Trophic role and predatory interactions between the blue crab, *Callinectes sapidus*, and native species in open waters of the Ebro Delta

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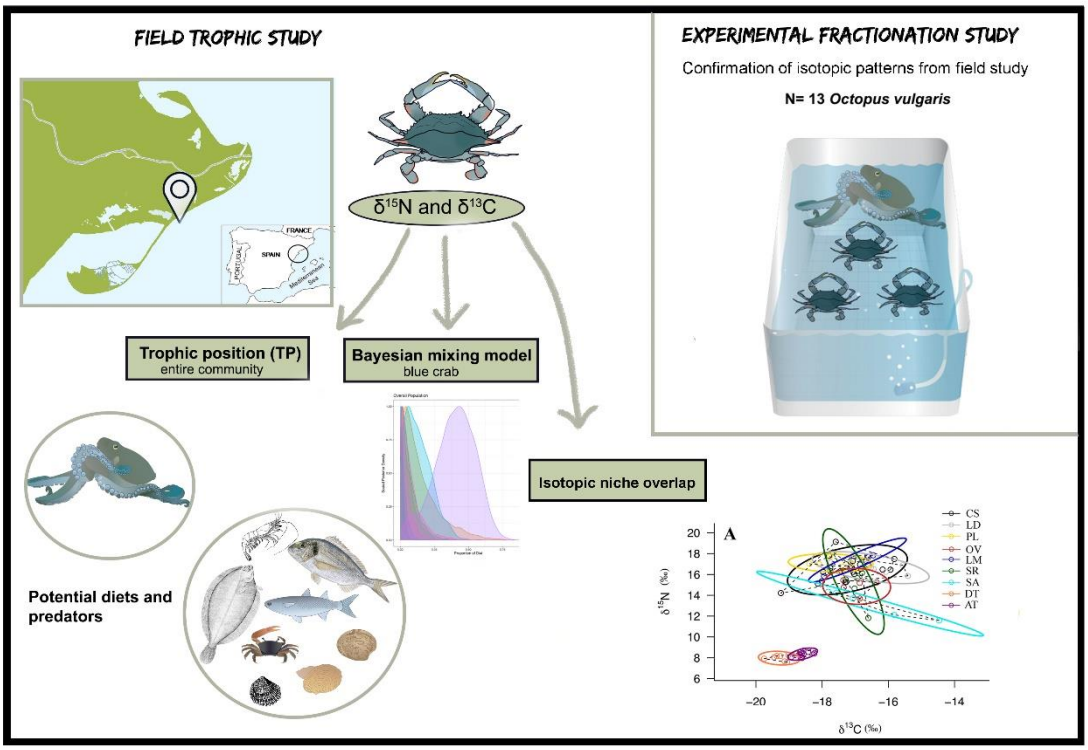
Graphical abstract



ABSTRACT

The Ebro Delta has become a major blue crab (*Callinectes sapidus*) fishery area in the NW Mediterranean, but there is limited information on factors controlling the abundance of populations in open waters, a crucial habitat for ovigerous females. Here, we use a stable isotope approach ($\delta^{15}N$ and $\delta^{13}C$), to assess blue crab trophic position, the potential consumption of food items using mixing models, and the isotopic niche overlap with local commercial species. For *Octopus vulgaris*, a potential blue crab key predator, a trophic enrichment experiment was also conducted to further assess predation control in wild populations. The blue crab showed 1.6 times higher trophic position than in other habitats of the Ebro Delta, and similar to that found in the harbor crab, *Liocarcinus depurator*, and several predatory fish. Additionally, the isotopic niche of blue crabs showed overlaps from 46.2 to 14.9% with native predators, and mixing models also suggest even dietary contributions throughout the food web. For *O. vulgaris*, field results showed a trophic position of 3.93, lower than that of blue crab, and lower $\delta^{15}N$ signatures were also obtained in a captivity experiment drawing negative fractionation (-1.1‰). We conclude that high dietary contribution of animal prey might provide a high protein diet that could be crucial for allowing the maintenance of a large local population, but the overall functional trophic similarity could also disfavor local native species. The similarity between experimental fractionation and field differences between predator and prey (-1.6‰) suggests that predation of blue crab is possible, but further research is needed to clarify the metabolic routes involved in octopus $\delta^{15}N$ fractionation.

