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ORIGINAL ARTICLE

Invasion of freshwater systems by the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 – new insights from Italian regions

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Abstract: The Atlantic blue crab, *Callinectes sapidus* Rathbun 1896, is a highly invasive species in the Mediterranean Sea, posing significant ecological and socio-economic threats in marine and estuarine environments. This study investigates the occurrence and potential impacts of *C. sapidus* in Italian inland waters, specifically examining specimens collected from an irrigation canal in Pomposa on the Adriatic coast and from the Ombrone River, flowing into the Tyrrhenian Sea. Morphometric analysis revealed that males from Pomposa had a mean carapace width (CW) of 172.15 mm (±23.77 mm) and a mean weight of 282.10 g (±98.34 g), while females had a mean CW of 160.68 mm (±25.76 mm) and a mean weight of 173.26 g (±56.11 g). In contrast, males from the Ombrone River were significantly smaller, with a mean CW of 73.36 mm (±10.11 mm) and a mean weight of 25.33 g (±8.86 g). The sex ratio in Pomposa was heavily skewed towards males (3.26:1), suggesting a dominance of adult males in this freshwater habitat. A similar trend was observed in the Ombrone River, though based on a smaller sample size, with only 1 female among 10 specimens. The condition factor (K) was significantly higher for females (1.960) than for males (0.116), while growth coefficient (*b*) for males had a higher value (2.401) than that of females (1.788), indicating differing growth patterns. This study provides new ecological and biological data on *C. sapidus* in freshwater systems, underscoring the need for targeted monitoring and management to mitigate the spread and impact of this invasive species in Italian inland waters.
***Keywords*:** alien invasive species; Italian waters; biological invasions; morphometric analysis; ecological traits

Sažetak: INVAZIJA ATLANTSKOG PLAVOG RAKA *CALINECTES SAPHIDUS RATHBUN*, 1896 U SLATKOVODNE SUSTAVE: NOVE SPOZNAJE IZ TALIJANSKOH REGIJA. Atlantski plavi rak, *Callinectes sapidus* Rathbun 1896, izrazito je invazivna vrsta u Sredozemnom moru te predstavlja značajne ekološke i socio-ekonomske prijetnje u morskim i estuarijskim ekosustavima. Ovo istraživanje proučava prisutnost i potencijalni utjecaj vrste *C. sapidus* u talijanskim kopnenim vodama na osnovi uzoraka prikupljenih iz irigacijskog kanala u Pomposi na području jadranske obale i iz rijeke Ombrone koja se ulijeva u Tirensko more. Morfometrijska analiza pokazala je da su mužjaci iz Pompose imali prosječnu širinu oklopa CW=172,15 mm (±23,77 mm) i prosječnu težinu 282,10 g (±98,34 g), dok su ženke imale prosječnu CW=160,68 mm (±25,76 mm) i prosječnu težinu 173,26 g (±56,11 g). Nasuprot tome, mužjaci iz rijeke Ombrone bili su znatno manji, s prosječnom CW=73,36 mm (±10,11 mm) i prosječnom težinom 25,33 g (±8,86 g). Omjer spolova u Pomposi bio je izrazito u korist mužjaka (3,26:1), što ukazuje na dominaciju odraslih mužjaka u ovom slatkovodnom staništu. Sličan trend zabilježen je u rijeci Ombrone, iako na manjem uzorku, sa samo jednom ženkom među 10 primjeraka. Koeficijent kondicije (K) bio je značajno viši kod ženki (1,960) nego kod mužjaka (0,116), dok su se koeficijenti rasta (*b*) razlikovali među spolovima, pri čemu su mužjaci pokazali višu vrijednost (2,401) u usporedbi sa ženkama (1,788), što ukazuje na različite obrasce rasta. Ovo istraživanje pruža nove ekološke i biološke podatke o plavom raku *C. sapidus* u slatkovodnim sustavima, naglašavajući potrebu za njegovim ciljanim praćenjem i upravljanjem kako bi se ublažilo širenje i utjecaj ove invazivne vrste u talijanskim kopnenim vodama.
***Ključne riječi*:** strane invazivne vrste; talijanske vode; biološke invazije; morfometrijska analiza; ekološke značajke

INTRODUCTION

Biological invasions are a major threat to global biodiversity, particularly impacting freshwater ecosystems (Strayer, 2010). These environments, though covering a small fraction of the earth's surface, host a rich diversity of life. However, their isolated nature makes them vulnerable to invasive species which can outcompete native species, introduce diseases or parasites, and alter habitats (Bellard *et al*., 2016; Jones, 2017; Pyšek *et al*., 2020;

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Najberek *et al*., 2022; Henry *et al*., 2023). In freshwater ecosystems, invasive species also pose economic risks, impacting fishing, tourism, and related activities (Gherardi, 2007; Johnson *et al*., 2008; Hanley and Roberts, 2019).

In Europe, rivers and lakes have been particularly affected by biological invasions. An emblematic example is the red swamp crayfish, *Procambarus clarkii* (Girard, 1852), which has colonized many European waterways, competing with native crayfish species and altering aquatic plant communities (Souty-Grosset *et al*., 2016). Interestingly, this species has also recently been observed in purely marine waters, underlining its great ability to adapt to different environments and, like the blue crab, its high euryhalinity (Nota *et al*., 2024a).

Callinectes sapidus Rathbun, 1896 (Decapoda: Brachyura: Portunidae), commonly known as the Atlantic blue crab, is considered one of the 100 most invasive alien species in the Mediterranean Sea (Streftaris and Zenetos, 2006). Native to the western Atlantic coast, it is known for the ability to colonize new environments due to its high fecundity and aggressive behavior. Introduced to European waters in the 20th century, *C. sapidus* is now widely distributed throughout the Mediterranean and Black Sea (Mancinelli *et al*., 2017; Windsor *et al*., 2019; Mancinelli *et al*., 2021). Its range is continuously expanding both westwards (Lipej *et al*., 2018) and eastwards (Snigirev *et al*., 2020). In Italy, the presence of this species has been extensively documented in marine and brackish waters, initially especially in the Adriatic (Mizzan, 1993) and later in the other Italian seas, with more recent reports also in the Tyrrhenian Sea which was the last basin to be entirely colonized (Tiralongo *et al*., 2021). The most probable vector of introduction is ballast water (Nehring, 2011).

Callinectes sapidus is a highly euryhaline species, meaning it can thrive in a wide range of salinities. It is commonly found in upper estuaries and lower reaches of rivers, and it has been recorded under fully freshwater conditions in coastal lakes and rivers as far as 195 km from the sea (Nehring, 2011). This adaptability enhances its potential as an invasive species, allowing it to colonize diverse habitats and rapidly expanding its range. In this regard, the discovery of the blue crab in freshwater environments represents a significant novelty for Italy. Indeed, only recently, the species was observed in inland waters of Latium, Tuscany, Basilicata, and Sicily, raising concerns about its impact on these newly colonized ecosystems. Vecchioni *et al*. (2022) have documented the presence of *C. sapidus* in Sicilian rivers, such as the Irminio and the Imera Meridionale rivers, at considerable distances from the river mouths. Meanwhile, Scalici *et al*. (2022) documented the presence of the species in freshwater systems of Latium, Tuscany, and Basilicata. However, no detailed data on population structure and growth parameters were provided. More recently, Bedmar *et al*. (2023) demonstrated that in some Spanish rivers the probability of occurrence of the blue crab decreases significantly in the first few kilometers upstream; however, it remains concerning even several kilometers from the river mouth. They observed the species in river over 100 km from the sea, indicating that the blue crab can easily penetrate far into freshwater systems. This expansion poses a new threat to the biodiversity of small coastal river basins, which are often valuable and highly threatened. Finally, it is worth noting that the first record of the species in freshwater within the Mediterranean region occurred in May 2013 in Vransko Lake (Croatia). This freshwater ecosystem, however, is connected to the Adriatic Sea *via* a narrow artificial channel (Župan *et al*., 2016).

