



Innate feeding plasticity and animal prey support invasiveness of aquatic species in a southwestern European estuary

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Received: 14 August 2024 / Accepted: 11 December 2024 / Published online: 23 December 2024
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Abstract Non-indigenous species often rely on trophic plasticity to adjust to available food sources and even to avoid interspecific competition while overcoming environmental constraints during the establishment phase and, eventually, as they become invasive. The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 is expanding quickly in southwestern Europe and northwestern Africa, raising concerns about its impacts. Its feeding ecology in non-native areas is poorly understood, so this study aimed to 1) unveil the diet and feeding strategy used by the Atlantic blue crab in a highly invaded European estuary,

2) evaluate if their invasiveness was facilitated by an invasion meltdown process concerning trophic facilitation, and 3) determine its trophic position. Metagenomic analyses of gut content and stable isotopes showed that the species relied on opportunistic and carnivorous feeding traits and preyed mainly on native animal species, such as fish, shrimps, and oysters. We did not observe evidence of a widespread invasion meltdown process through trophic facilitation mediated by other invaders. The Atlantic blue crab's trophic niche overlapped with two native crab species, particularly the European green crab *Carcinus maenas* (Linnaeus, 1758), while its high trophic position (4.3 ± 0.5) reflected the reliance on animal prey. These evidence suggests that trophic plasticity likely contributed to the invasiveness of the Atlantic blue crab because of its ability to exploit readily available prey. The Atlantic blue crab metapopulation is expanding and increasing over a vast region, and unfortunately a series of cascading effects throughout the food web can still be expected, as observed elsewhere.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10530-024-03515-y>.

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Keywords *Callinectes sapidus* · Invasive species · Food web · Niche overlap · Stable isotopes · DNA sequencing

Introduction

Food web studies of invasive aquatic species disclose the trophic levels they could directly impact but also

provide a deeper understanding of putative cascading effects within and across compartments of an ecosystem (Dias et al. 2014; Maceda-Veiga et al. 2018) and, eventually, impacts upon fringing ecosystems (Kimbrow et al. 2009; Wainright et al. 2021). Invasive aquatic species can disrupt food webs at different levels, modulate predator–prey interactions (Savidge 1987; Levine et al. 2003; Green et al. 2012), compete for food and habitat resources with native or non-indigenous species (Hudina et al. 2011; Britton et al. 2018), and reshape nutrient exchange rates between the sediment and water through changes in bioturbation (Figueredo and Giani 2005; Sousa et al. 2009). Understanding the potential ecological effects of a non-indigenous species upon an ecosystem requires an accurate determination of its feeding habits.

The invasive and fast-spreading nature of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 in Mediterranean and eastern Atlantic ecosystems (Encarnação et al. 2021; Mancinelli et al. 2021; Clavero et al. 2022) urges for comprehensive insights into their feeding ecology along the non-native range. In the native area, which extends along the western Atlantic from Nova Scotia in North America to Argentina in South America (Mancinelli et al. 2021), this species plays a significant ecological role and supports a large fishery industry across North America. In Chesapeake Bay (Maryland, United States), the heart and soul of the Atlantic blue crab fishery, landings exceeded 53,000 metric tons and 240 million US\$ in 2021 (Millikin and Williams 1984; Guillory et al. 2001; NOAA 2023).

The Atlantic blue crab links the benthic and pelagic compartments, particularly in estuarine food webs, by feeding on a variety of benthic prey, but also being preyed on by, for example, striped bass *Morone saxatilis* (Walbaum, 1792) or redfish *Sciaenops ocellatus* (Linnaeus, 1766) (Laughlin 1982; Scharf et al. 2000; Overton et al. 2009). The Atlantic blue crab is an eurythermal and euryhaline species with high osmoregulatory (Tagatz 1968, 1971; Epifanio 2019) and swimming capabilities that allow them to inhabit a variety of coastal and estuarine habitats, including low salinity habitats (Orth and van Montfrans 1990; Williams et al. 1990; Posey et al. 2005; Scalici et al. 2022). These traits enable the species to explore multiple food sources throughout its life and ecosystems. As an omnivorous, detritivore, scavenger, and cannibalistic species, the Atlantic blue crab is an

opportunistic predator with a high feeding adaptiveness (Laughlin 1982; Hines et al. 1990; Stoner and Buchanan 1990; Hsueh et al. 1992; Seitz et al. 2011). Its animal prey may include fish, bivalves (oysters, clams, mussels), polychaetes, and other crustaceans (crabs, shrimps, amphipods), while consumption of vegetation material includes mangrove plants, algae, or seagrass (Laughlin 1982; Ryer 1987; Stoner and Buchanan 1990; Fitz and Wiegert 1991; McClintock et al. 1991; Hsueh et al. 1992; Perkins-Visser et al. 1996; Reichmuth et al. 2009).

Traditionally, diet studies have relied on direct observations during feeding events or visual analyses of gut content or feces (Cherel et al. 2000; Balestrieri et al. 2011; Amundsen and Sánchez-Hernández 2019). These techniques are laborious, especially for small consumers, and require a large number of samples, while only providing a snapshot of the recently consumed prey. Analysis of gut contents with DNA sequencing has overcome part of such constraints. In the past decade, shotgun metagenomics expanded the taxonomic resolution already achieved with traditional DNA sequencing techniques by metabarcoding and provides a more accurate description of an individual's recent meal (Taberlet et al. 2012; Sriwathsan et al. 2015; Paula et al. 2016). Complementary, stable isotopes cannot accurately identify prey to the species level but reveal the origin and relative contribution of organic matter sources incorporated by consumers over a broader timeframe (Prado et al. 2022; Dias et al. 2023). Carbon ($\delta^{13}\text{C}$: $^{13}\text{C}/^{12}\text{C}$) and nitrogen ($\delta^{15}\text{N}$: $^{15}\text{N}/^{14}\text{N}$) stable isotopes ratios are the most widely used tracers to study energy flows in aquatic food webs (Hobson 1999; Fry 2002; Middelburg 2014; Prado et al. 2022) and are a time-integrated reflection of the physical and metabolic processes occurring in natural ecosystems (Peterson and Fry 1987; Boecklen et al. 2011). Stable isotope ratios increase by about 0.4‰ in $\delta^{13}\text{C}$ and 3.4‰ in $\delta^{15}\text{N}$ per trophic level, a process called trophic fractionation that reflects the enrichment in ^{15}N and ^{13}C between consumers and their diets (Post 2002). For this reason, $\delta^{15}\text{N}$ is very useful in determining the trophic position of species across the food web and establishing relations between consumers and prey along with $\delta^{13}\text{C}$ (Minagawa and Wada 1984; Post 2002). The isotopic composition reflects short- and long-term diets depending on the analyzed tissue and turnover times (Peterson and Fry 1987; Dalerum

and Angerbjörn 2005). Stable isotopes may also help to distinguish the origins of consumed organic matter between ^{15}N -depleted terrestrial sources and ^{15}N -enriched aquatic sources (Peterson and Fry 1987; Cloern et al. 2002). The $\delta^{13}\text{C}$ gradients may help distinguish between marine and freshwater food sources, as consumers in marine locations are, on average, more ^{13}C -enriched (Fry 2002; Dias et al. 2016).

