity gradient [18, 78]. According to Berglund (1981) [77], males generally show a reduction in energy investment for growth in order to reduce pre-birth risk. In our study, the population was in favor of males which was in accordance with the high percentage of matures males all over the year which was consistent with similar studies in Italian [70, 79], Croatian [80] and Egyptian [57] lagoons, but diverged from sex ratio in a lagoon in southern Turkey, where the population was in favor of females [81]. A seasonal transition in sex ratio as a function of water temperature has been reported for *C. sapidus*, with a positive response in the case of male crab abundance, and negative in the case of females [82]. These conclusions were applicable to the results obtained in our study since the monthly variation of the sex ratio showed an almost total absence of females (dominance of males) at the end of summer (September-October) for both years studied, certainly due to the migration of adult females to waters of higher salinity, where they release their larvae [83]. This hypothesis was confirmed by the ovigerous females in the sea observed by fishermen (pers. obs.) and showing a high salinity tolerance/growth of females observed in the CW-WW slopes of our results.

Spawning periods and female fecundity

Spawning of *C. sapidus* occurs throughout the year, with a predominance during the warmer months in its native sub-tropical range, pattern observed in most tropical brachyurans [52, 84, 85]. This reproductive pattern of *C. sapidus* can be classified as "seasonal-continuous" in Brazil, meaning a higher incidence in certain seasons of the year [86], possible because of the continuity of the physiological growth and reproductive process throughout the year. This pattern does not occur in temperate regions (native and non-native), where ovigerous females are present for a few months, being classified as discontinuous, which is related to the favorable environmental conditions that prevail during these periods [85]. This was the case in our study where ovigerous females were captured only in late summer/early autumn (September-October). These results were consistent with the results obtained in Italy in Lesina Lagoon [63] and in Northeast Aegean Sea [64]. This seaward migration is very important in the life cycle of the species, in which mature and ovigerous females move to higher salinity waters for gonad maturation. Saltwater provides lower larval mortality than waters with salinity variations; in addition, proposed benefits to marine larval development include reduced predation and increased survival in high salinity waters due to physiological limitations [52, 87–90]. Indeed, the blue crab's salinity and temperature ranges for hatching fluctuate with geographic distribution and life history. Egg hatching generally proceeds between 19 and 29°C and for optimal

Table 3. Studies on the size at first maturity for *Callinectes sapidus*.

Country	Study site name	Native / Introduced	Sex	Size at maturity (cm)	Reference
Italy	Trapani saltmarshes	Introduced	Males	11.75	Our study
Brazil	Babitonga Bay	Native	Males	8.9	[69]
USA	Sarah's Creek and Purtan Bay	Native	Males	10.7	[49]
USA	Chesapeake Bay	Native	Males	11.2	[18]
Italy	Trapani saltmarshes	Introduced	Females	12.00	Our study
Greece	Evros River	Introduced	Females	12.39	[64]
Turkey	Beymelek Lagoon	Introduced	Females	11.85	[81]
Brazil	Lagoon-Estuarine of Iguape and Cananéia	Native	Females	10.33	[52]
Brazil	Babitonga Bay	Native	Females	10.2	[69]
USA	St. Johns River	Native	Females	15–16	[72]
USA	Tampa Bay	Native	Females	13.0	[71]
USA	-	Native	Females	12.5	[101]
USA	Texas bay	Native	Females	12.0	[102]
USA	Chesapeake Bay	Native	Females	14.7	[73]
USA	Chesapeake Bay	Native	Females	12.0	[74]
USA	Chesapeake Bay	Native	Females	11.2	[18]

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salinity between 23 and 28 psu [91]. In our study, temperature and salinity were between 22.0 and 27.0°C and 32.5 and 37.3 respectively in late summer, period in which females were in advanced embryonic stages (II-intermediate and III-pre-hatching). Considering the embryonic stages of the eggs recorded in our study, it is certainly possible that the females were ovigerous earlier in the summer, as in the species' native range [92], but that a high density migrated out of the study area before extruding the eggs onto the abdomen, which would explain the low number of ovigerous females captured in our study.

