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# Physiological responses of the invasive blue crabs *Callinectes sapidus* to salinity variations: Implications for adaptability and invasive success

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## ABSTRACT

This study provides a comprehensive analysis of the eco-physiological responses of the blue crab (*Callinectes sapidus*) to variations in salinity, shedding light on its adaptability and invasive success in aquatic environments. Gender-specific differences in osmoregulation and Electron Transport System (ETS) activity highlight the importance of considering sex-specific aspects when understanding the physiological responses of invasive species. Females exhibited increased ETS activity at lower salinities, potentially indicative of metabolic stress, while males displayed constant ETS activity across a range of salinities. Osmoregulatory capacity which depended on gender and salinity, was efficient within meso-polyhaline waters but decreased at higher salinities, particularly in males. These findings provide valuable understandings into how *C. sapidus* specimens in an invaded area responds to salinity changes, important for considerate its distribution through saline pathways during tidal cycle fluctuations. This study shows the importance of interdisciplinary research for effective management of invasive species and conservation of affected aquatic ecosystems.

## 1. Introduction

The waters of the Atlantic have observed a subtle but substantial ecological transformation with the introduction of invasive species. One such species that has gained increasing attention is the Atlantic-native portunid, *Callinectes sapidus* Rathbun (1896), commonly known as the blue crab. Originally spanning from Nova Scotia to Argentina, this species has been introduced into the Eastern Atlantic, including the Mediterranean, Adriatic, North and Baltic seas, and the waters of Hawaii and Japan, through human-mediated means (Mancinelli et al., 2021; Schubart et al., 2023). Its invasion has a number of ecological, economic and scientific implications (Castriota et al., 2024; de Carvalho-Souza et al., 2024). With its important reproductive capacity (Darnell et al., 2009) and wide temperature tolerance (Marchessaux et al., 2022), this species has successfully invaded, expanded, and established itself in the non-native Atlantic and Mediterranean region over the past few decades (Mancinelli et al., 2021; González-Ortegón et al., 2022). The blue crab exhibits a widespread distribution along the eastern Atlantic coast, spanning both the Northern (Portugal and Spain) and Southern (Morocco) coasts (Encarnação et al., 2021; Mancinelli et al., 2021;

Chairi and González-Ortegón, 2022). This species is an opportunist predator with a large body size and particular aggressive behaviour, omnivorous diet, and strong swimming ability (Nehring, 2011). The consequential effects of its presence include significant impacts on native biodiversity, involving predation, competition and the potential for local species extinctions (Clavero et al., 2022; Ortega-Jiménez et al., 2024), as well as adverse consequences on artisanal fisheries, such as net destruction (Glamuzina et al., 2021).

The life history of *C. sapidus*, is characterized by its intricate adaptation to both oceanic and estuarine habitats, utilizing various salinity conditions throughout its life cycle (Mancinelli et al., 2017). Reproduction between male and female blue crabs takes place in low salinity waters, prompting female crabs to migrate to polyhaline zones for egg production and incubation (Aguilar et al., 2005). In contrast, adult males persist in low salinity waters. Following the maturation of eggs, females migrate to the sea (with salinity levels up to 30) to release planktonic zoeae larvae (Epifanio, 2019). Within the estuary systems along the southwestern European coastline, this species was identified within salinity levels ranging from 0.6 to 35 (Prado et al., 2022).

Salinity plays an important role in establishing biotic zones within

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estuaries in the marine environment. The capacity of organisms to adapt to changes in salinity varies widely, and fish and macroinvertebrates, including crabs, are posited to inhabit five or six biotic salinity zones (Wolf et al., 2009). Therefore, adaptability to salinity emerges as a crucial factor in resource management, particularly when assessing the risk of potential invasions by specific species.

One of the key areas of research is the effect of salinity on different genders of blue crabs, particularly concerning hemolymph composition and its implications for maturity, growth and metabolism. Hemolymph, the crab's circulatory fluid, plays a fundamental role in transporting oxygen and other nutrients throughout the body. The influence of salinity becomes evident, as it can significantly impact hemolymph composition, which in turn can affect the crab's overall physiology, including its reproductive and growth processes.