Several hypotheses have been proposed to explain the invasiveness of a species, including competitive advantage in feeding strategies, reproductive traits, release from native enemies, meltdown effect, or tolerance to a broader range of abiotic variables (Simberloff and Von Holle 1999; Levine et al. 2003; Colautti et al. 2004; Keller et al. 2011; Lenz et al. 2011). In this study, we explored which food sources might have supported the exponential increase of the Atlantic blue crab in a highly invaded European estuary (Guadiana estuary, SW Iberian Peninsula) since its first detection in 2017 (Morais et al. 2019; Encarnaç o et al. 2021). This study aimed to assess three main hypotheses. First, we hypothesized that the Atlantic blue crab would show omnivorous and opportunistic behavior, as in its native range, which might have contributed to its invasiveness. Second, we hypothesized that the numerous invasive species present in this estuary registered after a profound hydrological regime shift could promote the species invasiveness through a process coined ‘invasion meltdown’. This hypothesis postulates that the interactions between a group of non-indigenous species may facilitate their own invasiveness through synergistic interactions, increasing the likelihood of survival and resulting in an acceleration on the magnitude of ecological impacts (Simberloff and Von Holle 1999; Simberloff 2006). Third, we hypothesized that the trophic niche of the Atlantic blue crab overlaps with sympatric native crabs, as documented by the collapse of the European green crab *Carcinus maenas* (Linnaeus, 1758) in the Ebro Delta in eastern Spain (Clavero et al. 2022).

Material and methods

Study area

The Guadiana estuary in southeastern Portugal (SW Iberian Peninsula, Europe) is part of an ecosystem

that underwent profound abiotic and biotic changes since in 2002 the largest water reservoir in Europe was built in the river mainstream, approximately 140 km from the river mouth. Although characterized by a Mediterranean climate (i.e., periods of extended droughts and infrequent heavy floods), the freshwater inflow regularization of the Guadiana River also resulted in an increasing salinization of the estuary (Ch ıcharo et al. 2006; Morais 2008; Barbosa et al. 2010; Encarnaç o et al. 2013). This triggered a series of biotic responses, primarily the establishment of numerous non-indigenous species – mainly in the middle estuary (Morais 2008; Morais et al. 2009a, 2017, 2019; Ch ıcharo et al. 2009; Encarnaç o et al. 2024) – and the expansion/contraction of available habitats for native species (Ch ıcharo et al. 2006; Morais et al. 2009b; Barbosa et al. 2010; Encarnaç o et al. 2013).

Field sampling

Sampling took place in the middle Guadiana estuary, in the area of Foz de Odeleite (GPS: 37.3536 / -7.4408) about 20 km from the river mouth, in June 2021 and March 2022. Atlantic blue crab specimens were collected with benthic-baited traps and obtained from local fishers that operate in the same area. The traps were made of two 50 cm diameter steel rings, and 30 cm height when raised, joined and closed at the bottom by 1 cm diameter fishing net, with a bait box at the center (see Encarnaç o 2023). In both cases, specimens were frozen at $-20\text{ }^\circ\text{C}$ after collection and until sample extraction. A total of 42 Atlantic blue crabs were collected, 22 in 2021 and 20 in 2022, for metagenomic and stable isotope analyses. The average carapace width was 155.5 ± 31.8 mm in 2021 (12 males: 163.0 ± 27.9 mm; 10 females: 146.6 ± 35.3 mm) and 207.9 ± 16.7 mm in 2022 (20 males).

For stable isotopes, the sampling of potential food items was done one month before the Atlantic blue crab sampling to account for tissue turnover (Llewellyn and La Peyre 2011; Mancinelli et al. 2013; Carrozzo et al. 2014). The putative food items sampled included pelagic and benthic macrofauna, and plant material. Macrofaunal prey were selected based on the feeding habits of the Atlantic blue crab across its native range (Laughlin 1982; Stoner and Buchanan 1990; Fitz and Wiegert 1991; Hsueh et al.

1992), therefore including fish, bivalves, polychaetes, jellyfish, and crustaceans (shrimps, isopods, other crabs). Potential macrofaunal prey were mainly collected with baited benthic traps that were checked every 10 min, and the collected species were frozen at $-20\text{ }^{\circ}\text{C}$ until analysis. A minimum of three specimens were collected for each species. Plant material and vegetation detritus brought by the river current and entangled in the traps, and riparian vegetation was also sampled (*Juncus* sp.).

Most Atlantic blue crabs were captured by local fishers in the middle Guadiana estuary, along with specimens of the European eel *Anguilla anguilla* (Linnaeus, 1758) in 2022. In June 2021, together with the Atlantic blue crabs, ten specimens of three native crab species (*Afruca tangeri* (Eydoux, 1835), *Carcinus maenas*, and *Panopeus africanus* A. Milne-Edwards, 1867) were collected to compare trophic positions and quantify niche overlap.

Metagenomic analysis

DNA extraction of gut content samples was performed with the Quick-DNA™ Miniprep Kit (D3024, Zymo Research), following the manufacturer's protocol. After DNA extraction, samples were stored in a DNA elution buffer and kept at $-20\text{ }^{\circ}\text{C}$ until shipping to BGI Genomics in Hong Kong for shotgun metagenomic sequencing. Of the 42 Atlantic blue crabs collected in the Guadiana estuary, 37 had enough food content in their digestive tract for DNA extraction, and potentially for metagenomic analysis. Twenty samples were sequenced individually, but the other 17 samples were of low-grade quality for library construction and sequencing. To increase chances of positive results of these 17 low-grade samples, 11 samples from 2021 were pooled into three batches: Pool 1 (3 males), Pool 2 (4 males), Pool 3 (4 females). Six of the 20 individual samples failed library construction, so we present data from 17 samples – 14 individual samples and three pooled samples.

Data were initially processed with Geneious® to merge mate pair sequences. Then, each sequence was annotated in megablast with standard settings to return the best single hit of each sequence. The results of the annotation procedure were analyzed, filtered, and hand-curated individually in four steps. First, only hits of eukaryotic organisms were analyzed and filtered by sequence length (≥ 150 bp) and percentage

of identical basepairs ($\geq 98\%$). Also, only results with more than one hit in more than one sample or with three or more hits in the same sample were considered in the following steps. Second, the sequences of each taxon were checked manually in the Basic Local Alignment Search Tool (BLAST) of the US National Library of Medicine website (NCBI 2023). The sequences of each taxon were ordered by the higher percentage of identical sites and lower E-value (i.e., the number of hits expected by chance when searching the database). The top-ranked sequence was inserted in BLAST, or more than one sequence was tested when several sequences presented the same percentage of identical sites and E-values and whenever doubts emerged from the data analysis. Third, in the BLAST tool, the options “Nucleotide collection (nr/nt)” and “Highly similar sequences (megablast)” were selected. Fourth, BLAST hits with 100% similarity, hits with more than 99% similarity, or the higher available percentage were considered a priority for analysis. The species with the most hits was considered the best match. When hits signaled multiple species, we checked their status (presence/absence) in the Iberian Peninsula in the GBIF database (GBIF 2023) and, if in doubt, a classification by higher taxonomic level was considered, for example, in cases of many hits in many species of the same genus or family. Changes in taxonomic classification are available in Table S1 (Online Resource 1). One of the 14 individual samples had a small number of hits, and after the initial filtration, all the eukaryote taxa belonged to the host, so this sample was also discarded. The final dataset was analyzed to calculate each taxon's frequency of occurrence (FO) across individual samples, excluding the pooled samples. The average was used to summarize data, and the standard deviation was used to represent data dispersion.

Stable isotope analysis

In the laboratory, muscle samples were retrieved from fish (muscle without skin or bones), bivalves (adductor muscle or foot if the specimen was small), shrimps (tail muscle), and crabs (leg or claw muscle, depending on the specimen size). In the case of polychaetes, jellyfish, and isopods, the whole animal was used. These samples were dried at $60\text{ }^{\circ}\text{C}$ for 24 h and ground into a powder with a mortar and pestle. Plant material was cleaned with deionized water to

remove debris and epiphytes, dried at 60 °C for 24 h, and ground to a fine powder with a mixer mill. After being ground to a fine powder, all samples were stored in glass containers and kept airtight with silica gel until isotopic analysis.

