Feeding habits and short-term mobility patterns of blue crab, *Callinectes sapidus*, across invaded habitats of the Ebro Delta subjected to contrasting salinity

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Abstract

The blue crab *Callinectes sapidus* was first observed in the Ebro Delta in 2012 and since then captures have increased exponentially up to over 2 tons per day, while its presence remains low in other Catalonian estuarine areas. Here, we use a stable isotope approach ($\delta^{15}N$ and $\delta^{13}C$) to explore the dietary habits of adult blue crab in four different invaded habitats –bays, coastal lagoons, rice field drainage channels, and the Ebro River– in order to assess the strength of bottom-up forces and identify risks for native and aquaculture species, as well as patterns of site fidelity (male individuals). Mixing models showed average contributions of 35.89% from organic matter in sediments, 34.25% from animal resources (fish, crustaceans, gastropods, and bivalves), and 23.84% from vegetal resources (aquatic plants and algae), although there were important differences across habitat sites. In sites where bivalves were available, they can represent up to ca. 75% of the diet, thus threatening natural banks and local oyster and mussel farms. The average estimated trophic position of blue crabs in those sites was only 2.8, which confirms an omnivorous behavior but also can be attributed to the fact that mollusks were rare in the majority of the areas sampled in the Ebro Delta. Crabs from the same habitat site exhibited very little isotopic variability, suggesting that they remain in those environments long enough to reflect local salinity conditions. Overall, our results suggest that blue crabs are likely using all locally available resources and remain in certain sites, even when preferred animal preys are scarce and low-quality items are the main dietary option.

Key words invasive species; salinity gradient; trophic position; stable isotopes; mixing models

Introduction

The interplay between top-down and bottom-up forces is a fundamental driver behind population dynamics and energy flows in marine ecosystems (review by Hunt and McKinnell 2006). The abundance and nutritional features of food resources may constitute a limiting factor for productivity across trophic levels (Frederiksen et al. 2006), whereas the effectiveness of predation control can depend on the availability of intact food webs (Byrnes et al. 2007). These contributions from the field of population biology are vital for controlling the spread of invasive species, as they enable the identification of the coupling between species traits and ecosystem processes where the implementation of management strategies could be most effective (e.g., introduction of turtles and duck varieties to enhance predation rates on invasive populations of apple snails; Teo 2001; Dong et al. 2012). Although a universal set of traits exclusive to successful invaders have not been identified, an ample tolerance for a wide range of environmental conditions, high reproductive output, lack of predation control, and generalist consumption habits are often behind successful establishments (review by Keller et al. 2011). Thus, it is imperative to understand the impact that invasive species have on food-web dynamics, including temporal effects on predator-prey interactions and the effects of possible feedbacks on the fitness of populations (Carlsson et al. 2009; Belgrad and Griffen 2016).

The blue crab, *Callinectes sapidus* Rathburn 1896, is a commercially important species supporting million-dollar fisheries across its native distribution in the western Atlantic Ocean, ranging from Maine to the Río de la Plata (Paolisso, 2007). In the Mediterranean Sea, the species was accidentally introduced in 1948 (Zenetos et al. 2018) and it is currently considered as one of the 100 worst invasive species despite its commercial value (Zenetos et al. 2005). Since its first observation in Greece, the species has successfully spread to other Mediterranean countries including, among others, Albania (Beqiraj and Kashta 2010), Italy (Mancinelli et al. 2017a), Croatia (Dulčić et al. 2011), and more recently Spain and the Southern coast of Portugal (López and Rodon 2018; Vasconcelos et al. 2019). The blue crab has altered the functioning of ecosystems and impacted native fisheries and biodiversity in its invaded Mediterranean habitats (Zenetos et al. 2005; Mancinelli et al. 2017a). Notably, the Ebro Delta appears to be especially favorable for the species, being the only area in the Spanish Mediterranean coast where abundances are large enough to sustain a targeted fishery management approach aimed at controlling the species, reaching captures of over 2 tons per day (López and Rodon 2018; López 2020).Such differences in abundance compared to surrounding regions suggest that bottom-up forces may also be an important driver of high populations in the Ebro Delta.

The Ebro Delta is one of the largest deltas (320 km^2) in the northwestern Mediterranean Basin and features a complex combination of interconnected marine, estuarine, and freshwater habitats, all of which have been colonized by *C. sapidus*. Rice fields occupy around 70% of the delta surface and release freshwater discharges to coastal lagoons and marine bays through a network of drainage channels from April to September (Prado et al. 2013). The remaining 30% of the land contains numerous wetland habitats hosting diverse and abundant wildlife, which are protected by the European Union and by the Ebro Delta Natural Park and Biosphere Reserve. Ebro Delta bays feature lower salinities than the open Mediterranean Sea (ca. 35‰ in Alfacs Bay and 30-33‰ in Fangar Bay) and their waters support important bivalve cultures of *Crassostrea gigas* and *Mytilus galloprovincialis* (Prado et al. 2020), as well as extensive seagrass habitats in the surrounding shallow margins (Pérez and Camp 1986). Coastal lagoons feature varying salinities (5.5 to 32‰) and habitat types with submerged aquatic vegetation (mostly *Ruppia cirrhosa* and *Potamogeton pectinatus*; Prado et al. 2013) depending on the degree of connectivity with the sea and seasonal freshwater discharges. In the lower Ebro River, a rapid proliferation of macrophytes on the riverbed, mainly *P. pectinatus*, following upstream damming and low suspended sediment (Ibáñez et al. 2012) has occurred in the last decades and provides habitat for a large number of fish and invertebrates including *C. sapidus* (López and Rodon 2018; López 2020).

Although the blue crab is an euryhaline and eurythermal species that can inhabit estuarine environments, coastal lagoons, and other aquatic habitats (Carr et al. 2004; Beqiraj and Kashta 2010), the Ebro Delta hosts a complex mosaic of habitats across salinity gradients that are seldom available at once. The species is regarded as a generalist consumer that feeds on a wide range of food resources including mollusks, fish, crustaceans, and vegetal and detrital material, depending on resource availability and the stage of ontogenetic development (Laughlin 1982). This is coupled with the female's capacity to travel great distances (>150 km) for reproduction and larval release; on the other hand males do not show pronounced migrations other than during the early juvenile stages when moving up-estuary (Turner et al. 2003; Carr et al. 2004). Hence, the blue crab has the potential to affect benthic communities at multiple trophic levels across distant habitat types (Carrozzo et al. 2014; Mancinelli et al. 2016). Stable isotope analyses offer a powerful tool to simultaneously assess mobility and dietary issues. Dual $\delta^{15}N$ and $\delta^{13}C$ signatures are commonly used to determine the relative contributions of different food sources to an animal's diet using mixing models (Post 2002; Stock et al. 2018). $\delta^{15}N$ signatures can also provide an accurate estimate of trophic position within the hierarchy of local food webs (Post 2002). Additionally, because $\delta^{13}C$ signatures are typically more depleted in freshwater marshes and phytoplankton (<-25 to -19‰) compared and more enriched C4 saltmarshes, submersed macrophytes, or microalgae in estuarine sites (-18 to -12‰) (Estiarte et al. 2008; Obrador and Pretus 2012), isotopic patterns across salinity gradients –more enriched at higher salinities– are effectively transmitted through the entire food web (e.g., Deegan and Garritt 1997; Vizzini et al. 2005; Obrador and Pretus 2012; Prado et al. 2014) and can provide useful evidence of habitat use.

In this context, the aim of the present study was to investigate the dietary habits of the blue crab across Ebro Delta habitats, including marine bays, coastal lagoons, rice fields drainage channels, and the Ebro River, which are subjected to contrasting salinity conditions and therefore, feature contrasting habitats and associated biodiversity. The absence of physical barriers potentially allows the for free mobility of individuals and the use of resources across the different Delta habitats. Local variability in the $\delta^{13}C$ signatures of adult blue crab males was first used to assess site fidelity and short-term mobility patterns. Then, Isotope mixing models ($\delta^{15}N$ and $\delta^{13}C$) were used to evaluate trophic patterns and resource contributions in investigated habitat sites. This information is key to understanding how the species has become so successfully stablished in the Ebro Delta, elucidating possible implications for the overall fitness of populations, and effectively protecting local biodiversity.