Previous studies have indicated that salinity levels may affect the hormonal regulation of molting and mating in blue crabs (Hines et al., 1987; Shock et al., 2009). Variations in salinity can influence hemolymph osmoregulation processes, affecting the crab's ability to molt and mate successfully. Understanding how salinity affects hemolymph and, consequently, the metabolism pattern of different *Callinectes sapidus* genders is essential for understanding the adaptability and ecological impact of this invasive species in Atlantic waters.

Furthermore, exploring the activity of the electron transport system (ETS) as a potential indicator of respiration (Packard et al., 1971) in the context of fluctuating salinity levels is important. The ETS is a fundamental component of cellular respiration (Ikeda et al., 2000), known to exhibit variations over short timescales, often spanning a few days (Herrera et al., 2017; Ruiz-Delgado et al., 2019). This variation offers insights into metabolic dynamics and the adaptive capacity of marine organisms. Assessing the interplay among salinity, potential respiration, and the physiological responses of crabs to environmental shifts is vital for assessing the impact of invasive species on Atlantic ecosystems.

Understanding the osmoregulatory and the potential respiration responses of *C. sapidus* to rapid changes in salinity is important for elucidating its ability to thrive and disperse through saline pathways during fluctuations in tidal cycle salinity and also vital for assessing the impact of invasive species on Atlantic ecosystems. Our study examines the salinity tolerance on the hemolymph, sex, and respiration potential responses of *C. sapidus* under short-term exposure to salt stress. This research will also provide a comprehensive understanding of the adaptability and ecological impact of the invasive species in Atlantic invaded waters and will also compare the osmoregulatory capacity of *C. sapidus* in its native range versus the invaded areas. This knowledge is necessary for both ecological conservation and management of economically valuable resources.

## 2. Material and methods

Adults of the Atlantic blue crabs were obtained from the Guadalquivir estuary (36°89' 7 N, 6°22' 6 W) between August to September 2023. The blue crabs were captured using local traps strategically

positioned near the bottom substrate (at a depth of approximately 1–4 m) and baited with deceased fish, predominantly anchovies/sardines. These traps were left deployed for 16 h, encompassing a complete tidal cycle. Subsequent to retrieval, the captured animals were carefully transferred to plastic gallon containers filled with aerated water sourced directly from the collection site. Water salinity was checked (Hanna HI 96822 digital refractometer, Italy) after the animals were collected, demonstrated variations ranging from 9.64 to 9.69.

### 2.1. Experimental work

Blue crabs were acclimated to different salinities using five water baths (Table 1). Within each bath, adult males and non-ovigerous females were placed in 100 L of filtered seawater at a temperature of 26 °C. Hemolymph osmolality and ETS activity were measured in adults ( $n = 54$ ) with a total body length ranging from 48 to 77 mm. After 24 h, both hemolymph and potential respiration (electron transport system; ETS) were measured of each blue crab individual. For the ETS measurement, a piece of muscle from each individual was stored in liquid nitrogen (−196 °C).

### 2.2. Analysis assays

Hemolymph was detected as follows: A hemolymph sample (8–10  $\mu$ L) was drawn from each live blue crab between the sternites of the first and second pereonites with a 50 ML syringe. The osmolality of hemolymph and water was measured as mOsm  $\text{kg}^{-1}$  using a Wescor 5600 vapour pressure osmometer.

ETS activity was assessed in small samples of blue crab muscle at a temperature of 29 °C, following the method described by Owens and King (1975) with adaptations for microplate readings as explained in Ruiz-Delgado et al. (2019). Frozen samples were homogenised in Tris-HCl buffer (20 mM, pH 7.8) and centrifuged (10 min, 0 °C). ETS activity was corrected for in situ temperature by applying an activation energy of 15 kcal·mol<sup>−1</sup> (Packard et al., 1975) to the Arrhenius equation to obtain the in situ activity (ETS). And, to determine the ETS per unit biomass, the protein biomass (mg of protein) was analysed by the bicinchoninic acid method described by Smith et al. (1985), using a Pierce BCA protein assay kit and bovine serum albumin (BSA) as a standard.