Stable isotopes were determined at MARINNOVA (University of Porto, Portugal) with a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) via the ConFlo IV Interface. The raw data were normalized by three-point calibration using the international reference materials IAEA-N-1 ($\delta^{15}\text{N} = +0.4\text{‰}$), IAEA-NO-3 ($\delta^{15}\text{N} = +4.7\text{‰}$) and IAEA-N-2 ($\delta^{15}\text{N} = +20.3\text{‰}$) for nitrogen isotopic composition, and two-point calibration using USGS-40 ($\delta^{13}\text{C} = -26.39\text{‰}$) and USGS-24 ($\delta^{13}\text{C} = -16.05\text{‰}$) for carbon isotopic composition. Stable isotope ratios are reported in δ notation with Vienna Pee Dee Belemnite and air as standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. The analytical error, i.e., the average standard deviation of replicate reference material, was $\pm 0.1\%$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

The bulk $\delta^{13}\text{C}$ data were corrected for lipid content to avoid bias in data interpretation since lipids are depleted in ^{13}C compared to protein and carbohydrates (DeNiro and Epstein 1978; Logan et al. 2008). The correction proposed by Bodin et al. (2007; Eq. 1) for the spider crab *Maja brachydactyla* Balss, 1922, was applied to the $\delta^{13}\text{C}$ values of all crab species, as this is so far the only lipid correction available for decapods. Lipid content in fish, bivalves, polychaetes, and other crustaceans was corrected using the mass balance correction proposed by Hoffman and Sutton (2010; Eq. 6). This correction uses estimates of $\text{C:N}_{\text{protein}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ that are similar to those from the muscle tissue found in fish (e.g., Sweeting et al. 2006) and other taxonomic groups as shrimps and zooplankton (Fry et al. 2003; Smyntek et al. 2007). To test for differences in the average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the Atlantic blue crab specimens and the multiple sources of organic matter, a one-way analysis of variance (ANOVA) was conducted with post-hoc Tukey HSD comparison tests, after checking for the assumptions of normality and homoscedasticity of variance.

The most likely preys assimilated by Atlantic blue crabs in each year were identified using bi-plots, where Atlantic blue crabs' $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (after adjusting for trophic fractionation) were compared to isotope ratios of prey. The isotope ratios of Atlantic

blue crabs from this study were adjusted for one trophic level using the trophic fractionation estimates ($\Delta\delta^{13}\text{C} = 0.56 \pm 1.27\text{‰}$; $\Delta\delta^{15}\text{N} = 1.15 \pm 1.05\text{‰}$) derived from the results of McCann and Jenson (2018) of muscle tissue of Atlantic blue crabs fed with clams and black sea bass. Mixing polygon simulations were constructed to check the adequacy of the food sources selected after bi-plot inspection and trophic fractionation values used for each year (Smith et al. 2013). If consumers fell within the 95% mixing region, that indicates that a mathematical solution that satisfied the geometry of mixing models was found, while consumers falling outside the 95% mixing region, were not considered for the mixing models (see Smith et al. 2013). To quantify the relative contribution of the most likely food items to Atlantic blue crabs, the Bayesian stable isotope mixing model MixSIAR v3.1.12 was used (Stock and Semmens 2016). Taxonomic groups used as source inputs were chosen considering their position on the bi-plots and the results of metagenomic data obtained during this study. The number of input sources was kept low ($n = 3$) so the models would run with less uncertainty. To run the MixSIAR models, the stable isotope ratios of the Atlantic blue crab and their most likely food items were input as raw data, using non-informative priors. In the case of the 2021 data, sex of the Atlantic blue crab was considered as a factor in the model. Model convergence was assessed via Gelman–Rubin and Geweke diagnostics (Stock and Semmens 2016). Posterior distributions obtained from the MixSIAR analyses are expressed as median and 95% credibility intervals (CI).

The trophic position (TP) of the Atlantic blue crab and three other native brachyuran crabs was calculated with the equation proposed by Mancinelli et al. (2016). In this equation, $\delta^{15}\text{N}_{\text{Consumer}}$ is the stable isotope ratio of the Atlantic blue crab, and $\delta^{15}\text{N}_{\text{Baseline}}$ is the stable isotope ratio of the baseline indicator, in this case bivalves (oysters) with an average value of 11.73‰. $\Delta^{15}\text{N}$ is the trophic fractionation of $\delta^{15}\text{N}$ per trophic level (1.15‰, in this study; McCann and Jenson 2018). The λ is the trophic level of the baseline indicator, in this case $\lambda = 2$ for bivalves (oysters) as primary consumers (e.g., Fukumori et al. 2008; Briant et al. 2018).

To estimate the trophic niche width of the four brachyuran crabs, their stable isotope values were used to calculate the standard ellipse area (SEA)

using the SIBER (Stable Isotope Bayesian Ellipses in R) package (Jackson et al. 2011). To account for the small sample size, the corrected Standard Ellipse Areas (SEA_C; considering 40% of central data points) and the corresponding 95% Bayesian Standard Ellipse Areas (SEA_B) were estimated for each species (Jackson et al. 2011, 2012). When overlap was observed between the trophic niche of the Atlantic blue crab and the other crabs, the extent of overlap (%) was calculated using the SEA_C, which represents the overlap between the core dietary niches of any pair of species (Jackson et al. 2011, 2012).

All the analyses were performed using the open-source statistical language R with the RStudio software (RStudio 2023).

Results

Metagenomic analysis

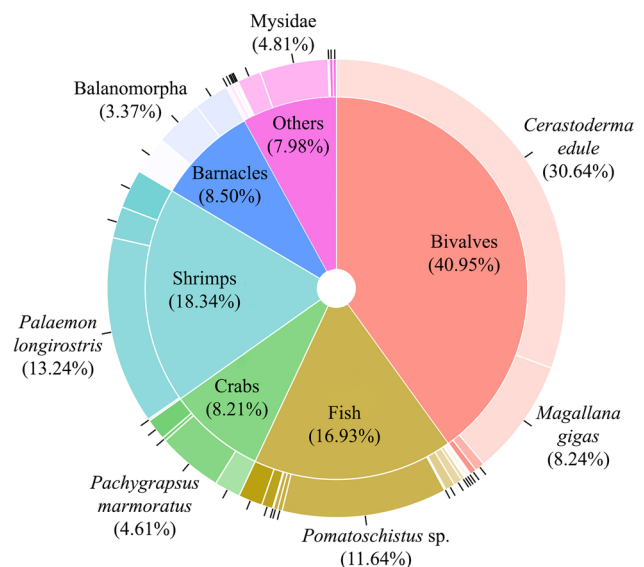
A very laborious hand-curing process was needed to filter the initial results (446,365 hits) to reach a final dataset resembling the diversity of prey taxa of this estuary. Firstly, hits that did not belong to eukaryotic organisms, including bacteria and viruses, were removed from the database (95.5%) as these were beyond the study's scope. The resulting database classified 80.2% of the hits as belonging to the host – the Atlantic blue crab. Only 32.9% were initially

classified as *Callinectes sapidus* and 3.1% as other *Callinectes* species. An additional 44.1% were initially classified into 39 crustacean taxa and, after the hand-curing process, were reclassified as hits likely derived from host genetic material.