Methods

Study sites

The study was conducted in four habitats of the Ebro Delta where the blue crab is commonly observed and/or where there are well-stablished fishery practices targeting the species (López and Rodon 2018). These habitats include the Ebro River, coastal lagoons, bays, and rice fields drainage channels permanently connected to the bays. Three sites were haphazardly chosen for each habitat type (Fig. 1). In the Ebro River, sampling was conducted in Tortosa (R1), St. Jaume d'Enveja (R2), and Amposta (R1) located at different distances from the mouth. All three sites feature oligohaline conditions but differ in exposure to the salinity wedge (see Annex 1 for detailed salinity values). Among the three lagoons chosen for this study, the Encanyissada (L1) and the Tancada lagoons (L2), both feature polyhaline conditions whereas Les Olles lagoon (L3) has oligohaline conditions resulting from agricultural freshwater discharges from surrounding rice fields. Drainage channels located along the north coast of the Alfacs Bay featured varying salinity conditions, such as mesohaline in Sèquia Gran (DC1) and oligohaline in Sèquia de l'Ala (DC2), both of which experience agricultural freshwater runoff into the bay, and polyhaline in El Canalot, which connects the Tancada lagoon with the bay. All three bay sites under study were polyhaline: one in the norther coast of the Alfacs Bay (B1) close to local mussel and oyster farms, one in the Trabucador sand bar in the western side of Alfacs Bay(B2) hosting natural banks of bivalves, and one in the southern coast of the Fangar Bay close to the mouth of the bay (B3). Due to its smaller size and recurrent incoming discharges of agricultural freshwater, salinity was ca. 5 units lower in the Fangar Bay compared to Alfacs Bay (Annex 1).

Collection and preparation of samples

Blue crab sampling

Blue crabs were captured in June-July 2019 by deploying 35 × 35 × 40 cm crab traps made of 8 mm mesh and with 14 cm diameter openings -similar to those used by local fishermen-, and measures of salinity were taken at the moment of deployment using a YSI 6660 multiparametric probe equipped with a 650 MDS data logger. Traps were placed in the bottom of the water column in the morning and collected 24 h after. During the period of May to August very few females can be captured across Ebro Delta habitats (López 2020), and for this reason only males were used for this study in order to eliminate sex as a confounding factor.

After collection, crabs were put into nylon nets with plastic labels and transported back to the lab in an ice cooler. From each site, N= 3 male individuals of similar large adult size (237.5 to 404.2 g WW; Prado et al. 2020), which were confirmed to be the most abundant, were selected, and a piece of the right claw musculature was extracted for isotope analyses. Tissue samples were dried at 60 ^{o}C until constant weight and reduced to powder with a mortar and pestle, which were carefully rinsed with ethanol 99% after each sample to remove any remains.

Food sources sampling

Three two-meter long fyke nets with 80 cm hoop diameter and 5 mm mesh size were set in adjacent areas fringing the Ebro River, lagoons, bays, and drainage channels in order to capture as many as possible of the benthic species (i.e., fish and small crustaceans using the vegetation for sheltering during the day) that were locally available to blue crabs during the study period. In one rare instance in which aquatic plants were not available (DC1, Sèquia Gran), fyke nets were deployed in the same manner, oriented towards the border of the channel. Fyke nets were set in late afternoon and hauled the next morning, hence having an average soak time of 12 h. Captured individuals were transported to the laboratory in an icebox for further sorting and identification to the lowest possible taxonomical level. Once in the lab, three individuals of the most abundant taxa were selected in order to be representative of local fish and small crustacean communities and used as replicates in stable isotope analyses (see later). Individuals of similar sizes were also selected to minimize possible changes in the diet due to age-dependent energy requirements. Fish specimens were skinned for the extraction of the entire musculature and rinsed with ultrapure water to prevent contamination with other tissues. For small crustaceans (*Palaemon* spp.), the cephalothorax was removed, and the exoskeletons was peeled to obtain the tail muscle.

Bivalve samples in sites close to mussel (*Mytilus galloprovincialis*) and oyster (*Crassostrea gigas*) farms in Alfacs and Fangar Bay were obtained from the local producers. Additionally, all sites were sampled with a hand rake coupled to a net of 0.5 cm mesh size in order to capture benthic communities associated with either vegetated and unvegetated patches. The rake effectively recovered bivalves and gastropods. Other small crustaceans (e.g., amphipods, isopods) and polychaetes were not captured but they are not expected to be a significant food resource for large blue crab individuals (Laughlin 1982). In the Alfacs Bay, where there is a very large abundance of the small anemone *Paranemonia cinerea* attached to seagrass leaves of *Cymodocea nodosa*, some shoots were collected to obtain samples of this species. Once in the laboratory, gastropods and bivalves were removed from their shells and rinsed with ultrapure water to remove sediments, and anemones removed from seagrass shoots using a razor blade.

The more representative plant and algae species in each habitat site were collected by hand and kept within labeled plastic bags. The top 2 cm of the sediment layer at each site was collected for the sediment samples (N= 3 per site) using plastic containers. Once in the lab, algae samples that could not be identified in situ were sorted to species level under the stereomicroscope. All vegetation samples were dry weighted as previously indicated for animal samples. For sediments (including an inorganic fraction mixed with detrital material and bacterial/microalgae biofilms), samples were homogenized and split in two for separate examination of isotope signatures. Inorganic carbon in sediment samples was removed following the ISO 10694 normative. Briefly, 1.5 ml of HCl 4M was added per 0.5-1 g of sample within a beaker. After 4 h, samples were placed in a heater, washed with distilled water, and dried again using a heater and a vacuum pump.

All animal and vegetal food sources were classified into 5 trophic groups: fish, prawns, bivalves, gastropods, anemones, plants, and algae. For each animal and vegetal taxon, a minimum finding of three individuals/vegetation patches was required for inclusion in a given group and assumed as an indicator of medium to high availability for blue crab consumption in the natural environment (no further quantitative assessment of prey items was conducted during the study). Taxa with the adequate number of individuals for replication were dried at 60^{o}C, reduced to powder, and weighed. For each site, an equal quantity of a given taxon was mixed together with other taxa belonging to the same trophic group, to obtain a total of N=3 replicates. Some trophic groups such as anemones were composed of just one species. In the Ebro River, top predators such as the catfish *Silurus glanis* (N= 1) and the European bass (*Dicentrarchus labrax*) (N= 3) were also captured and analyzed separately for SIA to assess potential predation on the blue crab. It is worth noting that our results are temporally constricted to an estimate isotopic turn-over in blue crab tissues of ca. 3 months (McCann and Jensen 2018) during the spring-summer season. Overall, the applied methodology was considered as a useful, cost-effective approach for an initial screening of the blue crab's dietary habits in invaded habitats of the Ebro Delta.

Stable isotope analyses

Samples were analyzed with a PDZ Europa 20-20 mass spectrometer connected to a PDZ Europa ANCA-GSL elemental analyzer at the UC Davis Stable Isotope Facility. Isotope ratios in samples were calculated from linear calibration curves constructed with standard reference materials of known composition and a blank correction. The difference in isotopic composition between the sample and reference material is determined by the equation:

$$\delta_{sample-standard} = [(R_{sample} - R_{standard})/\ R_{standard}]\ \mathrm{x}\ 1000$$

where R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample; $R_{standard}$ is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the calibration material and $\delta_{sample\text{-}standard}$ is the difference in isotopic composition of the sample relative to that of the reference (Vienna Pee Dee Belemnite for carbon, and atmospheric nitrogen for nitrogen). Experimental precision (based on the standard deviation of replicates of an atropine standard and/or IAEA or USGS intercomparison materials) was considered to be adequately high (±0.12 ‰ for $\delta^{13}C$ and ±0.09 ‰ for $\delta^{15}N$).

The possible variability in $\delta^{13}C$ signatures induced by uneven storage of lipids (typically depleted in ^{13}C) across tissues was corrected using the equations provided by Post et al. (2007). The threshold for the application of this correction was an increase in corrected signatures of at least 0.1‰ (Prado et al. 2012). $\delta^{13}C$ signatures of blue crab, small crustaceans, and fish were normalized using the relationship with C:N ratios indicated for aquatic animals (corrected $\delta^{13}C= \delta^{13}C - 3.32 + 0.99 \times C{:}N$), and macrophyte and algae with that indicated for plants (corrected $\delta^{13}C= \delta^{13}C + 1.25 – 0.00 \times C{:}N$). No correction values were used for riverine and marine sediments since the lipid content was assumed to be very low compared to living organisms. Elemental contents (C and N) in blue crab tissues and diets were given in total µg per sample and expressed as C:N.

Data analyses

Differences in the composition of $\delta^{13}C$ (normalized) and $\delta^{15}N$ signatures, and C:N ratios of blue crabs and potential food resources (including fish, small crustaceans, plants, algae, and sediments) across habitat sites featuring distinctive salinities and nutrient loads associated to agricultural discharges and/ or the salinity wedge effects in the Ebro River were investigated with a two-way nested ANOVA (Habitat = 4 levels, fixed factor, and Site = 3 levels, random factor) and SNK post-hoc analysis. For other resources that were only present at certain habitats (bivalves, gastropods, and anemones), the presence of significant differences in $\delta^{13}C$ (normalized), $\delta^{15}N$ and C:N ratios was assessed with a one-way ANOVA (Site, random factor).