The adaptability and invasiveness of *C. sapidus* are attributed to several biological and ecological traits. The species exhibits high reproductive output, with females capable of producing millions of eggs per spawning event (Prager *et al*., 1990). Furthermore, the zoeal larval stages of the blue crab are marine planktonic with active swimming ability and can be transported over long distances across the sea by currents, facilitating widespread dispersal (Epifanio, 2019). The last larval stage or megalopa settles down on the seabed undertaking a benthic lifestyle while maintaining some swimming ability (Forward *et al.*, 2004) Juvenile and adult crabs are omnivorous and opportunistic feeders, preying on a wide range of organisms, including mollusks, crustaceans, small fish, algae and detritus. This dietary flexibility enables them to exploit various food resources in different habitats (Kampouris *et al.*, 2019). The environmental tolerance of *C. sapidus* also contributes to its invasiveness. The species can survive in temperatures ranging from 5°C to 35°C and salinities from freshwater to full-strength seawater (Nehring, 2011). This broad environmental tolerance allows the blue crab to establish populations under diverse climatic and hydrological conditions, further enhancing its potential for invasion, a potential that appears even more enhanced in the context of global warming (Marchessaux *et al.*, 2022). Additionally, the species' ability to burrow and its strong swimming capabilities enable it to evade predators and withstand adverse environmental conditions as, in one case here reported, drying out of water in confined environments. The new data reported here integrate and expand the existing knowledge on the distribution and impact of *C. sapidus* in freshwater ecosystems, contributing to a better understanding of its invasiveness and potential effects on local ecosystems (Marrone and Naselli-Flores, 2015; Mancinelli *et al*., 2017).

Therefore, we add new data to the existing literature, highlighting the presence and potential impact of *C. sapidus* in Italian freshwater environments, an aspect previously under-documented (Scalici *et al*., 2022; Vecchioni *et al*., 2022). Moreover, a further and recent study highlights the potential of the invasive blue crab to reach freshwater ecosystems through long-distance migrations in the Ebro and Guadiana rivers (Bedmar *et al*., 2023). These other observations underscore the

significance of our study, as it contributes new insights into the expansion of *C. sapidus* in inland waters, emphasizing the need for monitoring its presence and the potential impacts on native species and ecosystems. This spread highlights the invasive potential of *C. sapidus*, emphasizing the need for monitoring and management strategies to mitigate its impact on native ecosystems and economy of fishery and aquaculture. Addressing the invasion of *C. sapidus* requires a comprehensive approach that includes early detection, rapid response, and long-term management plans. Collaborative efforts between researchers, policymakers, and local communities are essential to effectively manage this invasive species and protect freshwater biodiversity.

MATERIAL AND METHODS

The blue crab (*Callinectes sapidus*) individuals were collected from two freshwater systems in Italy: an irrigation canal of Pomposa, a location situated at a distance of 10 km from the Adriatic Sea and 1 km from the Volano River (a former delta branch of the Po River, which diverges from the main river at Stellata and flows through the city of Ferrara). The second location was the Ombrone River, situated at a distance of 13 km from the Tyrrhenian Sea (Fig. 1).

In the irrigation canal of Pomposa (44°49'48.4"N, 12°10'32.9"E), a total of 81 crabs were collected using a hand net on 15th November 2023. The channel was partially dried up with no connection to the sea or the river, and with only a stretch of about 20 meters still having stagnant water. In this last stretch, the remaining live crabs were concentrated, while in the dry part of the channel there were dead crabs and some live crabs were observed in the transition area between the two, amidst the mud (Fig. 2B).

In the Ombrone River, a total of 10 blue crabs were obtained during sampling of fish fauna and macroinvertebrates carried out for conservation purposes and monitoring of invasive alien species. The samplings were carried out on 16 June 2024. The individuals were collected in the Grancia area, immediately downstream of the bridge over the Ombrone River (42°44'2.78"N, 11° 7'44.78"E), at a distance of 13 km from the sea (Fig. 2A). Sampling was carried out with a nylon lift net of 1500 mm side and 15 mm mesh with chicken meat bait. The net used for the catch of blue crabs was lowered into water approximately 1 m deep and retrieved approximately every 10 minutes. Salinity was measured with a Hanna Instruments HI9033 conductivity meter with automatic temperature compensation and checked with an optical refractometer Milwaukee MR100ATC with automatic compensation of the temperature between 10 and 30 °C and accuracy ±1‰. The temperature was measured with a Parkside PTI 380 C2 digital infrared thermometer with an accuracy of +/- 1.5°. Following the crab sampling, a checklist of the associated fish fauna was produced using the same lift net in the same location.

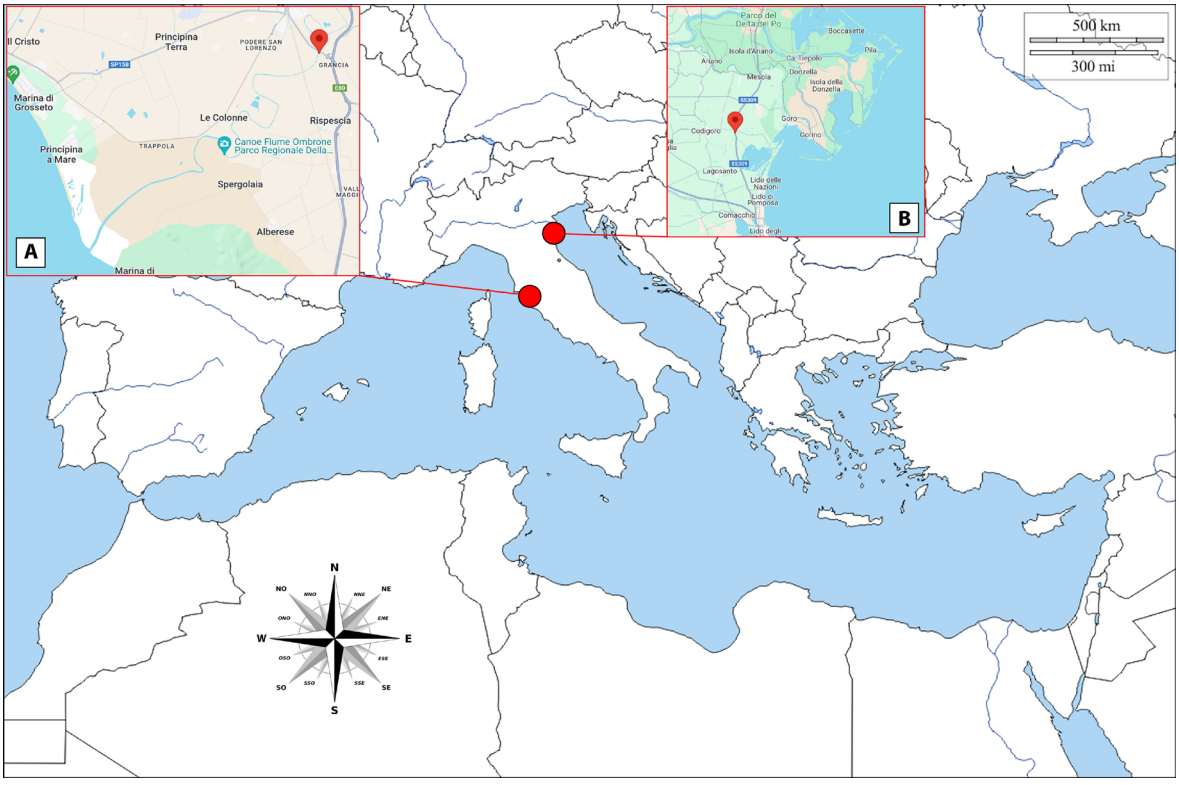

Fig. 1. Study areas in Italy (central Mediterranean Sea) indicated with red circles: Ombrone River (**A**) and Pomposa irrigation channel (**B**).