The metagenomic analysis of the final database of gut content of Atlantic blue crabs collected in the Guadiana estuary generated a total of 3,238 hits matching 40 taxa, including bivalves, fish, other crabs, shrimps, barnacles, cnidarians, a gastropod, an ascidian, terrestrial plants, but also terrestrial mammals and pond turtles. The bivalve *Cerastoderma edule* (Linnaeus, 1758) was the species with the highest percentage of hits (30.64%) for a total of 938 hits, followed by the shrimp *Palaemon longirostris* H. Milne Edwards, 1837 (13.24%, 405 hits), the goby *Pomatoschistus* sp. (11.64%, 456 hits), and the oyster *Magallana gigas* (Thunberg, 1793) (8.24%, 252 hits) (Fig. 1).

Frequency of occurrence on the presence of prey species across individual samples showed that the crab *Pachygrapsus marmoratus* (Fabricius, 1787) was the most frequent species, present in 12 out of 13 individual samples (FO=92.3%). Following, five taxa showed a frequency of occurrence of 84.6% (11 out of 13 samples) – Palaemonidae and Penaeus (shrimps), Grapsidae and *Panopeus* sp. (crabs), and Leptothecata or Ceriantharia (Cnidaria). Following, the pipefish *Syngnathus* sp. (FO=46.2%), the oyster *Magallana gigas* (FO=38.5%), the common cockle

Fig. 1 Percentage of hits (3238 in total) from shotgun metagenomic analysis of the intestine contents of Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896 collected in the Guadiana estuary in 2021 and 2022. The hits shown here are of the six larger groups and their respective taxa after data was analyzed, filtered, and hand-cured. Results include 13 individual samples and the three pooled samples



Cerastoderma edule (FO=30.8%), and the clam *Scrobicularia plana* (FO=7.7%). Considering larger taxonomic groups, other crabs showed the highest frequency of occurrence ($87.2 \pm 4.4\%$), followed by shrimps ($64.1 \pm 35.5\%$), unidentified fish (38.5%), and bivalves ($23.1 \pm 14.0\%$).

Carbon and Nitrogen stable isotopes analysis

The $\delta^{13}\text{C}$ values of the Atlantic blue crab varied between -27.62‰ and -19.47‰ , with an average ($\pm\text{SD}$) of $-24.19 \pm 1.63\text{‰}$. The $\delta^{15}\text{N}$ values ranged between 13.12‰ and 16.15‰ and averaged ($\pm\text{SD}$) $14.57 \pm 0.68\text{‰}$. In 2021, when Atlantic blue crabs of both sexes were analyzed, males were on average more ^{13}C -enriched than females ($\delta^{13}\text{C}$ males = $-23.13 \pm 0.92\text{‰}$, $\delta^{13}\text{C}$ females = $-24.70 \pm 1.60\text{‰}$; ANOVA: F-value = 8.678, $p = 0.007$), while no differences were detected for $\delta^{15}\text{N}$ ($\delta^{15}\text{N}$ males = $14.43 \pm 0.70\text{‰}$, $\delta^{15}\text{N}$ females = $14.35 \pm 0.56\text{‰}$; ANOVA: F-value = 0.099, $p = 0.756$).

Among the potential prey sources, fish had the highest $\delta^{15}\text{N}$ values, $16.12 \pm 0.86\text{‰}$ in 2021 and $16.32 \pm 1.44\text{‰}$ in 2022, above the Atlantic blue crab's overall $\delta^{15}\text{N}$ average (Fig. 2). The Atlantic blue crab $\delta^{15}\text{N}$ was higher ($p < 0.05$) than most taxonomic groups analyzed, including oysters ($12.74 \pm 1.16\text{‰}$), polychaetes ($12.51 \pm 1.06\text{‰}$), and isopods

($12.50 \pm 1.59\text{‰}$). The $\delta^{15}\text{N}$ values of most benthic species, except for *Afruca tangeri* ($11.10 \pm 0.31\text{‰}$), often overlapped with those of the Atlantic blue crab, including shrimps ($14.84 \pm 0.49\text{‰}$), other crabs (*Carcinus maenas* $15.71 \pm 0.47\text{‰}$, *Panopeus africanus* $14.91 \pm 0.36\text{‰}$), and most fish species (*Syngnathus abaster* Risso, 1827 $15.28 \pm 0.45\text{‰}$, *Anguilla anguilla* $15.57 \pm 1.22\text{‰}$) (Fig. 2; Table S2 of Online Resource 1). Differences in $\delta^{13}\text{C}$ between the Atlantic blue crab and potential food sources were not noticeable ($p > 0.05$), except Isopoda in 2022 ($\delta^{13}\text{C} = -19.28$, $p < 0.001$). Differences in $\delta^{13}\text{C}$ were observed between the Atlantic blue crab and *Afruca tangeri* in 2021 ($p < 0.001$) and between *Carcinus maenas* and *Palaemon longirostris* in 2022 ($p < 0.05$) (Table S2 of Online Resource 1).

Mixing models

Based on the stable isotope ratio bi-plot analysis, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the Atlantic blue crabs were intermediate between several potential prey groups, namely fish, bivalves, shrimps, or other crabs (Fig. 2). The $\delta^{15}\text{N}$ values of individuals analyzed in 2021 and 2022 suggest the assimilation of ^{15}N -enriched sources such as fish, shrimps and some other crabs. The range of $\delta^{13}\text{C}$ values suggests the assimilation of bivalves, isopods, and/or jellyfish. Given the results obtained by the metagenomics analyses, the

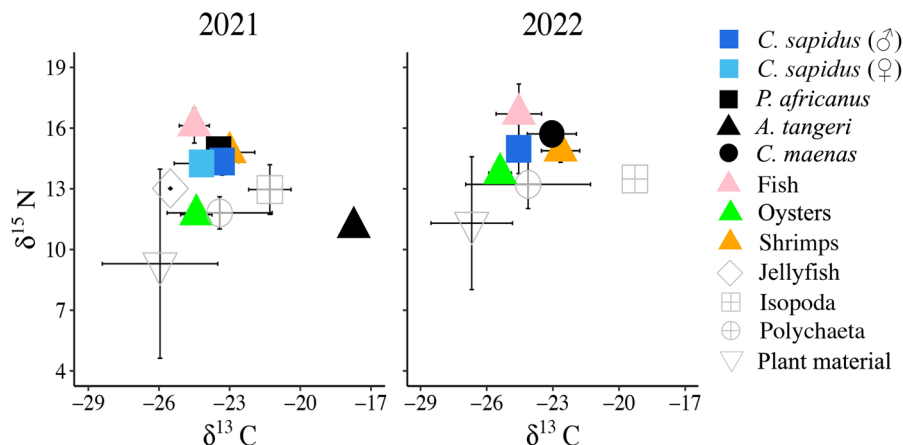


Fig. 2 Bi-plots of average ($\pm\text{SD}$) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the consumer species – the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 – and their potential sources sampled in 2021 and 2022. Values of animal samples were corrected for lipid content, and the consumer species also considering trophic

fractionation ($+0.56 \pm 1.27\text{‰}$ $\delta^{13}\text{C}$; $+1.15 \pm 1.05\text{‰}$ $\delta^{15}\text{N}$), obtained from McCann and Jenson (2018). Only the Atlantic blue crab specimens used in the MixSIAR models were included in this figure ($n = 21$ in 2021; $n = 15$ in 2022)

potential prey selected for the mixing models were bivalves (oysters), fish, and shrimps. This choice of prey represents a mathematical solution satisfying the geometry of mixing models for most of the individuals analyzed (Fig. 3; Smith et al. 2013). Eight Atlantic blue crab specimens were not included in the models (three from 2021 and five from 2022) because they were outside the 95% mixing region in the simulated mixing polygons (red dots in Fig. 3).