For all ANOVAs, normality (Chi-square test) and homogeneity of variances (Cochran's test) were tested, and when necessary (fish and small prawns), data was sineh and log transformed to meet these assumptions. All ANOVA were performed using Statistica v.12 software. The possible relationships between $\delta^{13}C$ (normalized) and $\delta^{15}N$, between C:N ratios and isotopic signatures and between isotopic signatures and local salinities was assessed using the Pearson's correlation analysis.

Isotope mixing models

To estimate the proportion of each locally available resource group in the diet of blue crab males we used the Bayesian MixSIAR model (V.3.1) implemented as an open-source R package (Stock et al. 2018) using a generalist prior distribution. Given that there were important differences in salinity within habitats that could strongly influence $\delta^{13}C$ signatures, models were conducted separately for each site (n = 3 blue crab samples).

Prior to the development of mixing models, an isospace plot was produced to visually inspect that consumer $\delta^{15}N$ and $\delta^{13}C$ values fell within the prey polygon in isospace (Stock and Semmens 2016). MixSIAR uses stable isotope signatures with their standard error (SE), and tissue-diet discrimination factors as input variables to estimate the probability distributions of each food item to a mixture within 95% confidence intervals and accounts for uncertainty associated with multiple sources. The estimated median contribution (i.e., the 50% quartile) represents the median source contribution value for each source and is usually given along with SD and/or confidence intervals for comparative purposes.

For food resources, the most abundant species at each study site were pooled in equal quantities (both number of individuals and mass) during sample preparation and used as a proxy of the value in the trophic group (see Annex 1). For fractionation, we applied the 0.4‰ $\Delta^{13}C$ and the 3.4‰ $\Delta^{15}N$ values derived from literature data on non-herbivorous aquatic consumers (Post 2002), as they have been largely used in other stable isotope works in invaded Mediterranean regions (Mancinelli et al. 2013, 2016, 2017b; Carrozzo et al. 2014; Aslan and Polito 2021). Although a recent study by McCann and Jensen (2018) provided experimental values of $\Delta^{13}C$ and $\Delta^{15}N$ for blue crab (0.6 ± 0.7‰ and 1.6 ± 0.6‰, respectively), dietary items were restricted to feeding with clams and black sea bass, and do not reflect the diversity of diets available for blue crab in the field. Since the quality of the diet in terms of protein content and assimilability has been shown to have a strong influence on fractionation (McCutchan et al. 2003; Prado et al. 2012), generic values were still preferred. Runs were also conducted using the 1.3‰ SD of $\delta^{13}C$ and 1‰ $\delta^{15}N$ indicated by Post (2002).

The Markov Chain Monte Carlo (MCMM) in MixSiar, a method for obtaining information and estimating posterior distributions in Bayesian inference, was set as follows: chain length: 100,000; burn-in: 50,000; thin: 50; and number of chains: 3. With these settings, convergence conditions for the Gelman–Rubin diagnostic was <1.05 in all cases; and in the Geweke diagnostic testing for the equality of the means in the first and last part of a Markov chain the number of variables falling outside the ±1.96 range was < 5 % for the three chains.

Estimates of trophic position

In order to confirm patterns obtained in previous mixing models, the trophic position (TP hereafter) of blue crab was calculated, when possible (see later), according to the methodology and equation proposed by Mancinelli et al. (2016, 2017b):

$$TP_{\delta 15N} = (\delta^{15}N_{Consumer} - \delta^{15}N_{Baseline})/\,\Delta^{15}N + \lambda$$

Where $\delta^{15}N$ Consumer is the nitrogen isotopic signature of the blue crab, $\delta^{15}N$ Baseline is the mean isotopic signature of available filter feeder bivalves, which are expected to be a preferred prey (Laughlin 1982) and a well-known primary consumer (primary producers= trophic level 1, primary consumers= trophic level 2, and so on). $\Delta^{15}N$ is the $\delta^{15}N$ isotopic enrichment for the blue crab, and λ the trophic level of the baseline indicator, respectively. Since bivalves were only present in bays and one of the coastal lagoons, TP was only estimated for those sites. Bivalves, and particularly the Mediterranean mussel, have been commonly used as food web baseline in previous stable isotope studies (Mancinelli et al. 2013, 2016, 2017b; Carrozzo et al. 2014; Aslan and Polito 2021) and therefore allow for a comparative approach.

Results

Patterns of $\delta^{13}C$, $\delta^{15}N$, and C:N ratios

***$\delta^{13}C$*:** At each study site, variability in $\delta^{13}C$ signatures of blue crabs was generally small, although higher SE were observed in drainage channels (average of 1.1) compared to the other habitats (0.27, 0.30, and 0.47, respectively in bays, Ebro River, and coastal lagoons).

Significant differences were found across habitats and among study sites in all investigated trophic groups (Table 1A; Fig. 2-3). Values of $\delta^{13}C$ were more enriched in bays, intermediate in drainage channels and lagoons, and more depleted in the Ebro River, consistent with the salinity gradient (Fig. 2-3). There were also important differences among sites within the same habitat type, which also reflected exposure to contrasting salinities (Annex 1). Overall, $\delta^{13}C$ signatures across habitats and sites ranged between -15.61 to -25.77 for blue crab, -16.35 to -28.32 for fish, -15.03 to -26.75 for prawns, -6.48 to -23.52 for plants, -8.20 to -26.45 for algae, and -8 to -26.67 for sediments.

Significant associations between $\delta^{13}C$ and salinity were found across the food-web, including blue crab ($R= 0.95$; $p<0.001$), small prawns ($R= 0.84$; $p<0.005$), fish ($R= 0.87$; $p<0.001$), plants ($R= 0.95$; $p<0.001$), algae ($R= 0.94$; $p<0.001$), and sediments ($R= 0.70$; $p< 0.05$). Other abundant items at certain habitat sites such as anemones (df=1, MS= 0.04, $F= 28.89$; $p<0.01$), bivalves (df= 3, MS= 9.20, $F= 42.67$; $p<0.001$), and gastropods (df=1, MS= 256.01, $F= 285.37$; $p<0.001$), also showed significant spatial differences with more enriched signatures at higher salinity sites. Ranges of variation for these groups were -16.95 to -19.90 for bivalves, -10.59 to -23 for gastropods, and -14.43 to -14.61 for anemones.

***$\delta^{15}N$*:** There were also significant effects of habitat and site for all investigated trophic groups (Table 1B; Fig. 2-3). However, patterns of habitat and site variation were not consistent across trophic groups (Table 1B). Ranges of $\delta^{15}N$ signatures across habitats and sites were 11.55 to 15.13

for blue crab, 13.75 to 18.28 for fish, 11.93 to 15.98 for prawns, 8.34 to 15.42 for plants, 8.62 to 11.88 for algae, and 4.75 to 8.32 for sediments.

δ^{15}N values of blue crab and fish were inversely related to salinity (*R*= -0.86; *p*<0.0001, and *R*= -0.63; *p*<0.05) and δ^{13}C signatures (*R*= -0.87; *p*<0.001, and *R*= -0.66; *p*<0.05), respectively. Sediments were only significantly associated with salinity (*R*= 0.81; *p*<0.005), and the other trophic groups did not display any significant association with either salinity or δ^{13}C values. For resources that were only present at certain habitat sites, significant spatial differences were detected for anemones in bay sites (df= 1, MS= 1.01, *F*= 34.02, *p*<0.005), and bivalves in bays and in the Tancada lagoon (df= 3, MS= 2.63, *F*= 63.37, *p*<0.0001) but not for gastropods (df=1, MS= 12.6, *F*= 5.72, *p*>0.05). Variability in δ^{15}N for these groups was 8.11 to 10.37 for bivalves, 9.87 to 12.77 for gastropods, and 11.26 to 12.08 for anemones. Only the anemone group displayed a significant relationship with δ^{13}C signatures (*R*= 0.84; *p*<0.05).

C:N ratios: There were significant effects of habitat and site in the C:N ratios of investigated groups of food resources, but not in the blue crab (Table 1C, Fig. 2-3). As with δ^{15}N, no coherent pattern of variation was observed across significant trophic groups (Table 1C). Variability ranges for C:N across habitats and sites were narrow for blue crab (3.19 to 3.43) and wider for potential food resources, particularly non-animal groups (3.23 to 4.46 for fish, 3.24 to 4.33 for prawns, 8.75 to 14.62 for plants, 8.18 to 14.62 for algae, and 5.07 to 10.25 for sediments).