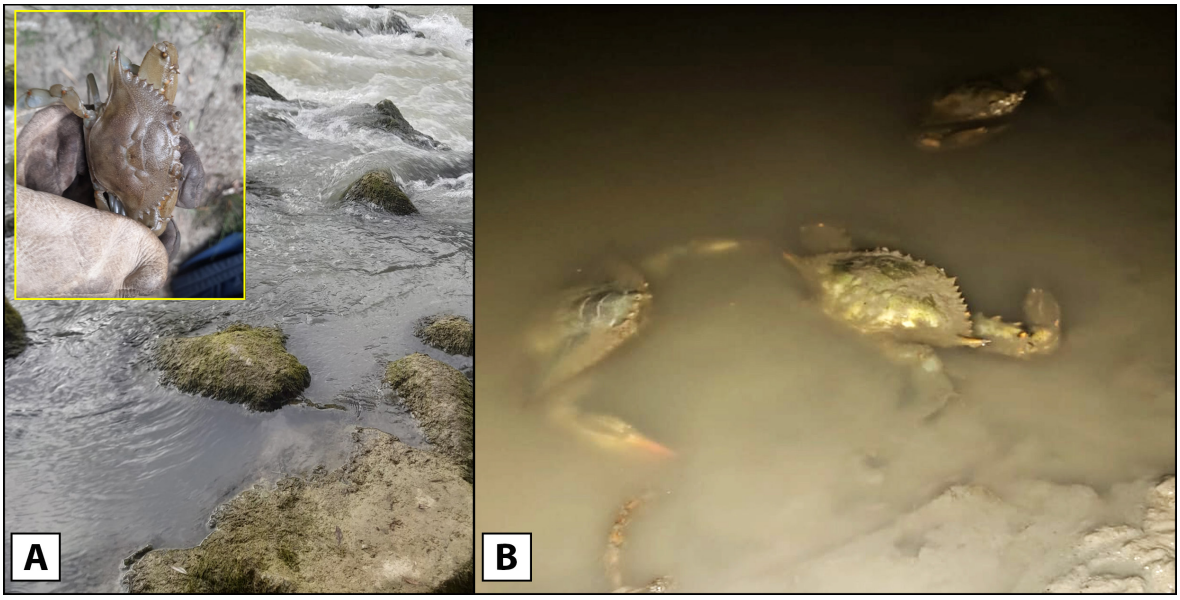

Fig. 2. Blue crabs sampled in the Ombrone River (**A**) and Pomposa irrigation channel (**B**).

For each captured specimen from both locations, sex was determined based on the morphological characteristics of the abdominal apron: wide in females and narrow and T-shaped in males. Carapace length (CL) and carapace width (CW) were measured using a digital caliper with a resolution of 1 millimeter (1 mm), and the weight was measured with a digital scale with a resolution of 1 gram (1 g). The collected measurements were statistically analyzed to assess differences between the populations from the two locations in terms of size and weight distribution, as well as sex ratios (M:F).

Data were compared using widely used and well-established statistical tests (e.g., ANOVA for differences in morphometric means). The weight-carapace width relationship of the crabs was modeled using the equation $W = aCW^b$, where W is the weight in grams, CW is the carapace width in millimeters, and a and b are the parameters to be estimated. The parameters a and b were estimated by fitting the logarithmically transformed data to a linear regression model, using the equation $\log(W)=\log(a)+b\ \log(CW)$. The parameters a and b provide insights into the growth patterns of the crabs. Specifically, the exponent b indicates the type of growth (isometric if $b \approx 3$, allometric if $b \neq 3$). Isometric growth means the crabs increase in weight proportionally to the cube of their carapace width, whereas allometric growth indicates a different proportional relationship (Tiralongo *et al*., 2020). The growth coefficient (b) between sexes in crabs from Pomposa and size between males from the two study areas (Ombrone River *vs*. Pomposa irrigation channel) were tested using a t-test.

The condition factor (K) was calculated for Pomposa's specimens only, because the Ombrone ones were in low number to perform this relationship. The estimation of the condition factor (K) helps in assessing the overall condition and health of the crab population. Deviations from expected growth patterns can indicate environmental stress, nutritional status or differences in habitat quality. It can be calculated following the formula:

$$K = \frac{W}{CW^b} X\ 100$$

where W is the weight of the crab in grams, CW is the carapace width in millimeters, and b is the exponent obtained from the weight-carapace width relationship. The carapace width-carapace length (CW-CL) relationships was calculated to analyze the morphological proportions of the crabs, between sexes for Pomposa and for male specimens only for Ombrone. This ratio provides insights into the shape and growth patterns of the crabs, which can vary between different populations and sexes. In other words, this relationship provides valuable information on the morphological variation and potential environmental influences on the crab populations. The equation representing this relationship is a simple linear equation of the form:

$$CL = a\ CW + b$$

where a is the slope and b is the intercept of the regression line.

Some large males collected in Pomposa are currently still alive at one of the freshwater aquariums of the '*Aquae Mundi*' zoological museum in Ravenna, of one of the authors (RG).

RESULTS

A total of 10 specimens of blue crab were sampled in the Ombrone River (CW = 59–90 mm, CL = 28–42 mm, W = 12–39 g for males; a single female with CW

= 84 mm, CL = 39 mm, and W = 33 g), while 81 in the Pomposa irrigation channel (CW = 114–218 mm, CL = 57–101 mm, W = 84–486 g for males; CW = 117–205 mm, CL = 58–84 mm, W = 75–268 g for females). No females with obvious egg masses were observed among the sampled animals. The analysis revealed significant differences in the size-weight (CW-W) relationship between male and female *Callinectes sapidus* individuals of Pomposa (Fig. 3). In particular, the allometric growth patterns, represented by the coefficients *a* and *b* between sexes suggested that for a given carapace width, males tend to be heavier than females, particularly at larger sizes (for males the *b* value was 2.401 vs. 1.788 of females; *t-value* = 18.26 and *p-value* <0.01). The value of *b* for sex combined was 2.512. The t-test performed on the weights of male and female blue crabs from Pomposa yielded the following results: t-statistic = 4.59; p-value = <0.01. The results showed a statistically significant difference in the weights of male and female blue crabs at Pomposa, indicating that their weights were indeed different.