Considering the MixSIAR models (95% CI), results from 2021 and 2022 showed similar yet

complementary results, with relevant contributions from all three potential food sources to the overall biomass of the Atlantic blue crab. While in 2021, shrimps composed a significant component of the Atlantic blue crab's biomass, with a median proportion of 63.3% for males and 51.6% for females, in 2022, the value decreased to 7.3% for males (Fig. 4). In 2021, the differences between sexes were small, and resulted from an increased contribution of shrimps to males, while oysters and fish contributed with similar proportions. Considering only the male

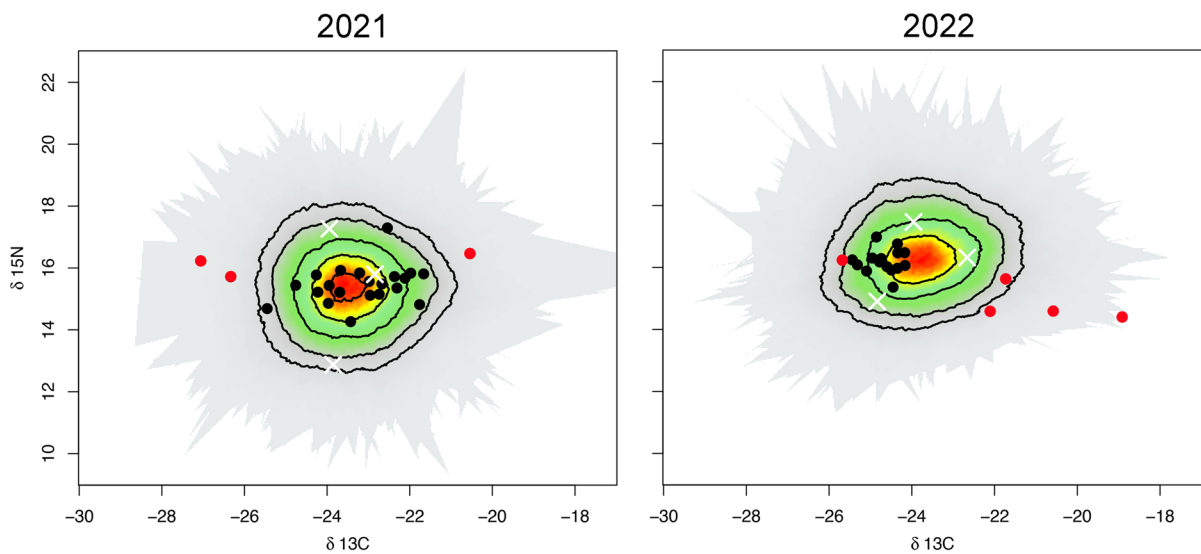


Fig. 3 Simulated mixing polygons calculated for each sampling year, presenting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ muscle values of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 (round symbols), and the most likely prey – fish, shrimps, oysters – based on bi-plot interpretation and metagenomics analysis (white crosses). Dark round symbols represent the Atlan-

tic blue crab's muscle data corrected for trophic fractionation ($\Delta\delta^{13}\text{C} = 0.56 \pm 1.27\text{‰}$, $\Delta\delta^{15}\text{N} = 1.15 \pm 1.05\text{‰}$) obtained from McCann and Jenson (2018), and red round symbols the specimens that fell outside the 95% mixing region and, therefore, not included in the mixing models

Fig. 4 Diet proportion of Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896 collected in the middle Guadiana estuary (southeast Portugal) in 2021 and 2022, estimated from MixSIAR models

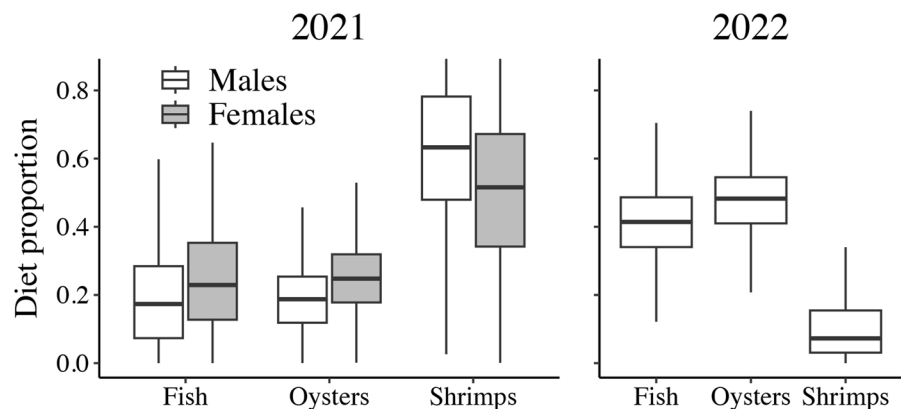


Table 1 Average (\pm SD) trophic position (TP) of the four species of brachyuran crabs collected in the middle Guadiana estuary in June 2021. The metrics to describe niche 'width' include the corrected standard ellipse areas (SEA_C; considering 40% of central data points) and the corresponding 95% Bayes-

ian ellipse areas (SEA_B; mode and the upper and lower 95% credibility intervals). The average (\pm SD) overlap area between ellipses and the percentage of niche overlap between the Atlantic blue crab and the other three brachyuran crab species are also presented

Crab species	Trophic position (TP)	Niche overlap		SEA _C (%c ²)	SEA _B
		(%c ²)	(%)		
<i>Callinectes sapidus</i>	4.3 \pm 0.5	NA	NA	3.0	2.8 (1.9 – 4.3)
<i>Carcinus maenas</i>	4.4 \pm 0.6	7.6 \pm 2.3	30.1 \pm 5.2	4.2	3.4 (1.7 – 6.9)
<i>Afruca tangeri</i>	1.6 \pm 0.4	0	0	0.6	0.5 (0.2 – 1.0)
<i>Panopeus africanus</i>	5.5 \pm 0.2	0.7 \pm 0.6	5.6 \pm 4.4	0.3	0.2 (0.1 – 0.5)

specimens, a decrease was observed in the contribution of shrimps in 2022 (7.3%) in relation to 2021, in opposition to an increase in the contribution of fish (41.4%) and oysters (48.3%).

Trophic position and niche overlap

Three out of the four brachyuran crabs analyzed for this study (Table 1), occupied a similarly trophic position – *Panopeus africanus* (5.49 \pm 0.20), *Carcinus maenas* (4.44 \pm 0.64), *Callinectes sapidus* (4.32 \pm 0.54) – while *Afruca tangeri* occupied a lower trophic position (1.61 \pm 0.37). The Atlantic blue crab niche overlapped with two native brachyuran crabs (Table 1). The highest overlap was with *Carcinus maenas* (30.1 \pm 5.2%) followed by *Panopeus africanus* (5.6 \pm 4.4%). The native *Carcinus maenas* was the crab species with the highest core niche width (SEA_C=4.2), followed by the non-indigenous *Callinectes sapidus* (Table 1).