Significant associations between C:N ratios and salinity and/ or δ^{13}C signatures were detected for prawns (R_{Sal}= 0.77; *p*< 0.01; R_{13C}= 0.84; *p*<0.01), algae (R_{Sal}= 0.81; *p*<0.005; R_{13C}= 0.77; *p*<0.005), plants (R_{13C}= 0.67; *p*<0.05), and sediments (R_{Sal}= 0.74; *p*< 0.01). Prawns also showed a significant association between C:N ratios and δ^{15}N (*R*= 0.72; *p*< 0.05). For food resources only present at specific sites, significant site differences were detected for bivalves (df= 3, MS= 0.28, *F*= 8.73, *p*< 0.01) and gastropods (df=1, MS= 0.66, *F*= 11.96, *p*< 0.05) but not for anemones (df=1, MS= 0.0004, *F*= 0.03, *p*> 0.05). Ranges of C:N ratios variations for these groups was 4.24 to 4.85 bivalves, 4 to 4.67 for gastropods, and only 5.01 to 5.03 for anemones. Both gastropods and bivalves displayed significant relationships between C:N ratios and $\delta^{13}C$ signatures ($R = 0.85$; $p < 0.05$, and $R = 0.60$; $p < 0.05$, respectively).

MixSIAR models

Model results were obtained separately for each habitat site to avoid confounding by variability in salinity within habitats (Table 2). In the Ebro River, crabs showed a relatively even consumption of available resources in the most upstream site (R1), although with dominance of benthic primary producers including both plants and algae (44.9%). In downstream sites (R2 and R3), the consumption of sediments increased considerably (from 17.6 to ca. 53-56%) and constituted the main food item. Other relatively important resources downstream were fish and algae (9.8 to 18.8%, respectively), although with important variation.

In coastal lagoons, those subjected to polyhaline conditions (L1 and L2) also showed a high contribution of sediments to the diet (32.7 to 40%). In L1, there was also an important contribution of plants and algae (42.4%), whereas in L2, fish and bivalves were more important (a combined 35.2%). In the oligohaline lagoon (L3), the relative weight of all resources was more equal (from 19.5 to 31.4%) but contributions tended to be higher from fish and lower from sediments.

In drainage channels, sediments were consistently the most consumed item (41.20 to 58.30% of diet). Vegetal resources were also important in DC1 and DC2, with overall contributions of 23.50 and 33.7%, but had much lower weight (8.7%) in DC3. Animal resources (fish and small prawns or gastropods) ranged from 12.8 in DC1 to ca. 27% in DC2 and DC3.

In bay sites B1 (adjacent to mussel and oyster farms) and B2 (natural bank area), bivalves were the most consumed resource (74.8 and 58.2%, respectively), followed by sediments (7.6 to 19.5%), with low contributions from other items. In contrast, a lower bivalve contribution (18.7%) was detected in B3, closer to the mouth of the bay. Instead, sediments were the most consumed item at this site with a contribution of 41.8%.

Food web trophic structure and blue crab trophic position

$\delta^{13}C$ and $\delta^{15}N$ signatures of the blue crab were often similar to those of other locally available crustaceans such as small prawns and showed a trophic position (TP) immediately below fish (Fig. 4). Where filter feeding bivalves were present (i.e., in the three bay sites and L2) and could be used as baseline indicator for the estimation of TP, blue crab showed values from 2.91 to 3.12 in bays and 3.21 in Tancada lagoon (L2). In the Ebro River, predatory fish showed the highest $\delta^{15}N$ signatures and $\Delta^{15}N$ fractionation values of 3.07 and 3.3 for *D. labrax* and *S. glanis*, respectively, that could be consistent with predation on blue crab (Fig. 4).

In the three bay sites and in the Tancada lagoon (L2), where bivalves were available for crab consumption, values of TP were coherent with former MixSIAR results. The highest TP (mean ± SE) of 3.11 ± 0.008 was obtained in B1 (northern Alfacs Bay) featuring the largest bivalve and animal consumption (74.8 and 84%, respectively). This was followed by a similar value in B2 (Trabucador) with 3.01 ± 0.06, which featured contributions of 58.2 and 67.5% from bivalves and total animals, respectively, according to mixing models. Similar lower values (TP of 2.67 ± 0.04 and 2.64 ± 0.20) were obtained in B3 (Fangar Bay) and L2, featuring contributions of 18.7 and 18.5 % of bivalves, and 35 and 40.6% total animals, respectively.

Discussion

Some decapod crustaceans such as majid and grapsid crabs are generally regarded as 'herbivorous,' having diets that are comprised of at most 50% animal material (Hines 1982; Kennish

1996; Lohrer and Whitlatch 1997). Our results show that the blue crab's total animal consumption values are below that limit in all habitat sites excepting two bay areas in which bivalve species are more abundant (Ramón et al. 2005) and the trophic behavior could be considered more skewed towards 'carnivore'. In fact, although ingestion of animal items such as mollusks and crustaceans is expected to increase in large size classes such as those used in the present work (Laughlin 1982; Rosas et al. 1994), individuals in the Ebro Delta seem to incorporate large quantities of detrital material from local sediments (an average of 35.9%; this study) or from vegetation (Gil Fernández 2018) depending on the site. High predation rates on different mollusks from local marine and freshwater ecosystems of the Ebro Delta were also experimentally demonstrated for individuals of similar large size (Prado et al. 2020), suggesting that overall patterns of field consumption are strongly shaped by local availability of food items. Our results showed a mean blue crab trophic position of ca. 2.8 in bays and lagoons (coherent with an average total animal contribution of ca. 57%), so lower values are expected in other Ebro Delta sites where bivalves were not available. Yet this value is considerably lower than the mean TP of 3.73 estimated by Mancinelli et al. (2016) in the Parila Lagoon (Adriatic Sea) using the same approach. According to a review by Aslan and Polito (2021), TP in invaded Mediterranean locations can range from ca. 2.2 to 4.5 and may be partly due to variability in resource exploitation. For instance, Mancinelli et al. (2013, 2017b) found comparatively lower TP in some study sites and hypothesized possible resource limitations and feeding on lower trophic levels such as plant material as potential explanations.

Animal contributions to blue crab diets and associated TP might be partly influenced by the timing of the invasion, which in the case of the Ebro Delta dates back to 2012 (Castejón and Guerao 2013). In the Ebro River, an important decrease of invasive species including apple snail (*Pomacea maculata*) and Asian clam (*Corbicula fluminea*) has been observed in the last few years (Gil Fernández 2018; Gallego et al. 2019), concurring with increasing abundances of the blue crab.

Predation of apple snail by the glossy ibis (*Plegadis falcinellus*) has been also reported in rice fields (Bertolero and Navarro 2018) and might contribute to observed trends. Both mollusk species, particularly the apple snail, have been shown to be readily consumed by blue crabs (Prado et al. 2020) and are currently so rare in the lower Ebro River that no individuals could be found for stable isotope analyses in the present study in riverine areas hosting blue crabs. A similar situation could have occurred in coastal lagoons, where natural banks of bivalves such as *Cerastoderma glaucum* and *Scrobicularia plana* were abundant (Prado et al. 2014), but are currently very scarce (P. Prado, pers. observation). Also, in the Fangar Bay, the hatchery production of the Japanese clam *Ruditapes philippinarum* was completely lost due to high predation losses by the blue crab (Vongole 2000 S.L., pers. communication from the manager, P. López to P. Prado) evidencing that the dietary contribution from animal items increases when preferred quality prey is available. Hence, a decrease in animal contributions and in TP might have occurred in these habitats compared to earlier invasion periods when those prey items were more available.

At present, the highest abundances of bivalves in the Ebro Delta are located in natural banks along the Trabucador sand bar, and in suspended cultures of the Mediterranean mussel (*Mytilus galloprovincialis*) and the Pacific oyster (*Crassostrea gigas*) (Ramón et al. 2005), which were the two points of the Alfacs Bay exhibiting higher consumption. Our results confirm those reported by Prado et al. (2020), who found that excepting oysters, most local mollusks might be at risk due the lack of an effective escape size from predation. Besides, differences in the mobility of available prey types (sessile and slow-moving bivalves or gastropods vs. fast-moving fish and crustaceans) and habitat complexity could also influence predation rates and shape the use of resources across investigated habitats (Florido and Sanchez 2010), thus accounting for decreased predation in drainage channels where low mobility species were scarce. This effect might be also amplified by the mediating influence of contaminated environments on predator behavior. Reichmuth et al. (2009) found that blue crabs from polluted environments had reduced ability to capture active prey (conspecific juveniles and adult mummichogs) and although they consumed less active preys (ribbed mussels and fiddler crabs), this resulted in larger amounts of algae, plant material, detritus, and sediments within stomach contents (ca. 60%). Although this study did not identify specific contaminants, the Ebro Delta has been deeply influenced by rice cultivation and associated agrochemicals for the last 150 years (Brossa et al., 2005; Prado et al. 2013), so the possible effect of contaminants on predation success cannot be excluded, particularly in agricultural drainage channels where sediment consumption was high.