Keywords: Stable isotope analyses; isotopic niche overlap; MixSiar; ^{15}N fractionation; *Octopus vulgaris*

1. Introduction

The blue crab *Callinectes sapidus* Rathbun 1896 is a member of the Portunidae native to the Western Atlantic from Uruguay to Nova Scotia (Mancinelli et al. 2017a). The species was recorded in Europe for the first time in 1901 on the Atlantic coast of France (Bouvier 1901), and then appeared in the Mediterranean Sea in 1948 probably introduced by ballast waters (Serbetis 1959; Zenetos et al. 2018). Since then, it has notoriously expanded throughout the entire basin and has become one of the 100 worst invasive species despite its commercial interest (Zenetos et al. 2005). In Spain, it was first detected in the Tancada Lagoon in the Ebro Delta, Catalonia in 2012 (Castejón and Guerao 2013), and since then, it has rapidly spread towards the Atlantic Sea reaching the Southern coast of Portugal (López and Rodon 2018; Vasconcelos et al. 2019).

The species is a voracious generalist consumer capable of using a wide range of animal resources including mollusks, fish, crustaceans, and vegetal (plants and algae) and detrital material in the sediment, depending on availability and the stage of ontogenic development, and local availability (Laughlin 1982; Rosas et al. 1994; Prado et al. 2021). Hence, it can affect benthic communities at multiple trophic levels, altering the functioning and biodiversity of ecosystems, and impacting native fisheries in invaded areas (Zenetos et al. 2005; Nehring 2011; Mancinelli et al. 2017a). For instance, in the Ebro Delta, blue crab has been indicated to prey on locally abundant mollusk species, decreasing the effective size of populations until they become very rare and there is a forced trophic shift to towards other less nutritional resources such as vegetal and detrital material (Prado et al. 2021). Trophic positions (hereafter TP) of blue crab in sites already depleted from animal prey could be lower than 2.6 (Prado et al. 2021), whereas estimates in other Mediterranean regions using the same methodology can reach values as high as 4.5 (Mancinelli et al. 2016; Aslan and Polito 2021).

Poor nutritional conditions may have negative consequences for long-term population traits including survival and growth rates, age at first maturity, and/ or fecundity (review by Metcalfe and Monaghan 2001). Blue crab juveniles when fed on lower levels of dietary protein show lower

76 growth rates and molting frequency than treatments featuring higher protein contents (Millikin
77 et al. 1980). However, the Ebro Delta is the only area in the Spanish Mediterranean where
78 abundances are large enough to sustain a targeted fishery that may reach over 2 tons of captures
79 per day (López and Rodon 2018; López 2020). Hence, fishing captures also suggest a locally
80 important reproductive output for the species. Ovigerous females, usually gather on the Ebro
81 River mouth and open sea in front of the Ebro Delta (López and Rodon 2018; López 2020), an
82 area that remains unexplored for the trophic structure and resource use of *C. sapidus*. The area
83 hosts a diverse fishery of other commercial species, particularly benthic predatory and
84 omnivorous fish that could overlap the functional trophic niche of blue crab, and behave either
85 as potential predators, food resource, and/ or as an active competitor (see Laughlin 1982;
86 Guillory and Elliot 2001), depending on the size. For instance, the harbor crab, *Liocarcinus*
87 *depurator*, has been reported to feed mainly on other decapod crustacea followed by small
88 fishes and small cephalopods (Abelló and Cartes 1987). Common predatory flatfish such as
89 *Pegusa lascaris*, *Scophthalmus rhombus*, and *Solea senegalensis* might already undergo certain
90 overlap in their trophic niche which is mostly comprised of different types of crustaceans,
91 bivalves, polychaetes and fish (Cabral et al. 2002). Sparid fishes such as *Lithognathus mormyrus*,
92 *Pagellus erythrinus,* and *Sparus aurata*, are also common opportunistic predators feeding on
93 benthic invertebrates, mainly crustaceans, polychaetes, and bivalve mollusks (Kallianiotis et al.
94 2005; Taieb et al. 2013) that may be affected by the progressive spread of blue crab. Hence,
95 comparative evaluation of the isotopic niche overlap and TP of these species relative to the blue
96 crab can also provide an adequate proxy to assess functional trophic similarity.
97 Top-down control of blue crab has been extensively studied in native ecosystems (e.g., Heck
98 and Coen 1995; Guillory and Prejean 2001; Hayes et al. 2022), but is still an unexplored ground
99 in invaded Mediterranean regions. In the comprehensive review conducted by Guillory and Elliot
100 (2001), authors provide a list of 93 species capable of preying on all blue crab life stages,
101 although very few would be capable of preying on adult crabs of commercial size (ca. ≥ 150 g