### 2.3. Analysis data

A permutational multivariate analysis of variance (PERMANOVA) was performed using a design for which the factors were: (1) size, (2) sex (two levels: male and female) and (3) salinity (five levels: 3, 7, 15, 25 and 35), and the response variables hemolymph concentration expressed in  $\text{mmol Kg}^{-1}$  and ETS expressed in  $\mu\text{LO}_2\cdot\text{h}^{-1}\cdot\text{mg prot}^{-1}$  (Anderson, 2001). Data were analysed based on the modified Euclidean distance dissimilarity of the hemolymph concentration and ETS, and the statistical significance of permutational variance components was tested

**Table 1**

Summary of the correlation between hemolymph concentration in female and male blue crabs relative to water osmolality. N=Number of individuals per treatment. Isosmotic point was calculated using equation by González-Ortegón et al., 2006.

	Salinity	Experimental osmolality	Regression coefficient	Isosmotic point	Length of carapace (Lc, in mm)		N	T (°C)
					Mean	SE		
Female	3–7	69–203	−0.007	814.15	15.3	1.4	3	26
	7–15	203–440	0.225		14.6	2.2	10	26
	15–25	440–709	0.186		14.4	3.4	5	26
	25–35	709–991	0.451		15.3	1.3	6	26
Male	3–7	69–203	−0.032	981.08	13.3	1.6	3	26
	7–15	203–440	0.217		15.3	1	6	26
	15–25	440–709	0.099		14.7	1.6	5	26
	25–35	709–991	0.772		15.1	1	4	26

using 9999 permutations. Linear regressions were used to fit and explore the relationships between hemolymph osmolality and the experimental salinities (3, 7, 15, 25, and 35).

### 3. Results

The hemolymph concentration of *Callinectes sapidus* was maintained hyperosmotic to that of the external medium (hyperosmoregulator) in salinities between 3 and 25 (69–709 mmol Kg<sup>-1</sup>). The isosmotic point occurred at 29.96 (814.15 osmolality) in females and at 35.93 (981.08 osmolality) in males, which were acclimated to salinities between 3 and 35 (Fig. 1 and Table 1). At the high salinities 25 and 35 (709–991 mmol Kg<sup>-1</sup>), the hemolymph was hypo-osmotic to the medium, mainly in females and slightly hyperosmotic in males (paralleling the isosmotic line). Within the salinity range where adults acted as osmoregulators, the slopes of the regression lines ( $\Delta$  hemolymph osmolality versus  $\Delta$  medium osmolality) indicated that blue crabs (both females and males) in salinities between 3 and 25 (69–709 mmol Kg<sup>-1</sup>) present high efficiency in osmoregulatory capability ( $\Delta$  below 0.24). Meanwhile, in salinities between 25 and 35 (709–991 mmol Kg<sup>-1</sup>) presented low efficiency in osmoregulatory capability ( $\Delta = 0.45$  in females) and mainly in males ( $\Delta = 0.77$ ). The PERMANOVA analysis showed a significant difference in hemolymph concentration, both in relation to salinity ( $p < 0.05$ ) and gender ( $p < 0.05$ ), as shown in Table 2.

Gender-specific differences were observed in the Electron Transport System (ETS) activity, representing potential respiration rates of both female and male *C. sapidus*, indicating distinct metabolic responses to varying salinities ( $t$ -test,  $p < 0.05$ , substantial evidence for unequal means). The ETS analysis also showed significant differences primarily related to gender ( $p < 0.05$ ) rather than salinity ( $p > 0.05$ ), as observed in Table 2. Females showed increased ETS ( $\mu\text{LO}_2\cdot\text{h}^{-1}\cdot\text{mg prot}^{-1}$ ) activity at salinities ranging from 3 to 7 (69–203 mmol Kg<sup>-1</sup>), suggesting potential metabolic stress in environments less conducive or adaptive to their metabolism. In contrast, males maintained consistent ETS activity values across the studied salinity range of 3 to 35 (69–991 mmol Kg<sup>-1</sup>), indicating a more stable metabolic response irrespective of salinity levels (Fig. 2).