Poor nutritional conditions may have many potential negative consequences for long term population traits including survival and growth rates, age at first maturity, fecundity, and reproductive outputs (see review by Metcalfe and Monaghan 2001). Since animal prey such as bivalves, crustaceans, and fish, usually feature higher protein and energy content than vegetal or detrital resources (Renaud and Luong-Van 2006; Hantoush et al. 2015; Venugopal and Gopakumar 2017), decreasing contributions from animal sources to blue crab diets could have negative effects on the fitness of the population in the long-term. For instance, in experiments conducted with the sea urchin, *Lytechinus variegatus*, individuals fed diets with higher protein contents featured the largest test diameters, total wet weights, production efficiencies, and gonad production efficiencies (Hammer et al. 2012; Prado et al. 2012), which might partially account for reported population explosions (Rose et al. 1999). Similarly, blue crabs exclusively fed with an animal diet showed lower mortality, along with enhanced long-term energy stores (hepatopancreas lipid content) and reproductive efforts as well as decreased aggressiveness compared to individuals fed seaweed diets (Belgrad and Griffen 2016). However, overall captures of blue crab keep still increasing (over 2 daily tons; López 2020), so the required limit for the presence of bottom-up regulation of populations effects remains uncertain. Yet, the bulk of commercial fisheries captures were recorded in Alfacs

Bay (51% of the total), followed by Fangar Bay and Ebro River (19 and 18%, respectively), Coastal lagoons (mostly Encanyissada and Tancada; 9%), and outer sea in front of the estuary (3%) (López 2020). Therefore, feeding in higher proportion from animal prey in different areas of the Alfacs Bay might partly account for observed patterns. Aside selective fisheries, our δ^{15}N results for of fish predators suggest the possible consumption of blue crab by *D. labrax* and *S. glanis*, although their abundance may be insufficient to exert an efficient top-down control (Vila-Martínez et al. 2019).

Differences in salinity showed no evidence for influence on feeding habits but had an enormous repercussion on patterns of δ^{13}C across habitats in all trophic groups. Overall, more enriched values were observed in higher salinity environments such as bays, the Encanyissada and Tancada lagoons (L1 and L2) and El Canalot drainage channel (DC3), and more depleted in freshwater/low-salinity sites of the Ebro River, Les Olles lagoon (L3) and the Sèquia Gran and Sèquia de l'Ala agricultural drainage channels (DC 1 and DC2). Higher δ^{13}C values at higher salinities is partly attributed to the supply of δ^{13}C-enriched marine phytoplankton (approx. -19 to -20‰; Dehairs et al. 2000), compared to that of rivers (-30 to -25‰; Anderson and Arthur 1983; Boutton 1991) and to the transmission of these patterns of zooplankton (Giorgio and France 1996). In addition, the main macrophyte species in Ebro Delta Bay, the seagrass *Cymodocea nodosa*, is one the most enriched sources of vegetal carbon (Vizzini and Mazzola 2003) and generates depleted POM that is partly incorporated *in situ* and partly exported in various degrees to adjacent coastal lagoons, depending on available connections (Prado et al. 2014). In saltier coastal lagoons, the dominant macrophyte species, *Ruppia chirrosa*, also features high δ^{13}C signatures (Chappuis et al. 2017), although comparatively lower than *C. nodosa* (-14 to -10.67‰ vs. -10 to -6.5‰, respectively). Additionally, the C4 plant, *Spartina versicolor*, is also common in areas adjacent to bays and coastal lagoons (Menéndez and Sanmartí 2007) and provides an additional source of enriched δ^{13}C. Conversely, C3 freshwater plants, including the most common species in the Ebro River (*Potamogeton* spp., *Myriophyllum spicatum*, and *Vallisneria spiralis*) are more depleted in δ^{13}C, with values typically ranging from <-25 to -19‰ (Estiarte et al. 2008; Obrador and Pretus 2012). An association between δ^{15}N and/or δ^{13}C and salinity patterns was observed only for some species and trophic groups – namely blue crab, fish, and sediments – suggesting than other factors such as effects of organic wastewater (usually enriched in δ^{15}N by 3-5‰) could be obscuring basal patterns (Vizzini and Mazzola 2004; Vizzini et al. 2005). Nitrogen-rich agricultural freshwater from rice cultivation is discharged into bays and coastal lagoon habitats through a network of drainage channels (Prado et al. 2013; Prado et al. 2014), but the site, volume and discharge frequency follow uneven agricultural practices that prevent the finding of coherent patterns across trophic groups. No coherent pattern of variation along the salinity gradient could be detected for C:N ratios across considered trophic groups, suggesting that they were not appropriate indicators of local trophic fluxes.

Male crabs captured at a given habitat site featured reduced isotopic variability, which varied according to local sanity conditions, suggesting restricted mobility for a period of time long enough to incorporate local signatures. McCann and Jensen (2018) assessed Δ^{13}C and Δ^{15}N in blue crab fed clams or black sea bass over a 3-month period and found that stable isotopes turnover could be in the order of days to months depending on the examined tissue, which is coherent with findings in other studies (Tieszen et al. 1983; Cerling et al. 2007). Female blue crabs are known to migrate 10s to 100s of km in spring to fall, from lower salinity, estuarine regions to high salinity areas near the ocean to release larvae after the mating season (Turner et al., 2003; Carr et al. 2004). The residence time within native estuaries of the female spawning stock has been estimated to be ca. 3 weeks (Seitz et al. 2001) while males are expected to be more sedentary. Ramach et al. (2009) conducted capture-and-recapture experiments in a high-salinity embayment near Beaufort, NC and found residence times of up to 92 d in males, although with large variability that could be attributed to size differences. To our knowledge, no research on site fidelity related to the defense of a habitat or territory has been conducted for adult males, although this type of behavior is common in other crab species (e.g., Barber and Cobb 2009). Territorial behavior could account for similar ranges of SD in $\delta^{13}C$ signatures across coastal lagoons, bays, and Ebro River habitats. Yet, observed SD were ca. 3-4 times higher in agricultural drainage channels featuring a lower diversity of food resources compared to other habitats, which suggests that the abundance and diversity of food resources could be also a factor conditioning residence time across habitats.

Conclusions

The use of stable isotopes has been reported to have a number of limitations related to the physiology and behavior or study organisms, environmental gradients, sample processing and/or analysis, and data analysis and interpretation that may compromise the validity of trophic evaluations (review by Shipley and Matich 2020). In our study, two main sources of uncertainty are identified. First, the use of low consumer sample sizes in Bayesian mixing models (N= 3 per site) has been suggested to enhance dispersion in estimates of dietary contributions (Brett et al. 2014), although it might be compensated by low within-site variability in blue crab stable isotope signatures. Second, the lack of adequate knowledge of fractionation under different quality diets may also promote isotopic variability and patterns could not be contrasted with other techniques for dietary evaluation such as stomach content analysis (Prado et al. 2012; Petta et al. 2020; Shipley and Matich 2020).

Despite these limitations, our work suggests that the current diet of blue crab in Ebro Delta habitats includes a large component of detrital and vegetal material (mean of ca. 36 and 24%, respectively) and only about 34% contribution from animal resources, which may have decreased overtime. Only at a few sites the available trophic resources might still provide an optimal fitness to populations, while in other sites, initially abundant resources during early invasion periods are no longer available (Gil Fernández 2018; Gallego et al. 2019; P. Prado, pers. observation). Hence, the coming years maybe critical to determine the possible effect of combined top-down (ongoing fisheries) and bottom-up pressures (resource constraints decreasing the performance of populations) in controlling the invasion of the species. Unfortunately, that process may involve an additional depletion of remaining natural banks of bivalves and aquaculture farms, particularly mussels (Prado et al. 2020).