WW) and/ or are present in a large quantity in the open waters of the Ebro Delta. Among potential candidates, the common octopus *Octopus vulgaris*, usually reaching sizes of 3-4 kg and locally abundant, appear as a good model predator based on general knowledge of decapods being a favorite prey item of octopuses (e.g., Guillory and Elliot 2001; Leite et al. 2009; Pech-Puch et al. 2016) and on local fishermen reports of attacks to blue crab fishing traps (personal communication to P. Prado).

In this context, the main aim of this study was to determine the isotopic niche width, the TP, and the contribution of major prey items to adult female blue crab diet in the open sea of the Ebro Delta, an area that is of key importance for hatching success (females are nearly the exclusive sex in the area) in order to obtain a comprehensive picture of resource uses across the territory (provided only for males in estuarine habitats by Prado et al. 2022) that could help to assess the overall fitness of the population. A second aim was to assess the isotopic niche overlap and compare the TP of the blue crab with other commercial marine species in order to evaluate the potential effect of its introduction in the local food-web structure. A final objective was also to assess the possible role of *O. vulgaris*, in blue crab predation using a controlled manipulative experiment to assess isotopic fractionation. *O. vulgaris* has been indicated as an active predator of both the native green crab, *Carcinus mediterraneus* (Fiorito et al. 1990) and the blue crab in natural (fishermen report to P. Prado) and captivity conditions (P. Prado, personal observation). Hence, information about the specific patterns of fractionation could shed some light on the importance of trophic relationships.

2. Materials and methods

2.1. Study sites, sample collection and processing

The study was conducted in coastal waters ca. 12-16 km south from the Ebro River mouth, an area that is commonly fished for blue crab and other commercial species and features typical Mediterranean marine salinity ranges (ca. 36-37 ppt; Fig. 1). Due to enhanced salinity, the local

sex composition of blue crab in this area is extremely biased towards adult females which constitute the bulk of the captures (López and Rodon 2018).

Fieldwork was conducted in five different dates from mid-October 2020 to late February 2021 to ensure that captures were representative of the abundance of local species. In particular, adult females were collected in a ca. 1.5-month period (two of the sampling occasions), from the beginning of December 2020 to mid-January 2021. Sampling was conducted at ca. 1-10 m depth, using a small-scale fishing boat equipped with three different fishing gears to collect benthic organisms: (1) Mechanized clam dredges (typically 3-4 per boat) were used to collect available infauna such as bivalves, and crustaceans, and some fishes at 1 to 5 m depth; (2) gill nets were used to collect *Callinectes sapidus* and other locally abundant fishes at 1 to 10 m depth; and (3) basket traps were used to capture *Octopus vulgaris* at 5 to 10 m depth.