### 4. Discussion

The eco-physiological responses of blue crabs (*Callinectes sapidus*) to varying salinity levels offer insights into their adaptability and invasive success in aquatic environments. Our study showed that the blue crab is a strong osmoregulator, supporting a successful invasion in estuaries of the South European Atlantic. There are significant gender differences in osmoregulation and ETS activity, taking into account the need to consider sex-specific responses in the study of invasive species, as was previously identified in the feeding responses of European green crabs (*Carcinus maenas*; Kattler et al., 2023).

The invasive success of the blue crab in the northeastern Atlantic Ocean and the Mediterranean Sea can be attributed to its significant physiological adaptability, allowing it to thrive in changing conditions driven by temperature increases (Nehring, 2011; Epifanio, 2019). The osmoregulatory capability of *C. sapidus* adults in the Guadalquivir estuary indicates that it is an euryhaline species and a strong hyperosmoregulator, displaying an osmoregulatory pattern similar to that of other blue crab populations (Towle, 1997). This has been demonstrated in postmetamorphic *C. sapidus*, which hyperregulate in diluted seawater (Kinsey et al., 2003; Li et al., 2006). Concurrently, the studied population was mainly located in the meso-polyhaline waters (salinity 10–25) of the outer zone (< 20 km from the river mouth), at the salinity in which the osmotic stress was minimum (isosmotic point~20), as suggested by the salinity-related ETS activity pattern obtained experimentally. The blue crab *C. sapidus* native habitat osmoregulation reflects a remarkable ability to thrive in fluctuating salinity environments. Blue crabs in these natural habitats demonstrate robust hyperosmotic regulation, particularly in low-salinity waters, which is necessary for their survival (Mangum et al., 1985; Cameron, 1978). For instance, the ability of blue crabs to osmoregulate efficiently in low-salinity conditions has been linked to the functionality of their gill Na<sup>+</sup>/K<sup>+</sup> + -ATPase activity, which varies between genders and developmental stages (Neufeld et al., 1980). The blue crab, a strong osmoregulator, has successfully colonized waters with varying salinities, leading to its widespread invasion across many parts of Europe, similar to other invasive crustaceans worldwide (González-Ortegón et al., 2010).

ETS activity as indicator of potential respiration, provide a perspective on the physiological adaptations of *C. sapidus* to variations in salinity. ETS activity generally showed higher values at lower