The result of the independent samples t-test for the carapace width (CW) - carapace length (CL) ratio between males and females *C. sapidus* in Pomposa showed a *t-value* = -4.91 and a very low *p-value* (<0.01). The low p-value indicated a statistically significant difference in the CW-CL ratio between male and female *C. sapidus* in Pomposa (Fig. 4). The sex ratio was approximately 3.26 in favor of males, with 62 male individuals (76.5%) versus 19 female individuals (23.5%). From the Chi-square statistical test, we obtained a *p-value* <0.01. This result indicated that the difference in the distribution of sexes is statistically significant.

The mean K value for females (1.960) was significantly higher than that for males (0.116). This might indicate that females in Pomposa are generally in better physical condition compared to males. Moreover, the standard deviation of K is much higher for females (0.554) compared to males (0.014), suggesting greater variability in physical condition among females.

At the Ombrone River, the allometric growth patterns for males, represented by the coefficients *a* and *b* was: $W = 0.000535CW^{2.5}$. This equation indicated that the weight of male blue crabs at Ombrone increases with carapace width at a rate proportional to $CW^{2.5}$, although the data were obtained from a limited number of specimens (but with similar size). This value was similar to that obtained for male specimens sampled in Pomposa (Fig. 3). The mean CW/CL ratio for males from Ombrone (2.068) was slightly lower than that for males from Pomposa (2.129). The standard deviations were similar (0.090 for Pomposa specimens vs. 0.085 for Ombrone specimens), indicating that the variation within each group is comparable (Fig. 4). Out of a total of 10 specimens from Ombrone, only 1 was female, indicating a strong male-biased sex ratio equal to 9. Moreover, the measurements of salinity indicated that the waters of the Ombrone River are typical of freshwater systems: conductivity was 0.8 mS/cm, with salinity of 0.392 ppm. The measured temperature during sampling was 22.7 °C. The associated fish fauna collected comprised seven species in total, of which four were non-indigenous and two non-primary freshwater species (Table 1).

The analysis revealed distinct differences in the average sizes and weights between the male and female crabs from Pomposa, as well as between the

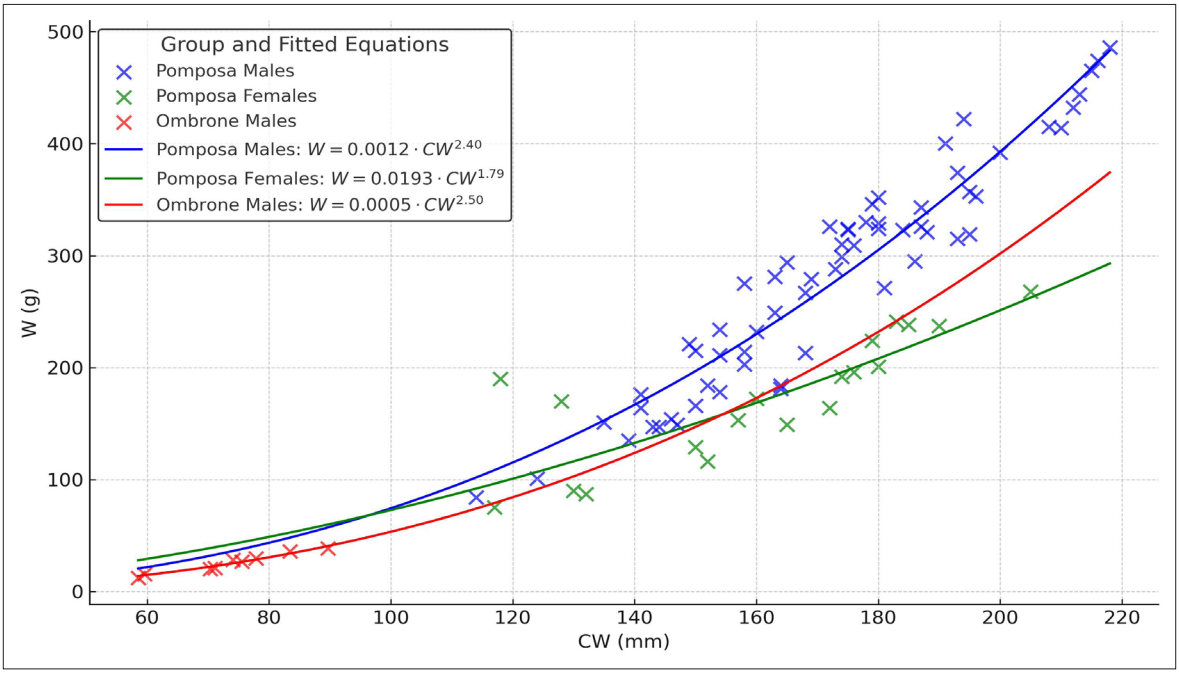

Fig. 3. Carapace width (CW) – Weight (W) relationships for blue crabs sampled in Ombrone River and Pomposa irrigation channel.

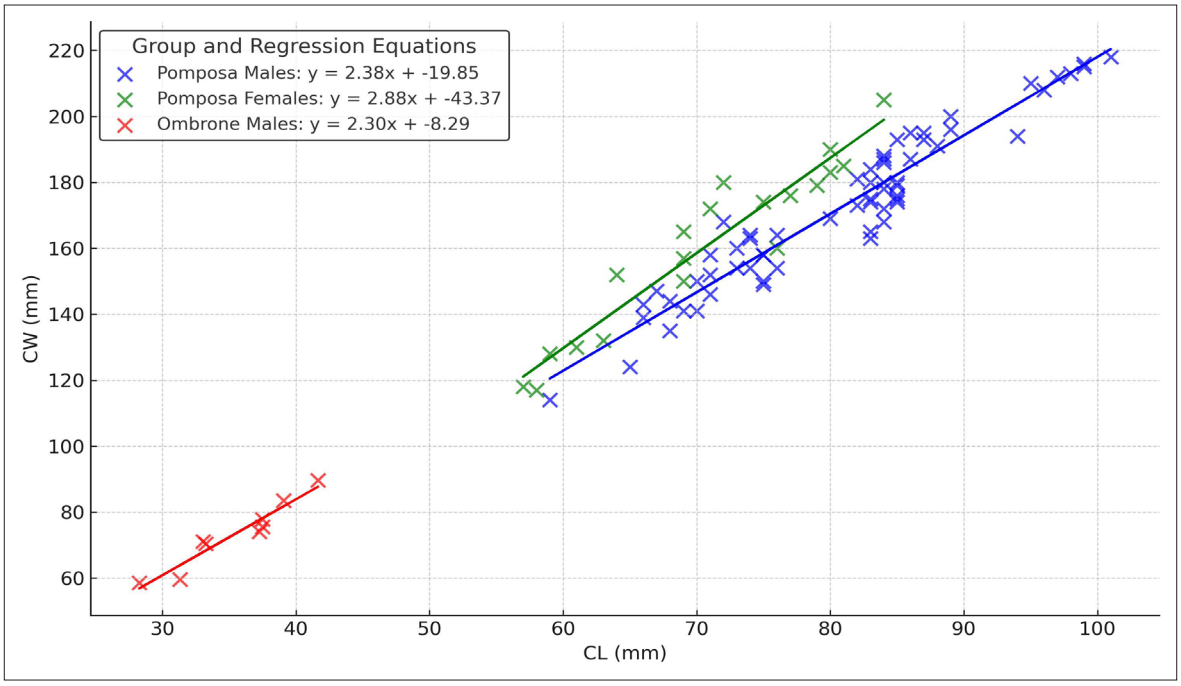

Fig. 4. Carapace width (CW) – Carapace length (CL) relationships for blue crabs sampled in Ombrone River and Pomposa irrigation channel.

males from Pomposa and Ombrone (Fig. 5). Although the sampling methods differed, the significant size difference and the fact that, during sampling at the Ombrone River, all observed specimens, including those not captured, were small, suggested a real and notable size difference between the two populations. Specifically, the male crabs from Pomposa exhibited a mean carapace width (CW) of 172.15 mm (±23.77), and a mean weight of 282.10 g (±98.34). In contrast, the female crabs from Pomposa had a slightly smaller mean carapace width of 160.68 mm (±25.76), along with a mean weight of 173.26 g (±56.11). Meanwhile, the male crabs from Ombrone were significantly smaller (*t-value* = 21.77 and *p-value* <0.01), with a mean carapace width of 73.36 mm (±10.11). Their mean weight (W) is also much lower, averaging 25.33 g (±8.86). These differences indicated that the crabs from Pomposa, particularly the males, were larger and heavier than those from Ombrone.