Discussion

Our metagenomic analysis confirmed the hypothesis of opportunistic nature of the Atlantic blue crab in the Guadiana estuary, with a diverse array of prey items, while stable isotope analyses pinpointed the prey they mostly rely on to support their biomass – namely shrimps in 2021, and fish and bivalves in 2022. So far, we have not observed evidence of a widespread invasion meltdown process through trophic facilitation. The trophic position of the Atlantic blue crab (4.3 \pm 0.5) was relatively high, while the comparison with native crab species showed a considerable niche

overlap, particularly with the European green crab *Carcinus maenas*, which nevertheless presented a larger trophic core niche than the Atlantic blue crab. These results confirm data from other parts of the world showing that Atlantic blue crab was previously known omnivorous predator with an opportunistic feeding behavior (Laughlin 1982; Hines et al. 1990; Stoner and Buchanan 1990; Hsueh et al. 1992; Seitz et al. 2011), while the present results show a predominantly carnivorous feeding trait. This adaptiveness results in a capacity to explore the resources that are most available at any given time, which may change seasonally or across ontogeny (Laughlin 1982; Mancinelli et al. 2017; Prado et al. 2022). In the following sections, these aspects are discussed in more detail.

Disclosing diet through metagenomic analyses

DNA-sequencing-based studies that infer ecological interactions in terrestrial, freshwater, and marine ecosystems, including feeding habits, are becoming increasingly popular (Srivathsan et al. 2015; Paula et al. 2016; Pan et al. 2021; Dou et al. 2023). A major advantage of these over other methods is allowing the identification of more species than what could be achieved by other methods. However, the data analyses to reach meaningful results is filled with challenges. Our study disclosed a diverse list of prey consumed by the Atlantic blue crab in the Guadiana estuary, which included bivalves, shrimps, and benthic and benthopelagic fish. The final database had 3,238 hits (16.1% of the initial eukaryote database), which still included three crab taxa, namely Grapsidae and *Panopeus* sp. (both with FO=84.6%), and *Pachygrapsus marmoratus* (FO=92.3%). The high

frequency of occurrence of these three decapod taxa, plus the large number of other crab taxa discarded as host hits, shows that these results should be carefully interpreted because they could belong to the consumer. *Pachygrapsus marmoratus* (Family Grapsidae) is a common intertidal species found in rocky shores and lower estuaries (Silva et al. 2009; Pires et al. 2020), thus its predation by the Atlantic blue crab would likely occur towards the lower Guadiana estuary end only.

The frequencies of occurrence of three taxa reached 84.6%, namely Palaemonidae and Penaeus (shrimps), and a taxon that could either be a Leptothecata (Order) or Ceriantharia (Subclass) – both belonging to Phylum Cnidaria. Palaemonidae is represented by at least three species in the middle Guadiana estuary (*Palaemon longirostris*, *P. macrodactylus* Rathbun, 1902, *P. serratus* (Pennant, 1777)), hence they are likely to be important prey for the Atlantic blue crab, namely *Palaemon longirostris* which is one of the most abundant shrimp species, along with the brown shrimp *Crangon crangon* (Linnaeus, 1758) (Encarnação et al. 2013). The latter was also detected with four hits in the pooled samples.

Representatives of the Order Leptothecata (Phylum Cnidaria) in the Guadiana estuary include the invasive *Blackfordia virginica* (Chícharo et al. 2009). The Subclass Ceriantharia, commonly known as tube-dwelling anemones, are not well studied within the Guadiana estuary, but in the nearby coastal zone of Algarve, at least two species have been documented – *Cerianthus membranaceus* (Gmelin, 1791) and *Pachycerianthus solitarius* (Rapp, 1829) (BioDiversity4All 2023). In the study zone – the middle Guadiana estuary – the most likely representative of these two taxonomic groups is the non-indigenous *Blackfordia virginica*. From the twelve samples where Cnidarians were detected, three samples were from June 2021 (21 hits) and nine from March 2022 (30 hits). Although blooms of free-living medusae are typically observed in latter summer months, such as the non-indigenous *Blackfordia virginica* or the native *Catostylus tagi* (Muha et al. 2017; Cruz et al. 2021), they also have a benthic polyp stage (Wintzer et al. 2011; Gueroun et al. 2021), which, despite their small size, can still be consumed by benthic predators such as the Atlantic blue crab. In June 2021, *Blackfordia virginica* medusae were already abundant in the estuary (Encarnação 2023), increasing the likelihood

of becoming prey for the Atlantic blue crab, as suggested previously for the same area (Cruz 2020).

The metagenomic data also highlight the impact of Atlantic blue crab on species of conservation concern. The occurrence of hits on fish was also considerable with 16.93%, with *Syngnathus* sp. showing the highest frequency of occurrence among fish (46.2%), followed by other non-identified fish (38.5%). The Black-striped pipefish *Syngnathus abaster* is an abundant benthic fish in the middle Guadiana estuary (Encarnação 2023), therefore these results agree with the potential prey species for the Atlantic blue crab. Additionally, such predatory behavior raises questions on potential impacts on other Syngnathids in the Algarve region, particularly the endangered seahorses *Hippocampus* spp. that were once common in the nearby Ria Formosa lagoon (Correia 2022), and where the Atlantic blue crab is present since 2016 and becoming highly abundant (Morais et al. 2019; Vasconcelos et al. 2019; Encarnação et al. 2021).

Bivalves had an average frequency of occurrence of $23.1 \pm 14.0\%$, with the oyster *Magallana gigas* having the highest frequency of occurrence among bivalves (38.5%). Oysters and the clam *Scrobicularia plana* inhabit the Guadiana estuary (Conde et al. 2013; López-Sanmartín et al. 2016), but the high frequency of occurrence of *Cerastoderma edule* (30.8%) in specimens caught in the middle estuary is likely if considering fast migrations to downstream areas. Typically, *Cerastoderma edule* is abundant in the sandy areas of the lower estuary, while *Scrobicularia plana* is common towards the middle estuary (Conde et al. 2013). There might be two explanations for the similar frequencies of occurrence of these two bivalve species. First, simultaneous acoustic telemetry study showed that Atlantic blue crabs performed long and fast migrations between the middle and low estuary (Barra 2022), where they may feed on *Cerastoderma edule*. Second, several species once only common in the low estuary expanded their distribution into the middle estuary due to a substantial reduction of river flow and salinity increase in the middle and upper estuary following the construction of the largest European water reservoir (Chícharo et al. 2006; Morais 2008; Morais et al. 2009b; Encarnação et al. 2013). Therefore, species once typical from the low estuary might have become potential prey for Atlantic blue crabs inhabiting in the middle estuary. Additionally, some hits may result from secondary predation,

i.e., positive identification of prey genetic material in the stomachs of Atlantic blue crab. Hypothetically, this could happen, for example, with the gobies *Pomatoschistus* sp., Pleuronectidae fish, or the brown shrimp *Crangon crangon*, which can pick on the siphons of bivalves (Moreira et al. 1992; Mendonça et al. 2007), carrying the genetic material of bivalves such as *Scrobicularia plana* or *Cerastoderma edule* with them. Interestingly, we did not find evidence of consumption upon the non-indigenous Asian clam *Corbicula fluminea*, although the species is abundant along the middle and upper estuary (Morais et al. 2009a) and its consumption being already documented in the Ebro River in Spain (Ventura et al. 2018).

An advantage of metagenomic data is that they can be re-analyzed in the future when better sequence reference databases become available. Overall, the shotgun metagenomic data disclosed an already high diversity of potential prey from different taxonomic groups. Although some results remain uncertain after data filtration and hand-curation, our study provides a better taxonomic resolution of the Atlantic blue crab diet in the non-native range, often to the species level, therefore expanding on the important ecological information retrieved by stomach content diet studies that rarely reach such level of taxonomic resolution (Rady et al. 2018; Kampouris et al. 2019; Ortega-Jiménez et al. 2024). The increase of reference databases available for metabarcoding studies in the coming years, along with solid local baseline biodiversity assessments, will improve this methodological approach to studying the diet of aquatic species in the Gulf of Cadiz and elsewhere in the world.