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A)	**δ^{13}C**				**B)**	**δ^{15}N**				**C)**	**C:N**			
Blue crab	**df**	**MS**	**F**	***p***	**Fish**	**df**	**MS**	**F**	***p***	**Fish**	**df**	**MS**	**F**	***p***
Habitat	3	69.60	55.46	**0.0000**	Habitat	3	6.29	10.76	**0.0001**	Habitat	3	0.007	0.65	0.5878
Site (H)	8	28.75	22.91	**0.0000**	Site (H)	8	2.41	4.13	**0.0032**	Site (H)	8	0.015	1.40	0.2483
Error	24	1.25			Error	24	0.58			Error	24	0.010		
SNK (H)	B> L> DC > R				SNK (H)	R> DC> L= B				SNK (H)	NS			
Fish	**df**	**MS**	**F**	***p***	**Fish**	**df**	**MS**	**F**	***p***	**Fish**	**df**	**MS**	**F**	***p***
Habitat	3	0.162	132.49	**0.0000**	Habitat	3	0.015	94.3	**0.0000**	Habitat	3	0.019	139.81	**0.0000**
Site (H)	8	0.053	43.38	**0.0000**	Site (H)	8	0.014	86.3	**0.0000**	Site (H)	8	0.002	18.26	**0.0000**
Error	24	0.001			Error	24	0.001			Error	24	0.001		
SNK (H)	B> DC> L> R				SNK (H)	L= R> DC> B				SNK (H)	DC> R= L> B			
Prawns	**df**	**MS**	**F**	***p***	**Prawns**	**df**	**MS**	**F**	***p***	**Prawns**	**df**	**MS**	**F**	***p***
Habitat	3	0.211	130.93	**0.0000**	Habitat	3	0.024	24.85	**0.0000**	Habitat	3	0.0239	737.8	**0.0000**
Site (H)	6	0.026	16.44	**0.0000**	Site (H)	6	0.001	1.95	0.1199	Site (H)	6	0.0021	65.3	**0.0000**
Error	21	0.001			Error	21	0.001			Error	21	0.0002		
SNK (H)	B> L> DC> R				SNK (H)	R> B= DC= L				SNK (H)	R> L= DC= B			
Bivalves	**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***
Site	3	9.20	42.6	**0.0000**	Site	3	2.65	135.9	**0.0000**	Site	3	0.284	8.73	**0.0000**
Error	8	0.21			Error	8	0.02			Error	8	0.032		
SNK	L2= B2> B3= B1				SNK (H)	L2> B1> B3> B2				SNK (H)	B3= B1≥ B2= L2			
Plants	**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***
Habitat	3	286.2	2278.49	**0.0000**	Habitat	3	13.25	248.01	**0.0000**	Habitat	3	9.42	10.35	**0.0001**
Site (H)	7	152.8	1216.47	**0.0000**	Site (H)	7	13.31	249.16	**0.0000**	Site (H)	7	13.42	14.76	**0.0000**
Error	22	0.12			Error	22	0.05			Error	22	0.90		
SNK (H)	B> L> DC> R				SNK (H)	DC> R> L= B				SNK (H)	B= R> L= DC			
Algae	**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***
Habitat	3	222.4	434.9	**0.0000**	Habitat	3	10.93	16.90	**0.0000**	Habitat	3	18.30	16.70	**0.0000**
Site (H)	8	70.7	138.4	**0.0000**	Site (H)	8	3.38	5.23	**0.0007**	Site (H)	8	12.91	11.79	**0.0000**
Error	24	0.51			Error	24	0.64			Error	24	1.09		
SNK (H)	B> DC= L> R				SNK (H)	DC= R= B> L				SNK (H)	B> L=R= DC			
Sediments	**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***		**df**	**MS**	**F**	***p***
Habitat	3	130.9	70.1	**0.0000**	Habitat	3	6.89	4.75	**0.0096**	Habitat	3	19.42	7.45	**0.0010**
Site (H)	8	72.1	38.59	**0.0000**	Site (H)	8	1.27	0.88	**0.5473**	Site (H)	8	5.32	2.04	0.0845
Error	24	1.87			Error	24	1.45			Error	24	2.60		
SNK (H)	B> L> DC= R				SNK (H)	R= DC= L≥ B				SNK (H)	R> L= B= DC			

Table 1 Two-way nested analysis of variance (ANOVA) and post-hoc Student-Newman-Keuls (SNK) for: a) lipid-corrected $\delta^{13}C$ and, b) $\delta^{15}N$ signatures; and c) C:N ratios of blue crab and potential diets (fish, small prawns, bivalves, plants, algae, and sediments) across the different study habitats (DC= Discharge Channel; B= Bay; R= River Ebro; and L= Lagoons). For bivalves, which where only found in bay sites and one of the lagoons, one-way ANOVA was conducted to test differences among the four sites. Gastropods and anemones were only abundant in two sites (DC2 and B2, and B1 and B2, respectively) and results are indicated in the results section. Significant values are indicated in **bold**.

Table 2 MixSir results (median and SD) showing relative contributions of food resources to blue crab diets across habitats and sites. We used the $\Delta^{15}N$ of 3.4 and a $\Delta^{13}C$ of 0.4 given for non-herbivorous aquatic consumers (Post et al. 2002), and previously used in blue crab (Mancinelli et al. 2016, 2017). Not Available (NA) food items at the site are indicated.

Habitat/Site	**Fish**	**Small Shrimp**	**Gastropods**	**Bivalves**	**Anemones**	**Plants**	**Algae**	**Sediment**
R1	0.173 (0.138)	0.171 (0.138)	NA	NA	NA	0.245 (0.146)	0.204 (0.16)	0.176 (0.13)
R2	0.184 (0.136)	0.072 (0.098)	NA	NA	NA	0.045 (0.066)	0.092 (0.115)	0.528 (0.146)
R3	0.013 (0.021)	0.014 (0.021)	NA	NA	NA	0.069 (0.124)	0.284 (0.103)	0.559 (0.134)
L1	0.091 (0.084)	0.127 (0.121)	NA	NA	NA	0.157 (0.146)	0.267 (0.153)	0.327 (0.08)
L2	0.167 (0.165)	0.054 (0.085)	NA	0.185 (0.152)	NA	0.037 (0.024)	0.049 (0.046)	0.400 (0.121)
L3	0.314 (0.048)	NA	NA	NA	NA	0.205 (0.155)	0.260 (0.150)	0.195 (0.143)
DC1	0.047 (0.131)	0.081 (0.113)	NA	NA	NA	NA	0.337 (0.118)	0.412 (0.163)
DC2	0.158 (0.141)	NA	0.120 (0.122)	NA	NA	0.13 (0.104)	0.105 (0.087)	0.438 (0.071)
DC3	0.110 (0.125)	0.164 (0.161)	NA	NA	NA	0.044 (0.050)	0.043 (0.049)	0.583 (0.096)
B1	0.042 (0.045)	0.032 (0.036)	NA	0.748 (0.096)	0.018 (0.046)	0.011 (0.014)	0.027 (0.032)	0.076 (0.061)
B2	0.015 (0.027)	0.014 (0.030)	0.036 (0.055)	0.582 (0.067)	0.028 (0.032)	0.040 (0.043)	0.030 (0.041)	0.195 (0.103)
B3	0.087 (0.062)	0.076 (0.057)	NA	0.187 (0.120)	NA	0.082 (0.054)	0.098 (0.062)	0.418 (0.081)

Fig. 1 Map of the Ebro Delta (NW Mediterranean) showing the position of the study sites selected across the four habitat types: River (R1 to R3), Lagoons (L1 to L3), agricultural Drainage Channels (DC1 to DC3), and Bays (B1 to B3).