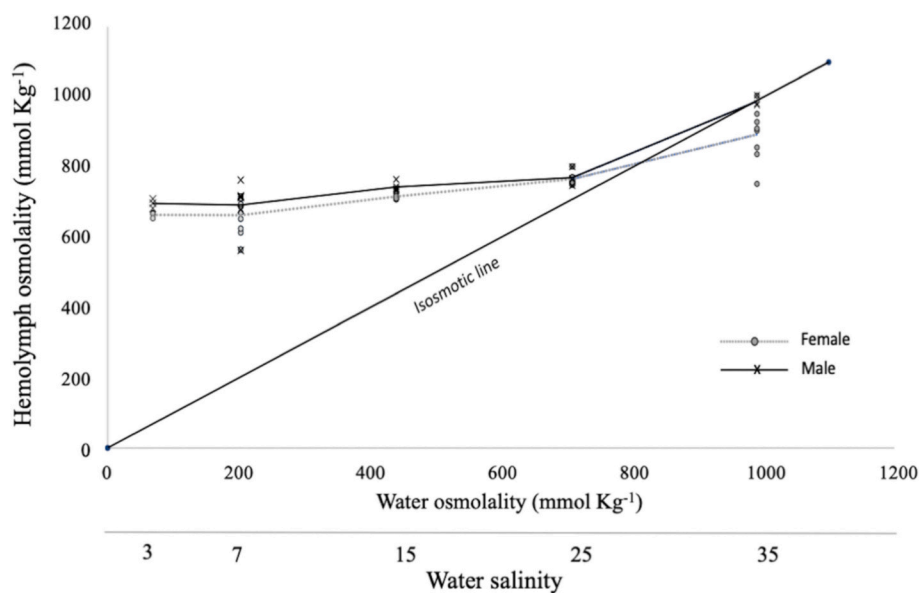
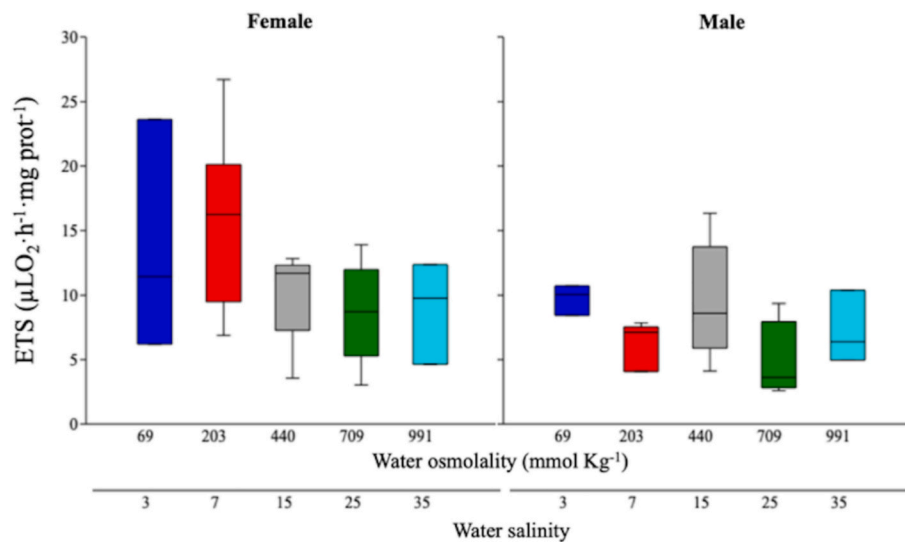


Fig. 1. Hemolymph osmolality (mmol Kg<sup>-1</sup>) activity of females (grey dash line) and males (black line) blue crab in relation to water osmolality (mmol Kg<sup>-1</sup>) and water salinity. Showing the isosmotic line in a dark line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**

PERMANOVA tests to evaluate the effects of the fixed factors salinity (Sal: 3, 7, 15, 25 and 35), sex (female and male) and the covariate size on osmolality expressed in  $\text{mmol Kg}^{-1}$  and electron transport system expressed in  $\mu\text{LO}_2\cdot\text{h}^{-1}\cdot\text{mg prot}^{-1}$  and the combination of both variables of *Callinectes sapidus*. MS = mean squares of factors; p (MC) = Monte Carlo p-value. Significant terms are highlighted in bold. The analysis is based on the modified Euclidean distance dissimilarity (9999 permutations). Significant terms are highlighted in bold.