Sources of organic matter for the Atlantic blue crab biomass

Variations in $\delta^{13}\text{C}$ found on Atlantic blue crabs, namely standard deviations of $\pm 0.9\text{‰}$ in males and $\pm 1.6\text{‰}$ in females during 2021, and $\pm 1.7\text{‰}$ in males in 2022, highlight the opportunistic nature of prey consumption and likely reflect regular movements along the estuarine salinity gradient. Atlantic blue crabs are excellent swimmers and take advantage of selective tidal-stream transport for longer migrations (Forward et al. 2003; Hench et al. 2004). Movements along the estuary's salinity gradient are likely reflected in a higher variation of $\delta^{13}\text{C}$, as food

sources in areas of higher marine influence tend to be more ^{13}C -enriched than areas under freshwater influence (Dehairs et al. 2000; Fry 2002; Prado et al. 2022; Dias et al. 2023). Individuals with isotope ratios further away from the group average, namely the individuals excluded from the models, may have moved recently to the sampling area and were not in equilibrium with the local sources of organic matter. Although less likely, given the prey coverage and the metagenomics results, the Atlantic blue crab may also rely on other food sources not sampled in the present study.

The three groups of potential prey included in the MixSIAR models – fish, oysters, shrimps – reflected their carnivorous feeding strategy. The changes observed in the males' diet between the two sampling years, likely reflect interannual prey variability. These three groups of prey were chosen due to the higher likelihood of integrating the diet of the Atlantic blue crab, either because they reflect their known feeding habits (Laughlin 1982; Hines et al. 1990; Stoner and Buchanan 1990; Hsueh et al. 1992; Seitz et al. 2011), or because they were the most common putative prey in the study area. Complementarily, the metagenomic analysis confirmed the consumption of these three groups of prey – those with the most hits – and therefore providing solid information when selecting MixSIAR model inputs.

The spread of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios also suggests that Atlantic blue crabs may have used other sources of organic matter. Other crab species were not included as potential prey in the models, although literature refers to such predation by the Atlantic blue crab (e.g., Laughlin 1982; Fitz and Wiegert 1991; Kampouris et al. 2019; Ortega-Jiménez et al. 2024). When considering the use of only *Panopeus africanus* in 2021 or *Carcinus maenas* in 2022, in both cases, the isotopic ratios of these species overlapped with shrimp species, thus they would only increase the models' uncertainty. This ratio similarities also suggests that *Panopeus africanus* and *Carcinus maenas* can be food sources for the Atlantic blue crab. As adults, the size differences between the Atlantic blue crab (180.5–36.7 mm carapace width) and native crab species (<80 mm carapace width) increases the predation potential. On the other hand, *Afruca tangeri*, with its ^{13}C -enriched and ^{15}N -depleted signature, is not likely to be an important prey for the Atlantic blue crab.

Plant material was not identified as an important food source assimilated by the analyzed specimens. The sampling area has high turbidity, and the availability of this food source is negligible. Only fragments of fresh plant material were collected with the benthic traps, while the majority of plant material carried by tidal currents consisted of a mix of degraded material, which is neither nutritious nor an appealing food source for the Atlantic blue crab even when available (Prado et al. 2022). Moreover, the importance of vegetation for the Atlantic blue crabs decreases with age (Dittel et al. 2006), and in this study, only adults were sampled. Exceptions occur when individuals are unfit for capturing live prey (Reichmuth et al. 2009).

A taxon group that needs further clarification on its potential role as a food source for the Atlantic blue crab in the Guadiana estuary are the jellyfish. This taxon group was represented by the non-indigenous *Blackfordia virginica*, which showed an isotopic ratio that was slightly ^{15}N -depleted compared to the Atlantic blue crab, which could reflect its consumption. This hydrozoan is abundant in the summer, particularly following dry winters. The consumption of jellyfish by the Atlantic blue crab has already been documented in North and South America (Farr 1978; Vitória et al. 2021), and genetic analyses have identified *Blackfordia virginica* in the gut contents of specimens from the Guadiana estuary (Cruz 2020). Our study period (March–June) was earlier than the peak of jellyfish abundance, thus this should be further investigated since the seasonal availability of this resource in the estuary creates the opportunity for the Atlantic blue crab.

Invasion meltdown considerations

The exponential increase of non-indigenous species since the Alqueva dam began operating in 2002 reshaped the estuarine biotic and abiotic dynamics (Chícharo et al. 2009; Morais 2008; Morais et al. 2009a, 2009b, 2019; Morais and Teodósio 2016; Encarnação et al. 2024). The existence of synergistic effects among invasive species, *sensu* invasion meltdown, has not been assessed until now. In the particular case of the Atlantic blue crab, we did not find evidence of a widespread invasion meltdown process supporting its invasiveness despite the possible contributions of the non-indigenous *Blackfordia virginica* to their diet as disclosed by metagenomic data

– 51 hits corresponding to 1.6% of the total hits in the final database. Nonetheless, the invasion meltdown hypothesis should be further explored to encompass a broader timescale and area along the estuary, and inclusion of additional non-indigenous benthic species such as the Asian date mussel *Arcuatula senhousia* (W. H. Benson, 1842), newly discovered in the middle Guadiana estuary (Encarnação et al. 2024).

Trophic position and niche overlap

The trophic position estimate for the Atlantic blue crab (4.3 ± 0.5) in the Guadiana estuary is among the highest registered in the non-native area. Studies made along the Mediterranean Sea showed a wide array of trophic position values, varying between 2.98 ± 0.08 and 4.22 ± 0.19 in the Ionian Sea (Carrozzo et al. 2014), and averaging between 2.0 and 4.4 in the Aegean Sea (Aslan and Polito 2021), 3.73 ± 0.06 in the Adriatic Sea (Mancinelli et al. 2016), 3.25 ± 0.17 between the Ionian and Adriatic Seas (Mancinelli et al. 2017), and 4.40 ± 0.06 in North-East Spain (Prado et al. 2024).

The high proportion of shrimps and fish as primary sources of organic matter, as also disclosed by metagenomic data (FO: $16.7 \pm 13.5\%$ and 697 hits for fish; FO: $64.1 \pm 35.5\%$ and 561 hits for shrimps), sustained the high trophic position of the Atlantic blue crab. Such a high trophic position suggests a higher reliance on animal prey, rather than on plant material and detritus (Germain et al. 2013; Mancinelli et al. 2016, 2017; Prado et al. 2022). In the native range, an average trophic position of 2.59 ± 0.38 was estimated for a population in an estuary in Texas, where no changes in trophic position were found between large and small individuals (Hoeinghaus and Davis 2007). Such lower values agree with previous studies, as these Atlantic blue crabs inhabiting salt marshes, feed mainly on C3 plants, benthic algae, and plant-derived detritus (Hoeinghaus and Davis 2007). In two New England tidal rivers, Atlantic blue crabs were found to be more generalist carnivores–omnivores, thus displaying a higher trophic level of 3.50 ± 0.35 (Taylor et al. 2022).