DISCUSSION

The adaptations of invasive alien species are facilitated by the new environmental conditions they encounter in introduced areas. Genetic and epigenetic modifications, along with human influences such as multiple introductions, habitat alteration, and climate change, can accelerate this adaptive process (Nota *et al*., 2024b). This dynamic interaction leads to the development of new traits that enhance the survival and competitiveness of invasive species in invaded ecosystems (Havel *et al.*, 2015).

One of the most notable physiological traits of the blue crab is its euryhalinity, meaning it can thrive across a broad spectrum of salinities, from near freshwater to hypersaline environments (Tagatz, 1969; Nehring, 2011). *Callinectes sapidus* is expanding in European inland waters, where its euryhaline capabilities facilitate survival across a broad salinity gradient. Our study pro-

Table 1. Associated fish fauna collected during sampling at the Ombrone River. N = No, Y = Yes.

Species	Family	Origin	Number	Indigenous
Alburnus arborella	Leuciscidae	Padano-Venetian	2	N
Alosa fallax	Alosidae	NE Atlantic and Mediterranean	1	Y
Chelon ramada	Mugilidae	NE Atlantic and Mediterranean	2	Y
Ictalurus punctatus	Ictaluridae	Eastern North America	4	N
Luciobarbus graellsii	Cyprinidae	Ebro River (Spain)	1	N
Protochondrostoma genei	Leuciscidae	Padano-Venetian	1	N
Squalius squalus	Leuciscidae	PanItalian	3	Y

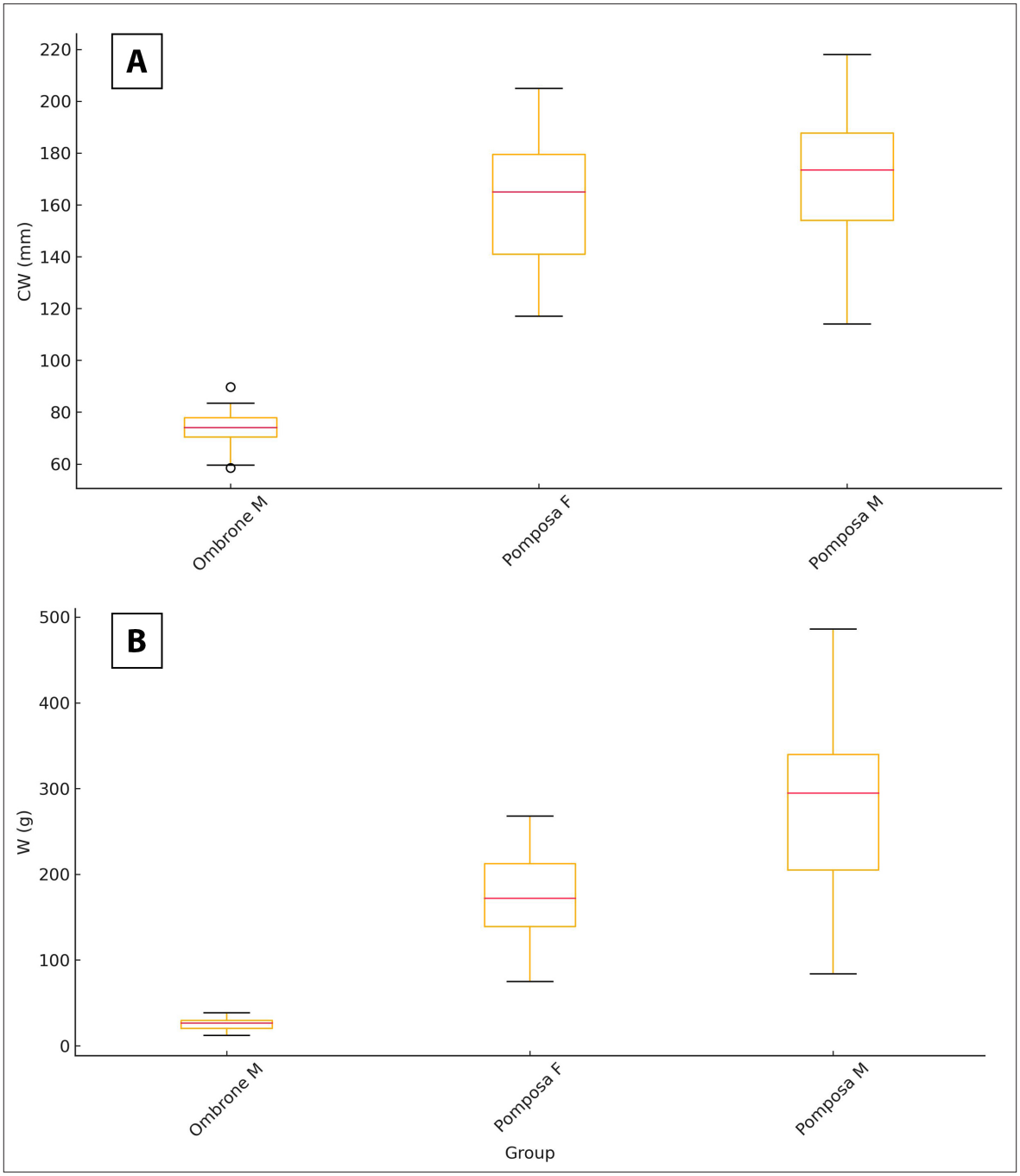

Fig. 5. Average sizes (**A**) and weights (**B**) between the male and female crabs from Pomposa, and between the males from Pomposa and Ombrone.

vides the first well-documented data on *C. sapidus* in freshwater habitats in Italy, in the sense that it includes not only observational records but also biological and ecological data. We observed sex-specific differences in growth and morphology, with male crabs showing greater relative weight gain and a shorter, wider carapace than females. These findings align with previous research on resource allocation and ecological role differentiation between sexes (Vermeiren *et al*., 2021). In comparing male specimens from Pomposa and Ombrone, a slight variance in carapace width-to-length ratios was noted, likely attributable to environmental conditions or genetic divergence between populations. This morphological variation, particularly in males from different locations, could provide adaptive benefits in distinct ecological contexts, such as increased stability or predator defense.