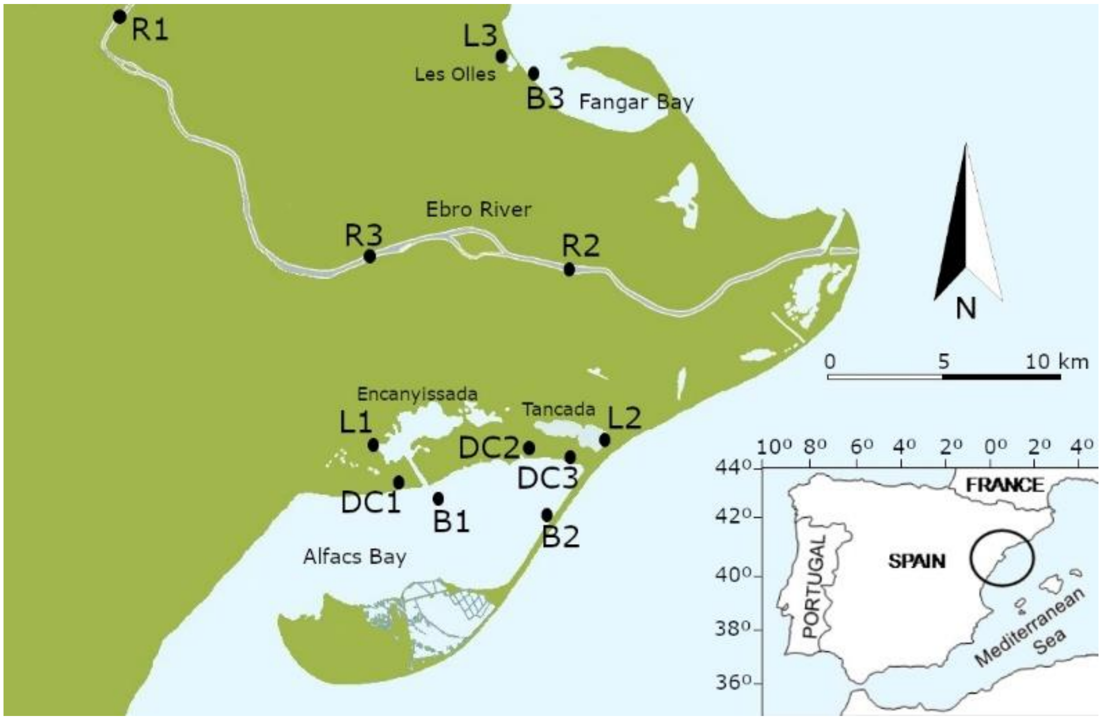


Fig. 2 δ^{13}C, δ^{15}N, and C:N ratios in blue crab (panels A to C), small prawns (panels D to F), and fish (panels G to I) in the four investigated habitats (each sampled at 3 different sites) of the Ebro Delta. R: Ebro River, L: Lagoons, DC: Drainage Channel, and B: Bays. Missing bars indicate a non-available resource at that site. Error bars are SE.

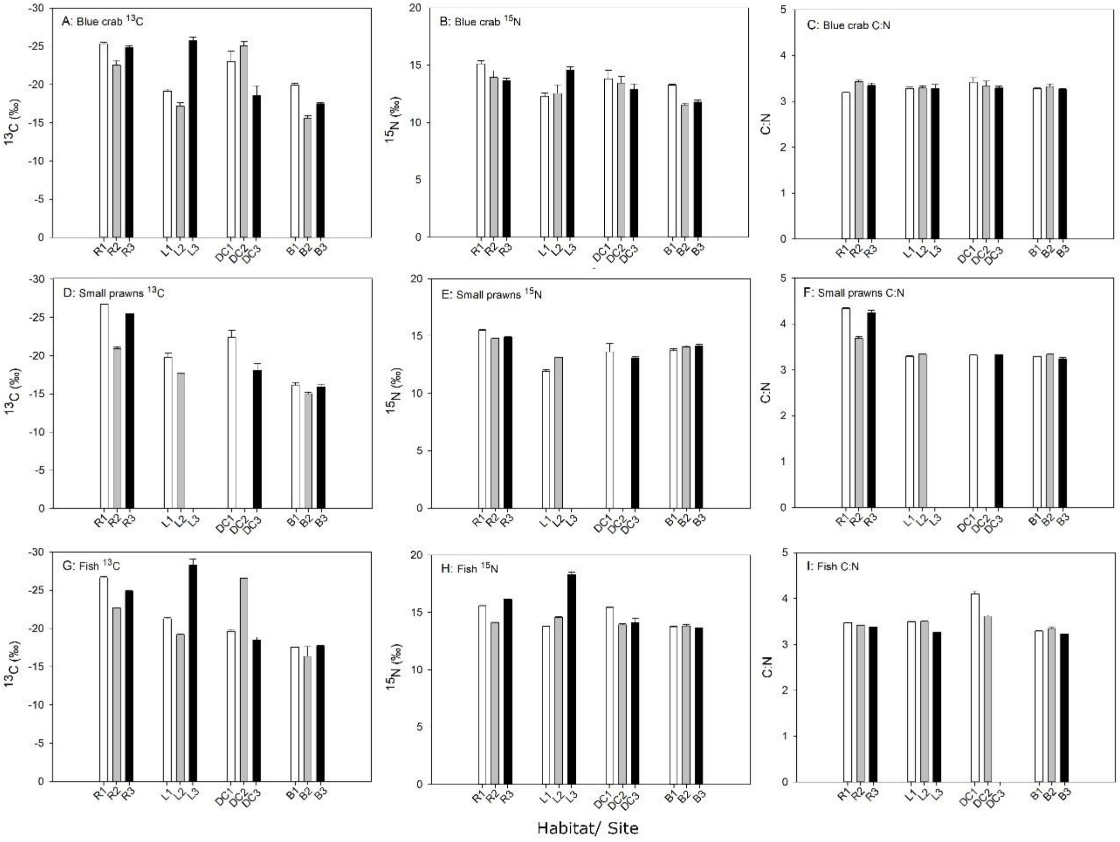


Fig. 3 δ^{13}C, δ^{15}N, and C:N ratios in plants (panels A to C), algae (panels D to F), and sediments (panels G to I) in the four investigated habitats (each sampled at 3 different sites) of the Ebro Delta. R: Ebro River, L: Lagoons, DC: Drainage Channel, and B: Bays. Missing bars indicate a non-available resource at that site. Error bars are SE.

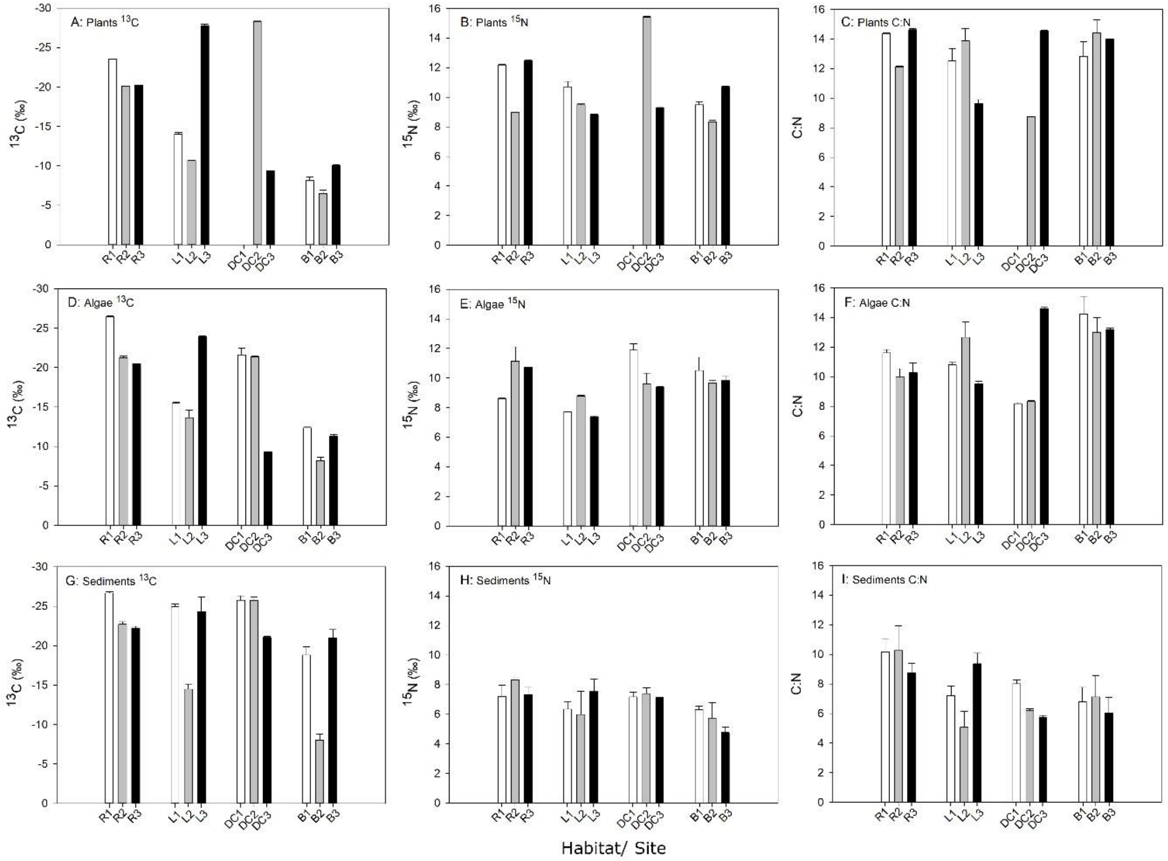
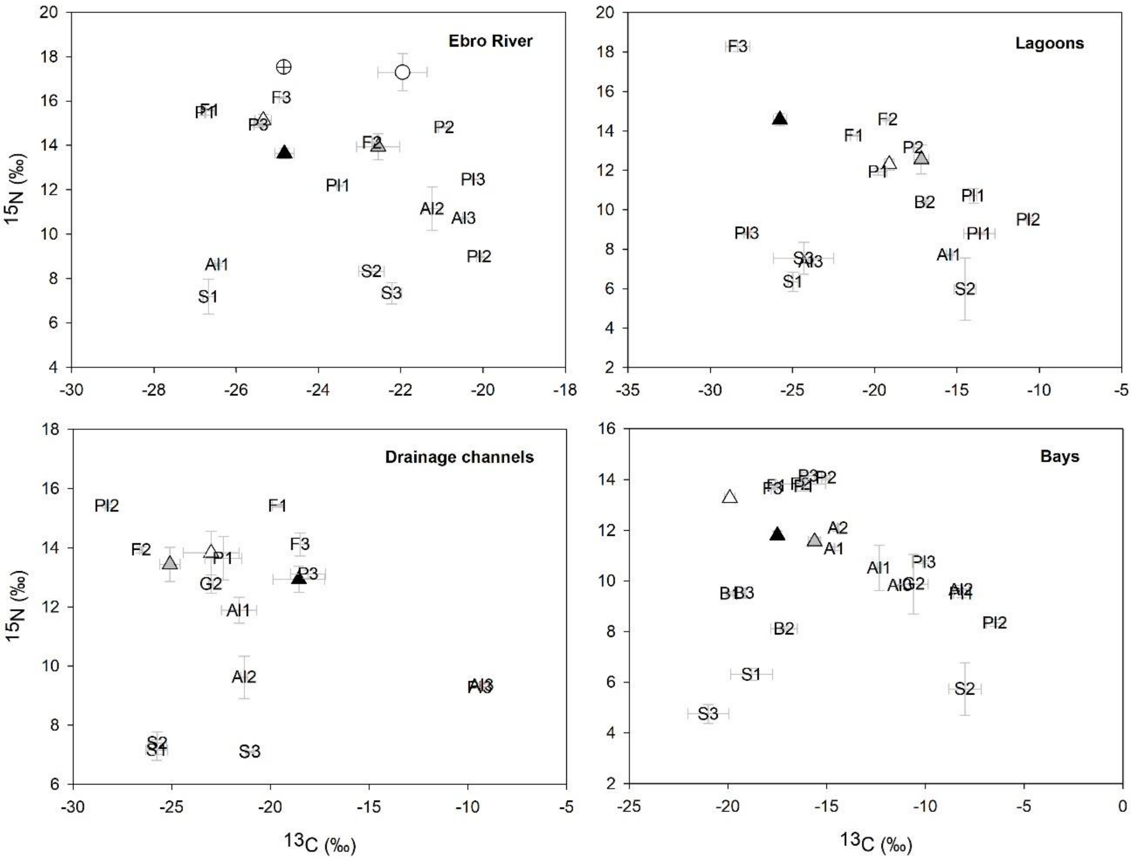