Additionally to the growth rates, sexual maturity, fecundity, associated with the number and diameter of eggs, gave an important information for estimating the reproductive potential of the species/population [74, 81]. Our results showed fecundity in the same range as that observed in the species native range in Chesapeake Bay (USA) [18] and Brazil [69] and in the Mediterranean Sea [93], but lower than populations from Mexico [94]. Such oscillations may be caused by several factors such as: the timing and stage of embryonic development of the eggs at the time of the count or the genetic characteristics of the populations [89]. However, our results remained consistent with the data available in the literature and provided new information on the fecundity of *C. sapidus* in Sicily, which has never been studied before.

Conclusion

To conclude, *Callinectes sapidus* is known to have negative ecological impacts on invaded habitats in the Mediterranean Sea. Our study emphasized a novel approach to study an invasive species, in particular the blue crab *C. sapidus*. Sicily being an island does not have brackish lagoons or areas with large variations in salinity such as estuaries that are favorable for *C. sapidus* and where the species is mostly observed. Our study showed that the blue crab *C. sapidus* has succeeded in establishing itself in microhabitats such as saltmarshes in Sicily. In these areas, known to be favorable for the species in its native area [95–97], the blue crab exhibits a life cycle involving spatio-temporal migratory movements between saltmarshes and the adjacent coastal areas, which is consistent with its complex life cycle [7, 22–24, 68, 98]. These microhabitats provide favorable habitat for juvenile growth with extensive areas of *Ruppia maritima* and *Cymodocea nodosa* as observed in the Monolimni Lagoon [64]. These habitats

are known to be productive and offer high abundances of macrozoobenthos [64]. On the other hand, we observed that large adults > 15 cm were rarely observed, which raises the hypothesis that the saltmarshes of Trapani would be a nursery area for the young stages of *C. sapidus*, which, once adult, would partially leave the saltmarshes in favor of other habitats such as the lagoon of Marsala where the species is observed in spring-summer (pers. obs.). The next step will be to determine the movements of adult crabs within the saltmarshes but also outside to confirm or not the hypothesis that the saltmarshes constitute a nursery area for *C. sapidus*. The results presented in this study provide evidence of the local adaptation of the species to invade different types of habitats, sometimes taking advantage of microhabitats such as saltmarshes, which would therefore be priority areas to study in Sicily.

Using a long-term and frequent population monitoring we were able to determine precisely how the environment (temperature and salinity) are influencing the males and females size spectra, combined with the periods of copulation and reproduction proved to be essential and useful to determine the periods suitable for the control of the species. Thus, thanks to our results the most sensitive periods for the species and therefore the periods when it is necessary to control the population should be in spring and summer when the highest percentage of mature males was recorded (more than 50% for 5 months). Females matured later and in smaller proportions (summer and autumn). Taking sexual maturity and the proportion of mature individuals into consideration, males would certainly be the key for an effective population control. But two hypotheses can be advanced. The first would be to fish intensively for males to reduce the sex ratio and prevent copulation. Intense, size-focused fishing of males may alter the male size structure and sex ratio of local populations (*sensu* Jivoff, 2003 [76]), especially for crustaceans species [99]. The second hypothesis is that harvesting mature females has the greatest impact on populations, and harvesting males is only effective if their numbers are such that females are unable to find a mate, as observed in North America [100]. These two hypothesis are to be explored in the near future. The most effective strategy would undoubtedly be to eliminate females before the period of sexual maturity and potential copulation. In our case, this would involve intensive control in spring.

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Supporting information

S1 Fig. Pictures of the traps specifically designed for our study with dimensions details, used to sample blue crabs. Copyright: @Marchessaux, CC BY 4.0 license.

(DOCX)

S2 Fig. Temporal evolution of (A) temperature and (B) salinity between July 2021 and December 2022. Copyright: @Marchessaux, CC BY 4.0 license.

(DOCX)

S3 Fig. Monthly evolution of the blue crab juveniles' density (ind m⁻²) during the monitoring period. Copyright: @Marchessaux, CC BY 4.0 license.

(DOCX)

S4 Fig. Relationships between immatures/matures males and females size (carapace width, cm) and linear regressions. Copyright: @Marchessaux, CC BY 4.0 license.

(DOCX)

S1 Table. Characteristics of the habitat of the saltmarshes of Trapani. Areas of each substrate (m²) and percentage of coverage [%] [34, 45]. (DOCX)

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