Osmolality								
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	p(MC)	
Size	1	224.31	224.31	0.10239	0.749	9807	0.7485	
Sal	4	<b>4.4625E+05</b>	<b>1.1156E+05</b>	<b>50.924</b>	<b>0.0001</b>	<b>9961</b>	<b>0.0001</b>	
Sex	1	<b>14,884</b>	<b>14,884</b>	<b>6.7941</b>	<b>0.0142</b>	<b>9838</b>	<b>0.0122</b>	
SalxSex	4	9213	2303.2	1.0513	0.3908	9952	0.3909	
Res	42	92,012	2190.8					
Total	52	5.6258E+05						
Electron transport system								
Size	1	8.9402	8.9402	0.39419	0.532	9841	0.532	
Sal	4	208.54	52.136	2.2988	0.0792	9954	0.0778	
Sex	1	<b>217.1</b>	<b>217.1</b>	<b>9.5722</b>	<b>0.0029</b>	<b>9819</b>	<b>0.0053</b>	
SalxSex	4	138.35	34.588	1.525	0.2248	9942	0.2152	
Res	36	816.48	22.68					
Total	46	1389.4						
Combination osmolality and electron transport system								
Size	1	0.29061	0.29061	0.32349	0.6771	9952	0.6677	
Sal	4	<b>46.105</b>	<b>11.526</b>	<b>12.83</b>	<b>0.0001</b>	<b>9938</b>	<b>0.0001</b>	
Sex	1	<b>7.3953</b>	<b>7.3953</b>	<b>8.2321</b>	<b>0.0042</b>	<b>9948</b>	<b>0.0032</b>	
SalxSex	4	4.7671	1.1918	1.3266	0.2596	9954	0.2538	
Res	35	31.442	0.89835					
Total	45	90						



**Fig. 2.** Electron Transport System (ETS;  $\mu\text{LO}_2\cdot\text{h}^{-1}\cdot\text{mg prot}^{-1}$ ) activity of females (left) and males (right) blue crab in relation to water osmolality ( $\text{mmol Kg}^{-1}$ ) and water salinity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

salinities, decreasing as salinity increased. A similar pattern was observed in the non-native crustacean *Syndotaea laticauda* (class Malacostraca) by Ruiz-Delgado et al. (2019), where high ETS values at low salinity suggest a high respiration rate of individuals under this osmoregulatory stress condition. These results also coincide with the information obtained through direct measurements of oxygen consumption in the crabs *Hemigrapsus crenulatus* (Urzúa and Urbina, 2017) and *Hemigrapsus takanoi* (Shinji et al., 2009), both of which showed the same pattern of high oxygen consumption values at low salinities. These authors suggest that this may be due to several metabolic functions, such as respiration, ammonia excretion, and  $\text{Na}^+$  regulatory capacity, which decrease as salinity increases. This is an important finding in relation to metabolic stress factors, as crustaceans must resort to other

physiological mechanisms to maintain hemolymph osmolality under low salinity conditions (Hudson et al., 2018). Although the species can strongly osmoregulate at different salinities, individuals that migrate to areas of maximum salinity likely gain an energetic advantage, as the need for ammonia excretion decreases (Weihrauch et al., 2004). This contrasts with the results obtained for *C. sapidus* in the present study, where a decrease in ETS activity was observed as salinity increased. This response is associated with an increase in osmotic work, especially at salinities where more energy is required to maintain the osmotic gradient between the internal and external environments of the organisms (Moreira et al., 1983; McNamara et al., 1986).

The successful establishment of *C. sapidus* in European estuaries could be partially explained by its tolerance to salinity changes,

demonstrating strong osmoregulatory compared to other non-native invertebrates (González-Ortegón et al., 2010). Since its first record in the Gulf of Cadiz (González-Ortegón et al., 2020), this species is likely to be capable to self-maintaining its estuarine population and becoming a naturalized species in this estuary. The role of salinity tolerance of invaders may explain their successful invasion, as was demonstrated by the European green crab *C. maenas*, a strong hyper-/hyporegulator and one of the world's most successful aquatic invaders (Darling et al., 2008). Their study revealed a particular ecological profile for invaders, with a strong influence of salinity tolerance. The Chinese mitten crabs, *Eriocheir sinensis* (Milne Edwards, 1853), in regions with higher salinity, specifically the invaded mesohaline waters of the Baltic Sea, experience more advantageous trophic conditions (Normant et al., 2012). This study suggests that these conditions are linked to increased diversity in both flora and fauna, highlighting the presence of abundant resources, such as sessile mussels. In Southern Brazil, the established Indo-Pacific swimming crab, *Charybdis hellerii* (Milne-Edwards, 1867), showcased extensive salinity tolerance, ranging from 10 to 40 saltwater in 24-h developed experiments (Occhi et al., 2019). This noteworthy tolerance is likely pivotal for ensuring the species' survival and contributing to the success of its invasion in environments characterized by salinity variations (~0–32 saltwater) during tidal cycles of approximately 6 h, as observed in the estuaries of this region. The invasive *Hemigrapsus sanguineus*, exhibited a robust osmoregulatory capability, with adult specimens demonstrating the capacity for hyper-osmoregulation in low salinities and hypo-regulation in concentrated seawater (39 saltwater; Torres et al., 2021). This species also showed no change in hemolymph osmolality for 48 h, highlighting the crab's remarkable survival capacity and its potential for successful invasion into new areas. These results align with the notion that salinity tolerance plays a crucial role in the establishment of non-native species (Hudson et al., 2018).