Fig. 4 δ^{13}C and δ^{15}N in blue crab (triangles; for each habitat white, grey, and black indicate sites 1 to 3, respectively) and food sources in the four investigated habitats of the Ebro Delta and subjected to contrasting salinities. Food sources abbreviations are indicated: F: Fish; P: Prawns; B: Bivalves; G: Gastropods; A: Anemones; Pl: Plants; Al: Algae; S: Sediments. In the particular case of the Ebro River, the stable isotope signatures of top predators are also indicated with circles (crossed for *S. glanis*, and empty for *D. labrax*). Error bars are SE.



Habitat	Site	Salinity	Trophic group	Species description
River			Predators	Silurus glanis (N= 1)
				Discentratus labrax (N= 3)
River	R1 (Tortosa)	0.63 ± 0.01	Plants	Potamogeton pectinatus
			Algae	Falta
			Fish	Alburnus alburnus, Leuciscus cephalus , Pseudorasbora parva, Carassius auratus, Liza ramada, Anguila anguila
			Crustaceans	Palaemon spp.
			Bivalves	No
			Gastropods	No
			Sediment	Yes
			Other invertebrates	No
	R2 (St. Jaume d'Enveja)	2.68 ± 0.04	Plants	Myriophyllum spicatum, Potamogeton nodosus, Potamogeton crispus
			Algae	Enteromorpha sp.
			Fish	Atherina boyeri, Discentratus labrax (juv), Pseudorasbora parva, Scardinius erythrophthalmus
			Crustaceans	Palaemon spp.
			Bivalves	No
			Gastropods	No
			Sediment	Yes
			Other invertebrates	No
	R3 (close to Amposta)	1.57 ± 0.02	Plants	Potamogeton pectinatus, Vallisneria spiralis
			Algae	Cladophora sp.
			Fish	Angulla anguilla, Atherina boyeri, Carassius auratus, Pseudorasbora parva, Rutilis rutilus, Scardinius erythrophthalmus, Sander lucioperca (juv)
			Crustaceans	Palaemon spp.
			Bivalves	No
			Gastropods	No
			Sediment	Yes
			Other invertebrates	No
Lagoons	L1 (Encanyissada)	27.26 ± 0.03	Plants	Ruppia cirrohosa
			Algae	Unidentified red algae
			Fish	Pomatochistus microps
			Crustaceans	Palaemon spp.
			Bivalves	No
			Gastropods	No
			Sediment	Yes
			Other invertebrates	No
	L2 (Tancada)	31.63 ± 0.02	Plants	Ruppia cirrohosa
			Algae	Chaetomorpha linum
			Fish	Atherina boyeri, Pomatochistus microps
			Crustaceans	Palaemon spp.
			Bivalves	Cerastoderma sp.
			Gastropods	No
			Sediment	Yes
			Other invertebrates	No
	L3 (Les Olles)	0.72 ± 0.03	Plants	Potamogeton nodosus, Lemna minor, Ceratophyllum demersum
			Algae	Green filamentous algae, and green folious algae
			Fish	Lepomis gibbosus
			Crustaceans	No
			Bivalves	No (lots of empty shells of Corvicula fluminea)
			Gastropods	No (lots of empty shells of Pomacea maculata)
			Sediment	Yes
			Other invertebrates	No
Discharge Channels	DC1 (Sèquia Gran)	5.28 ± 0.05	Plants	No
			Algae	Enteromorpha sp.
			Fish	Dicentrarchus labrax, Anguila anguila, Gobius paganellus
			Crustaceans	Palaemon spp.
			Bivalves	No
			Gastropods	No
			Sediment	Yes
			Other invertebrates	No
	DC2 (Sèquia de l'Ala)	1.11 ± 0.08	Plants	Lemna minor
			Algae	Enteromorpha sp.
			Fish	Cyprinus carpio, Gambusia holbrooki, Pseudospora parva
			Crustaceans	No
			Bivalves	No
			Gastropods	F. Physidae
			Sediment	Yes
			Other invertebrates	No
	DC3 (El canalot)	30.57 ± 0.07	Plants	Cymodocea nodosa
			Algae	Chondria tenuissima
			Fish	Anguilla anguilla
			Crustaceans	Palaemon spp.
			Bivalves	No
			Gastropods	No
			Sediment	Yes
			Other invertebrates	No
Bay	B1 (Northern Alfacs Bay)	30.02 ± 0.06	Plants	Zostera noltii
			Algae	Enteromorpha sp.
			Fish	Sparus aurata, Gobius paganellus
			Crustaceans	Palaemon spp., P. longirostris
			Bivalves	Mytilus galloprovincialis, Crassostrea gigas
			Sediment	Yes
			Other invertebrates	Paranemonia cinerea
	B2 (Alfacs Bay, Trabucador sand bar)	34.98 ± 0.05	Plants	Cymodocea nodosa
			Algae	Hypnea musciformis, Acetabularia acetatum, Chaetomorpha linum, Ulva sp., Anotrichium furcellatus, Stypocaulon scoparium
			Fish	Salaria pavo
			Crustaceans	Palaemon spp.
			Bivalves	Cerastoderma sp., Donax spp.
			Gastropods	Cerithium sp., Conus mediterraneus, Nassarius reticulatus, Hexaples trunculus
			Sediment	Yes
			Other invertebrates	Paranemonia cinerea
	B3 (Fangar Bay)	32.53 ± 0.04	Plants	Cymodocea nodosa
			Algae	Cladophora sp, Ulva sp, Dictyota sp
			Fish	Mullus surmulletus, Sparus aurata
			Crustaceans	Palaemon spp.
			Bivalves	Mytilus galloprovincialis, Crassostrea gigas
			Gastropods	Cerithium sp.
			Sediment	Yes
			Other invertebrates	No