Nonetheless, extreme winter conditions, characterized by temperatures below 3°C and salinity levels under 8, can lead to high mortality rates, particularly among smaller juveniles and mature female blue crabs

(Hines *et al*., 2010). Additionally, they demonstrate a remarkable ability to survive in low dissolved oxygen (DO) environments, tolerating levels as low as 1.3 mg/L under moderate water temperatures (Brill *et al*., 2015). These are precisely the conditions to which the Pomposa specimens were exposed, as they were crowded in an irrigation canal with limited water at the beginning of the cold season. The animals in the sections of the canal that still held water were still alive, while in the muddy, out of water area, both live and dead individuals were found, and only dead specimens were located in the completely dried-out area. This last group of survivors would likely have been saved by the next rain. Once the irrigation canal refills, conditions regarding oxygenation and temperature are expected to improve and return to be more suitable for the species.

The observed differences in growth patterns of species have important ecological implications (Lu *et al*., 2018; Alvarez *et al*., 2021). These differences may also be due to the significantly different average sizes between the males from Pomposa and those from Ombrone, as the males from Ombrone were significantly smaller because they were probably at juvenile or subadult stage (although only 10 were collected in total, the area was still 'infested' with specimens of similar size alone), while those from Pomposa were large-sized adult males. In fact, in crabs, these morphological differences in CW-CL ratios can change with growth too. From an allometric perspective, the coefficients obtained from blue crabs analyzed in this study suggest that the growth of weight (W) relative to carapace width (CW) differs between sexes in specimens from Pomposa. In males, a higher '*b*' value suggests a more pronounced weight growth relative to carapace width, while in females, a lower '*b*' value indicates a less pronounced weight growth with increasing carapace width. This greater weight of males is at least partly due to the fact that males tend to have longer, more robust, and therefore heavier claws (Schenk and Wainwright, 2001). Overall, these findings underscore the need for sex-specific considerations in ecological and conservation research. Moreover, values obtained for the CW/CL ratio in Pomposa specimens were lower in males than in females. This means that, on average, male individuals of *C. sapidus* tend to have a relatively shorter and wider carapace compared to females. Instead, the slight difference in the CW/CL ratio between Ombrone and Pomposa male specimens suggests that the crabs from Pomposa have a slightly wider carapace compared to those from Ombrone. This may reflect morphological adaptations to local environmental conditions or could be due to genetic variations between the two populations. In terms of ecology and behavior, a wider carapace might offer certain advantages, such as greater stability during movement on the seabed or improved defense against predators. However, these morphological differences should be interpreted with caution and may require further studies to determine the specific causes and ecological consequences.

The condition factor (K) analysis revealed significant differences between male and female crabs in Pomposa. Females exhibit a substantially higher mean K value (1.960) compared to males (0.116), indicating better overall physical condition among females. Additionally, the higher variability in K values for females suggests diverse individual health statuses within this group (Noori *et al*., 2015). These differences underscore the importance of considering sex-specific factors when assessing the health and condition of crab populations, as well as the potential influences of environmental and biological factors on these metrics.

The findings indicate a highly skewed sex ratio favoring males in *C. sapidus* population from Pomposa. The prevalence of males in Pomposa could result from several factors, including environmental pressures, behavioral tendencies, and physiological adaptations unique to males in this population. For instance, males of *C. sapidus* are often observed in less saline environments, which may correspond to the sampling areas in this study, thus leading to a higher representation of males (Taylor *et al*., 2021). In the Ombrone population, this skew was even more pronounced, with a sex ratio of 9:1 in favor of males. Despite the limited sample size (10 individuals), such a strong male bias suggests that similar environmental conditions or habitat preferences might be influencing the observed distribution in this area. The dominance of males could also be associated with selective pressures related to growth and maturation rates, as males often require different environmental conditions for optimal growth, while females migrate to marine habitats for reproductive needs (Aguilar *et al*., 2005; Sayekti *et al*., 2020). Indeed, male and juvenile blue crabs predominantly inhabit upriver oligohaline waters, while adult females are primarily found in the mesohaline and polyhaline sections of the rivers (Taylor *et al*., 2021). This distribution pattern is also the most likely explanation for why, in our study, the majority of specimens collected were males.

The presence of *C. sapidus* in inland waters represents a significant example of biological invasion with potentially extended ecological and socio-economic impacts, although it remains an understudied recently documented phenomenon for Mediterranean area (Župan *et al*., 2016; Scalici *et al*., 2022; Vecchioni *et al*., 2022; Bedmar *et al*., 2023). Indeed, although most of Mediterranean studies on the invasiveness and impacts of *C. sapidus* focus on marine and brackish environments (Tiralongo *et al*., 2021; Azzurro *et al*., 2024; De Giorgi *et al*., 2024), our study underlines the potential negative impact at ecological level of *C. sapidus* in various inland areas of Italy. This discovery is particularly concerning given the known impact of this species on invaded ecosystems, where it poses a threat to native species and local biodiversity. For instance, in Sicily, there is concern about the impact on already threatened species such as the Sicilian pond turtle (*Emys trinacris*) and the native

river crab (*Potamon fluviatile*) (Ottonello *et al*., 2021; Yousefi *et al*., 2022).

Managing this invasion requires effective monitoring and mitigation strategies. Continuous monitoring programs are essential to track the spread of the species and assess the effectiveness of control measures. Moreover, adopting integrated approaches involving the local community, including citizen science projects, can enhance early detection and prompt response to new invasions (Cerri *et al.,* 2020; Virgili *et al*., 2024).

In the context of this study, *C. sapidus* has also demonstrated the ability to reach irrigation channels from rivers, showcasing its remarkable capacity to further expand into inland areas. This phenomenon was observed in Pomposa, where the crab successfully penetrated irrigation channels, allowing the species to colonize both natural and semi-artificial or artificial basins. This ability to disperse through irrigation channels not only facilitates the expansion of the blue crab into inland areas but also poses a potential threat to local biodiversity and agricultural activities that rely on these water systems. The presence of the blue crab in these new habitats highlights the urgent need to implement targeted monitoring and management strategies to limit the further spread of the species and mitigate its ecological and socio-economic impacts.

CONCLUSIONS

This study provides the first well documented data from Italy on some biological and morphometric traits of blue crab specimens collected from freshwater habitats. The existing literature on *C. sapidus* in Europe and the Mediterranean basin largely addresses its records, distribution, and ecology within marine and estuarine environments (Mancinelli *et al*., 2021; Castriota *et al*., 2024). However, the blue crab's ability to migrate from river mouths to freshwater habitats poses a significant, yet under-researched, threat to endemic species in these ecosystems. Given the potential ecological impact, it is imperative to intensify monitoring efforts in freshwater systems to detect the presence of blue crabs, understand their population dynamics, and assess their impact on local freshwater biodiversity.