Regarding gender differences in osmoregulation in blue crabs, previous studies have observed various variations (Epifanio, 2019). One of the main reasons for adult segregation may be the difference in the ability of males and females to regulate sodium ions and/or sodium bound to certain organic constituents in the blood (Tan and Engel, 1966). Nutrient allocation towards eggs and the metabolic demands associated with reproduction may influence osmoregulation in females, whereas males may exhibit a more consistent response across a range of salinities (Clark et al., 2018). Engel and Eggert (1974) studied the gender-metabolic effects of salinity in isolated gills of the blue crab (*C. sapidus*), in both adult males and females, observing results similar to those in this study with ETS. That is, oxygen consumption decreased with increasing salinity. Although studies on gender-specific metabolic responses are scarce, differences have been observed between genders in relation to functional responses (e.g., feeding - consumption by predators in relation to prey density), specific in the life cycle of the species (*C. maenas*; Kattler et al., 2023). Additionally, comparing these differences in both the native and invaded areas, which could occur in males or females, could be associated with different adaptations in the new area for one of the genders. That could lead to changes in sexual selection pressures and impact population dynamics. Therefore, it is important to consider gender characteristics in an invasive species like *C. sapidus* to better understand its response and for predicting and managing the impacts of invasive species on native ecosystems.

Male individuals exhibit a higher tendency to migrate from the initial saline environment compared to females, possibly attributed to variations in overall activity levels between genders. This implies that males are more disposed to seek out alternative habitats in response to salinity fluctuations, whereas females may experience greater sensitivity to osmotic stress (*H. sanguineus*, Hudson et al., 2018). Additionally, mature male adults are generally found in the low-salinity waters of upper estuaries, while females spawn offshore, where the salinity regime favours larval survival (Meise and Stehlik, 2003). Therefore, males and females sometimes exhibit different spatial distributions (Millikin and Williams, 1984), with salinity playing an important role.

Experimental data indicate that this species is well adapted to environments with wide, short-term variations in salinity variations, and mainly inhabits the estuarine stretch in which the salinity range was coincident with that in which its adults hyperregulated (Unpublished). The information provided by this study can help to understand the mechanisms underlying the successful establishment and naturalization of this non-native species worldwide. In comparison to native species, blue crabs exhibit greater physiological plasticity, enabling them to adapt to a wider range of environmental conditions (Rilov and Crooks, 2009). This physiological flexibility may confer them a competitive advantage over native species and contribute to their invasive success in new habitats (Grosholz, 2002).

## 5. Conclusions

*Callinectes sapidus* showed a significant osmoregulatory capability, facilitating its invasive success in various aquatic habitats. Gender differences are evident in osmoregulation and ETS activity, with females showing greater sensitivity to osmotic stress compared to males, which presented a more consistent response across different salinity levels. The physiological plasticity of *C. sapidus* provides a competitive advantage over native species, enhancing its successful invasion. Our results contribute to the growing advancement in the eco-physiology of invasive species and underscore the ongoing need for interdisciplinary research to address the challenges associated with biological invasion and biodiversity conservation.

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## CRediT authorship contribution statement

**Inma Herrera:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Gustavo F. de Carvalho-Souza:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Enrique González-Ortegón:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known conflicts of interest or personal relationships that could have influenced the work reported in this paper.

## Data availability

Data will be made available on request.

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