Differences in the trophic position of the non-indigenous Atlantic blue crab populations go beyond the inherent autoecological and synecological factors within each ecosystem. The trophic position of this species tends to increase in larger individuals – we

only analyzed adult individuals (155.5 ± 31.8 mm in 2021; 207.9 ± 16.7 mm in 2022) – because the consumption of mollusks and crustaceans, including other crabs, is higher than during earlier life phases (Laughlin 1982). Data from the Ebro Delta (Spanish Mediterranean coast) showed how abundance fluctuations in primary food sources affect trophic position. There, the trophic position was lower when they relied on bivalves as their primary source of organic matter (2.64 ± 0.20) and higher when they relied on additional animal sources (3.11 ± 0.00) (Prado et al. 2022), attaining higher values closer to the coastal area (4.40 ± 0.06) where their diet mostly relied on animal prey (Prado et al. 2024). We opted to use a trophic enrichment factor explicitly calculated for the Atlantic blue crab, the only estimate available for this species (McCann and Jensen 2018). This trophic enrichment factor estimate was based on an experiment in which the Atlantic blue crab was fed with fish and clams to encompass a diverse diet. Aslan and Polito (2021) highlighted the potential effects of these methodological differences, namely on using benthic or pelagic baseline indicators and different trophic enrichment factors. Studies made along the Mediterranean Sea used distinct values, ranging between 1.15 and 3.4 in the case of ^{15}N . Therefore, an effort must be made to standardize data analyses to facilitate the comparisons between study areas.

Competition for space and resources is one of the most cited impacts of invasive species (Crooks 2002; Grosholz 2002; Davis 2003; Britton et al. 2018). Here, we found an overlap between the core trophic niches of the non-indigenous Atlantic blue crab and the native European green crab *Carcinus maenas* ($30.1 \pm 5.2\%$), while with the African mud crab *Panopeus africanus* the percentage was lower ($5.6 \pm 4.4\%$). In the Ebro Delta, the Atlantic blue crab also showed a considerable overlap of 46.2% with the harbor crab *Liocarcinus depurator* (Linnaeus, 1758) (Prado et al. 2024), while a steep and consistent decline of the European green crab has been correlated with the introduction of the Atlantic blue crab (Clavero et al. 2022). Nevertheless, the European green crab population from the Guadiana estuary showed a greater core niche width ($\text{SEA}_C = 4.2$) than the Atlantic blue crab ($\text{SEA}_C = 3.0$), indicating it may have some plasticity in the exploitation of available resources to cope with the competition with the Atlantic blue crab. Although we do not

have a baseline value of the niche size of the European green crab in the Guadiana estuary prior to the introduction of the non-indigenous Atlantic blue crab, some authors suggest that when facing an increase in competition for food, a native species may wide their trophic niches, in an effort to maintain their energy requirements (Svanbäck and Bolnick 2007). Interestingly, the European green crab is invasive inside the native range of distribution of the Atlantic blue crab. Across North America, the Atlantic blue crab has a biotic resistance role against the invasion of the European green crab, limiting its spread to areas where the native Atlantic blue crab is more abundant and where it readily preys upon the invader (DeRivera et al. 2005). Competition between similar-sized crabs can be comparable, while larger Atlantic blue crabs are better competitors at higher water temperatures, and the European green crab has a competitive advantage in lower temperature areas (Rogers et al. 2018).

The African mud crab *Panopeus africanus* and the west-African fiddler crab *Afruca tangeri* have narrower niches than the Atlantic blue crab. Although there is not much information on the feeding ecology of the African mud crab specifically, small mud crab species are known to feed on a variety of benthic invertebrates, gastropods, barnacles, and bivalves (Ladwig 1999; Silliman et al. 2004; Premo et al. 2013). In the case of the mud crab *Panopeus herbstii* H. Milne Edwards, 1834, the species can compete with the Atlantic blue crab to feed on oysters and mussels (Seed 1980; Bisker and Castagna 1987). In the Guadiana estuary, the African mud crab inhabits the same habitat and may exploit similar food sources as the Atlantic blue crab, thus partially sharing the same isotopic niche ($5.62 \pm 4.43\%$), becoming more prone to interspecific competition for food and habitat. The west-African fiddler crab is less susceptible to predation because fiddler crabs occupy primarily intertidal areas in riverbanks and feed mostly on sediment organic matter and detritus (Wolfrath 1992; Ens et al. 1993; Moruf and Ojetayo 2017), which was evident in the differences between isotopic ratios. To some extent, an increase in the abundance of the African mud crab and healthy populations of the European green crab may increase the biotic resistance against the invasion of the Atlantic blue crab, particularly towards juvenile individuals. Although we did not use other crab species in the MixSIAR models, their isotopic ratios were similar to shrimp

species, which contributed significantly to the diet of the Atlantic blue crab. This, along with the significant niche overlap with the European green crab and the African mud crab, and numerous literature evidence of consumption of other crab species in the non-native range (Kampouris et al. 2019; Ortega-Jiménez et al. 2024), we can only assume the Atlantic blue crab may also include other crab species of the Guadiana estuary on their menu, as also evidenced by the high frequency of occurrence of *Panopeus* sp. in the metagenomic results (FO = 84.6%).

Conclusions

The population of the non-indigenous Atlantic blue crab *Callinectes sapidus* in the Guadiana estuary relied on their innate trophic plasticity and a diverse diet composed mainly of native animal species to support their biomass. Metagenomic data showed that the Atlantic blue crab exploited diverse food sources, confirming their opportunistic trait, and a marked carnivorous feeding behavior since fish, mollusks, barnacles, and multiple crustaceans, including shrimps and likely other decapods, were their main prey items. These feeding traits were clear with the dispersion of stable carbon and nitrogen isotopic ratios. Fish, shrimp, and oysters, which are key food web nodes in estuaries, were the primary sources of organic matter assimilated by the Atlantic blue crab. Their reliance on animal prey, also disclosed by their high trophic position, highlights the potential negative impacts upon key groups of the food web and, consequently, putative cascading effects through the ecosystem. We did not observe evidence of a widespread invasion meltdown process through trophic facilitation. As hypothesized, the Atlantic blue crab's trophic niche overlapped with two native crab species, namely the European green crab *Carcinus maenas* and African mud crab *Panopeus africanus*. Multiple factors certainly support the invasiveness of the Atlantic blue crab and are still to be evaluated, but assessments of seasonal feeding adaptations and migrations between ecosystems could help to shed light on its invasion success among so many zones of Europe. From the present results, it is evident that this species will continue to invade and thrive among its non-native range, as long as it finds a diverse array of food sources. And will be particularly successful when introduced in

ecosystems under pressure of anthropogenic stressors and riverine ecosystems with empty niches. A more integrative approach is needed, both at national level, but also in a transboundary manner, as many waterways are connected between different countries and the Atlantic blue crab has a high dispersal capability along coastal zones as well.

Acknowledgements The authors acknowledge the fundamental help in the field work towards acquiring the necessary Atlantic blue crabs in the Guadiana estuary provided by local fishermen Mr. Ricardo Gonçalves, and the assistance of Dr. Tânia Aires with the protocol of DNA extraction. We also acknowledge the important comments made by the reviewers towards improving this manuscript.

Author Contributions All authors contributed to the study conception and design. Sample preparation and data collection were performed by João Encarnação. Data analysis was performed by João Encarnação, Ester Dias, and Aschwin Engelen. The first draft of the manuscript was written by João Encarnação and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study, its respective field work and sample analysis, were partially funded by the ATLAZUL project (Poctep/Interreg 0755_ATLAZUL_6_E – Impulso da aliança litoral atlântica para o crescimento azul). This study received Portuguese national funds from FCT—Foundation for Science and Technology through projects UIDB/04326/2020 (DOI: 10.54499/UIDB/04326/2020), UIDP/04326/2020 (DOI: 10.54499/UIDP/04326/2020) and LA/P/0101/2020 (DOI: 10.54499/LA/P/0101/2020), UIDB/04423/2020 and UIDP/04423/2020. Author JE was supported by Ph.D. scholarships (SFRH/BD/140556/2018; COVID/BD/153280/2023) and ED by a research contract (DL57/2016/CP1344/CT0021) funded by Foundation for Science and Technology, Portugal.

Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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