REFERENCES

Aguilar, R., Hines, A.H., Wolcott, T.G., Wolcott, D.L., Kramer, M.A., Lipcius, R.N. 2005. The timing and route of movement and migration of post-copulatory female blue crabs, *Callinectes sapidus* Rathbun, from the upper Chesapeake Bay. Journal of Experimental Marine Biology and Ecology, 319(1-2), 117-128. https://doi.org/10.1016/j.jembe.2004.08.030

Alvarez, P., Cotano, U., Estensoro, I., Etxebeste, E., Irigoien, X. 2021. Assessment of larval growth patterns: A comparison across five fish species in the Bay of Biscay. Regional Studies in Marine Science, 47, 101958. https://doi.org/10.1016/j.rsma.2021.101958

Azzurro, E., Bonanomi, S., Chiappi, M., De Marco, R., Luna, G. M., Cella, M., Guicciardi, S., *et al*. 2024. Uncovering unmet demand and key insights for the invasive blue crab (*Callinectes sapidus*) market before and after the Italian outbreak: Implications for policymakers and industry stakeholders. Marine Policy, 167, 106295. https://doi.org/10.1016/j.marpol.2024.106295

Bedmar, S., Oficialdegui, F.J., Clavero, M. 2023. Far-reaching blues: Long-distance migration of the invasive Atlantic blue crab. Aquatic Conservation: Marine and Freshwater Ecosystems, 34(4), e4136. https://doi.org/10.1002/aqc.4136

Bellard, C., Cassey, P., Blackburn, T.M. 2016. Alien species as a driver of recent extinctions. Biology Letters, 12, 20150623. http://doi.org/10.1098/rsbl.2015.0623

Brill, R.W., Bushnell, P.G., Elton, T.A., Small, H.J. 2015. The ability of blue crab (*Callinectes sapidus*, Rathbun 1886) to sustain aerobic metabolism during hypoxia. Journal of Experimental Marine Biology and Ecology, 471, 126-136. https://doi.org/10.1016/j.jembe.2015.06.003

Castriota, L., Falautano, M., Perzia, P. 2024. When nature requires a resource to be used – The case of *Callinectes sapidus*: Distribution, aggregation patterns, and spatial structure in northwest Europe, the Mediterranean Sea, and adjacent waters. Biology, 13(4), 279. https://doi.org/10.3390/biology13040279

Cerri, J., Chiesa, S., Bolognini, L., Mancinelli, G., Grati, F., Dragičević, B., Dulčic, J., Azzurro, E. 2020. Using online questionnaires to assess marine bio-invasions: A demonstration with recreational fishers and the Atlantic blue crab *Callinectes sapidus* (Rathbun, 1986) along three Mediterranean countries. Marine Pollution Bulletin, 156*,* 111209. https://doi.org/10.1016/j.marpolbul.2020.111209

De Giorgi, R., Bardelli, R., Cilenti, L., Falco, S., Fanizzi, F.P., Guerra, M.T., Katselis, G. *et al.* 2024. Opportunistic omnivory impairs the use of the Atlantic blue crab *Callinectes sapidus* as a trace metal biomonitor in invaded Mediterranean coastal waters. Marine Pollution Bulletin, 206, 116715. https://doi.org/10.1016/j.marpolbul.2024.116715

Epifanio, C. 2019. Early life history of the blue crab *Callinectes sapidus*: a review. Journal of Shellfish Research, 38(1), 1-22. http://doi.org/10.2983/035.038.0101

Forward, Jr. R.B., Cohen, J.H., Irvine, R.D., Lax, J.L., Mitchell, R., Schick, A.M., Smith, M.M., *et al*. 2004. Settlement of blue crab *Callinectes sapidus* megalopae in a North Carolina estuary. Marine Ecology Progress Series, 269, 237-247. https://doi.org/10.3354/meps269237

Gherardi, F. 2007. Biological invasions in inland waters: an overview. *In* Biological invaders in inland waters: Profiles, distribution, and threats (ed. F. Gherardi). Springer. pp. 3-25.

Hanley, N., Roberts, M. 2019. The economic benefits of invasive species management. People and Nature, 1(2), 124-137. https://doi.org/10.1002/pan3.31

Havel, J.E., Kovalenko, K.E., Thomaz, S.M., Amalfitano, S., Kats, L.B. 2015. Aquatic invasive species: challenges for the future. Hydrobiologia, 750(1), 147-170. https://doi.org/10.1007/s10750-014-2166-0

Henry, M., Leung, B., Cuthbert, R.N., Bodey, T.W., Ahmed, D. A., Angulo, E., Balzani, P. *et al.* 2023. Unveiling the hidden economic toll of biological invasions in the European Union. Environmental Sciences Europe, 35, 43. https://doi.org/10.1186/s12302-023-00750-3

Hines, A.H., Johnson, E.G., Darnell, M.Z., Rittschof, D., Miller, T.J., Bauer, L.J., Rodgers, P., *et al*. 2010. Predicting effects of climate change on Blue Crabs in Chesapeake Bay. *In* Biology and Management of Exploited Crab Populations under Climate Change (eds. G.H. Kruse, G.L. Eckert, R.J. Foy, R.N. Lipcius, B. Sainte-Marie, D.L. Stram, D. Woodby), Alaska Sea Grant; University of Alaska Fairbanks. pp. 109-127.

Johnson, P.T., Olden, J.D., Vander Zanden, M.J. 2008. Dam invaders: Impoundments facilitate biological invasions in freshwaters. Frontiers in Ecology and the Environment, 6(7), 357-363. https://doi.org/10.1890/070156

Jones, B.A. 2017. Invasive species impacts on human well-being using the life satisfaction index. Ecological Economics, 134, 250-257. https://doi.org/10.1016/j.ecolecon.2017.01.002

Kampouris, T., Porter, J., Sanderson, W. 2019. *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae): An assessment on its diet and foraging behaviour, Thermaikos Gulf, NW Aegean Sea, Greece: Evidence for ecological and economic impacts. Crustacean Research, 48, 23-37. https://doi.org/10.18353/crustacea.48.0_23

Lipej, L., Acevedo, I., Akel, E. H. K., Anastasopoulou, A., Angelidis, A., Azzurro, E., Castriota, L. *et al.* 2018. New Mediterranean biodiversity records (March 2017). Mediterranean Marine Science, 18(1), 179-201. http://doi.org/10.12681/mms.2068

Lu, H. L., Xu, C. X., Zeng, Z. G., Du, W-G. 2018. Environmental causes of between-population difference in growth rate of a high-altitude lizard. BMC Ecology, 18(1), 37. https://doi.org/10.1186/s12898-018-0194-8

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., Ribeiro, F. 2017. On the Atlantic blue crab (*Callinectes sapidus* Rathbun 1896) in southern European coastal waters: Time to turn a threat into a resource? Fisheries Research, 194, 1-8. https://doi.org/10.1016/j.fishres.2017.05.002

Mancinelli, G., Bardelli, R., Zenetos, A. 2021. A global occurrence database of the Atlantic blue crab *Callinectes sapidus*. Scientific Data, 8, 111. https://doi.org/10.1038/s41597-021-00888-w

Marchessaux, G., Bosch-Belmar, M., Cilenti, L., Lago, N., Mangano, M.C., Marsiglia, N., Sarà G. 2022. The invasive blue crab *Callinectes sapidus* thermal response: Predicting metabolic suitability maps under future warming Mediterranean scenarios. Frontiers in Marine Science, 9, 1055404. https://doi.org/10.3389/fmars.2022.1055404

Marrone, F., Naselli-Flores, L. 2015. A review on the animal xenodiversity in Sicilian inland waters (Italy). Advances in Oceanography and Limnology, 6(1-2), 2-12. https://doi.org/10.4081/aiol.2015.5451

Mizzan, L. 1993. Presence of swimming crabs of the genus *Callinectes* (Stimpson) (Decapoda, Portunidae) in the Venice Lagoon (North Adriatic Sea - Italy). First record of *Callinectes danae* Smith in European Waters. Bollettino del Museo civico di storia naturale di Venezia, 42, 31-43.

Najberek, K., Olszańska, A., Tokarska-Guzik, B., Mazurska, K., Dajdok, Z., Solarz, W. 2022. Invasive alien species as reservoirs for pathogens. Ecological Indicators, 139, 108879. https://doi.org/10.1016/j.ecolind.2022.108879

Nehring, S. 2011. Invasion history and success of the American blue crab *Callinectes sapidus* in European and adjacent waters. *In* In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts (eds. B. Galil, P. Clark, J. Carlton). Springer, pp. 607-624. https://doi.org/10.1007/978-94-007-0591-3_21

Nota, A., Santovito, A., Gattelli, R., Tiralongo, F. 2024a. From fresh to salt waters: First reports of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) in Mediterranean marine waters. Hydrobiology, 3(1), 1-10. https://doi.org/10.3390/hydrobiology3010001

Nota, A., Bertolino, S., Tiralongo, F., Santovito, A. 2024b. Adaptation to bioinvasions: When does it occur? Global Change Biology, 30(6), e17362. https://doi.org/10.1111/gcb.17362

Noori, A., Moghaddam, P., Kamrani, E., Akbarzadeh, A., Kalvani Neitali, B., Pinheiro, M.A.A. 2015. Condition factor and carapace width versus wet weight relationship in the blue swimming crab *Portunus segnis*. Animal Biology, 65(2), 87-99. https://doi.org/10.1163/15707563-00002463

Ottonello, D., D'Angelo, S., Marrone, F., Oneto, F., Spadola, F., Zuffi, M.A.L., Fritz, U. 2021. *Emys trinacris* Fritz, Fattizzo, Guicking, Tripepi, Pennisi, Lenk, Joger and Wink 2005 - Sicilian pond turtle, Testuggine Palustre Siciliana. *In* Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group (eds. A.G.J. Rhodin, J.B. Iverson, P.P. Van Dijk, C.B. Stanford, E.V. Goode, K.A. Buhlmann, R.A. Mittermeier). Chelonian Research Foundation and Turtle Conservancy. pp. 112.1-112.13.

Prager, M.H., McConaugha, J.R., Jones, C.M., Geer, P.J. 1990. Fecundity of the blue crab, *Callinectes sapidus*, in Chesapeake Bay: Biological, Statistical and Management Considerations. Bullettin of Marine Science, 46(1), 170-179.

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W. *et al.* 2020. Scientists' warning on invasive alien species. Biological Reviews, 95(6), 1511-1534. https://doi.org/10.1111/brv.12627

Sayekti, P.R., Fahrunnida, Cerniauskas, G., Robert, C., Retnoaji, B., Alam, P. 2020. The impact behaviour of crab carapaces in relation to morphology. Materials, 13(18), 3994. https://doi.org/10.3390/ma13183994

Scalici, M., Chiesa, S., Mancinelli, G., Rontani, P.M., Voccia, A., Nonnis Marzano, F. 2022. Euryhaline aliens invading Italian inland waters: The case of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896. Applied Sciences, 12(9), 4666. https://doi.org/10.3390/app12094666

Schenk, S.C., Wainwright, P.C. 2001. Dimorphism and functional basis of claw strength in six brachyuran crabs. Journal of Zoology, 255(1), 105-119. https://doi.org/10.1017/S0952836901001157

Snigirev, S.M., Bushuev, S.G., Kudriashov, S.S., Sylantyev, S.A. 2020. First records of blue crab *Callinectes sapidus* (Rathbun 1869) on the northwestern Black Sea shelf. Marine Ecological Journal, 1, 67-69. https://doi.org/10.47143/1684-1557/2020.1.09

Souty-Grosset, C., Anastácio, P.M., Aquiloni, L., Banha, F., Choquer, J., Chucholl, C., Tricarico, E. 2016. The red swamp crayfish *Procambarus clarkii* in Europe: Impacts on aquatic ecosystems and human well-being. Limnologica, 58, 78-93. https://doi.org/10.1016/j.limno.2016.03.003

Strayer, D.L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology, 55(S1), 152-174. https://doi.org/10.1111/j.1365-2427.2009.02380.x

Streftaris, N., Zenetos, A. 2006. Alien marine species in the Mediterranean - the 100 'worst invasives' and their impact. Mediterranean Marine Science, 7(1), 87-118. https://doi.org/10.12681/mms.180

Tagatz, M.E. 1969. Some relations of temperature acclimation and salinity to thermal tolerance of the blue crab, *Callinectes sapidus*. Transactions of the American Fisheries Society, 98(4), 713-716. https://doi.org/10.1577/1548-8659(1969)98[713:SROTAA]2.0.CO;2

Taylor, D.L., Fehon, M.M. 2021. Blue crab (*Callinectes sapidus*) population structure in Southern New England tidal rivers: Patterns of shallow-water, unvegetated habitat use and quality. Estuaries and Coasts, 44(5), 1320-1343. https://doi.org/10.1007/s12237-020-00867-1

Tiralongo, F., Arculeo, M., Yeo, M.D., Kakou, B.I., Adepo-Gourene, A.B. 2020. First data on population structure and growth parameters of *Ocypode cursor* (Linnaeus, 1758) along the Mediterranean and Atlantic coast. Cahiers de Biologie Marine, 61(4), 405-413. https://doi.org/10.21411/CBM.A.3AA1FF69

Tiralongo, F., Villani, G., Arciprete, R., Mancini, E. 2021. Filling the gap on Italian records of an invasive species: first records of the Blue Crab, *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: Portunidae), in Latium and Campania (Tyrrhenian Sea). Acta Adriatica, 62(1), 99-104. https://doi.org/10.32582/aa.62.1.8

Vecchioni, L., Russotto, S., Arculeo, M., Marrone, F. 2022. On the occurrence of the invasive Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: Portunidae) in Sicilian inland waters. Natural History Sciences, 9(2), 43-46. https://doi.org/10.4081/nhs.2022.586

Vermeiren, P., Lennard, C., Trave, C. 2021. Habitat, sexual and allometric influences on morphological traits of intertidal crabs. Estuaries and Coasts, 44, 1344-1362. https://doi.org/10.1007/s12237-020-00856-4

Virgili, R., Fasciglione, P., Tiralongo, F., Pannone, R., Palomba, M., Santoro, M., De Luca, P., et al. 2024. Interdisciplinary approaches at early invasion stages maximise the evidence on human-induced and climate-driven fish dispersal. Hydrobiologia, in press. https://doi.org/10.1007/s10750-024-05537-y

Yousefi, M., Naderloo, R., Keikhosravi, A. 2022. Freshwater crabs of the Near East: Increased extinction risk from climate change and underrepresented within protected areas. Global Ecology and Conservation, 38, e02266. https://doi.org/10.1016/j.gecco.2022.e02266

Windsor, A.M., Moore, M.K., Warner, K.A., Stadig, S.R., Deeds, J.R. 2019. Evaluation of variation within the barcode region of Cytochrome c Oxidase I (COI) for the detection of commercial *Callinectes sapidus* Rathbun, 1896 (blue crab) products of non-US origin. PeerJ, 7, e7827. https://doi.org/10.7717/peerj.7827

Župan, I., Karaga, A., Ŝarić, T., Kanski, D. 2016. Blue crab *Callinectes sapidus* Rathbun, 1896 continues invasion: first case of entering into freshwater ecosystem in the Mediterranean (Nature Park Vransko Lake, Adriatic Sea). Cahiers de Biologie Marine, 57(1), 81-84. https://doi.org/10.21411/CBM.A.2BD02BBF