After collection, blue crabs and other captured species were separately placed within aerated ice coolers and transported to the lab. For each species, a minimum of N= 3 individuals was considered in the study. Individuals of similar sizes were also selected to minimize possible changes in the diet due to age-dependent energy requirements, and except for octopus (ca. 2000 g WW), and mullet (ca. 500 g WW based on feasible predation according to previous observations by P. Prado) featured sizes that were similar or smaller to that of blue crab, in order to allow for possible predation. Individuals of crustaceans: *C. sapidus* (females, n= 25, 250-300 g WW), *Penaeus kerathurus* (n= 3), and *Liocarcinus depurator* (n= 10); bivalves: *Acanthocardia tuberculata* (n= 14), *Donax trunculus* (n= 7), and *Mactra stultorum* (n= 3); Octopoda: *Octopus vulgaris* (n= 5); and fishes: *Pegusa lascaris* (n= 5), *Trachinus draco* (n= 3), *Scophthalmus rhombus* (n= 6), *Sparus aurata* (n= 5), *Lithognathus mormyrus* (n= 5), *Pagellus erythrinus* (n= 3), *Mugil cephalus* (n= 4), *Bothus podas* (n= 3) and *Sciaena umbra* (n= 3) were obtained and processed for stable isotope analyses (SIA).

Fish specimens were filleted for the extraction of the entire musculature and rinsed with ultrapure water to prevent contamination with other tissues. For blue crab and harbor crab (*L.*

154 *depurator*) a piece of the right claw musculature was extracted, whereas *P. kerathurus* were
155 peeled to obtain the tail muscle, and bivalves were rinsed of sediments and processed as whole
156 individuals without the valves. For octopus, a tentacle fragment was dissected. All tissue samples
157 were dried at 60 ^{o}C until constant weight and reduced to powder with a mortar and pestle, which
158 were carefully rinsed with alcohol after each sample to remove any remains.
159 No plant or macroalgae sources were considered because no consistent material was
160 recovered in the study area during the surveys. Sediments were not included either because
161 within the study area they feature an extremely low organic content (0.011% N and 0.26% C) and
162 were considered as an unlikely food source for blue crab.
163
164 *2.2. Stable isotope analyses*
165 Samples were analyzed with a Flash 112 IRMS delta C series EA Thermo Finnigan mass
166 spectrometer connected to an elemental analyzer for the determination of C and N contents at
167 the isotopic ratio mass spectrometry facility in the University of Barcelona - Scientific and
168 Technological Center (CCiT-UB). Isotope ratios in samples were calculated from linear calibration
169 curves constructed with standard reference materials of known composition and a blank
170 correction. The difference in isotopic composition between the sample and reference material is
171 determined by the equation:
172 $$\delta_{sample-standard} = [(R_{sample} - R_{standard})/\, R_{standard}] \text{ x } 1000$$
173 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
174 calibration material and $\delta_{sample-standard}$ is the difference in isotopic composition of the sample
175 relative to that of the reference. The reproducibility of the stable isotope measurements was
176 ~0.1‰. The possible variability in $\delta^{13}C$ signatures induced by the presence of lipids, which are
177 typically depleted in ^{13}C, were normalized for crustaceans, mollusks, and fish using the equations
178 provided by Post et al. (2007). The threshold for the application of this correction was an increase
179 in corrected signatures of at least 0.1‰ (Prado et al. 2012).

180

181 *2.3. Isotopic niche of blue crab and native marine species*

182 We used the package SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011) to
183 analyze the structure of the food web. Standard ellipses corrected for small sample size (SEA_c)
184 were used to represent each species in the isotopic space (only those with N≥ 5 were included).
185 The Bayesian estimate of the standard ellipse and its area (SEA_b) was calculated for each
186 investigated species as a measure of their isotopic niche width or resource use area. SEA_b in units
187 of area ($‰^2$), captures all the same properties as SEA_c and the Total Area (TA) of the convex
188 polygon, but it is unbiased with respect to sample size and exhibits more uncertainty with smaller
189 sample size (Jackson et al. 2011). This Bayesian method based on Markov-Chain Monte Carlo
190 (MCMC) simulations uses posteriori randomly replicated sequences in the 95% confidence
191 interval for the distributions of both stable isotopes to correct the bivariate ellipses ($\delta^{13}C$ and
192 $\delta^{15}N$). The magnitude of isotopic overlap ($‰^2$) –as a measure of resource partitioning over time-
193 was obtained from SEA_b estimations and expressed as a % overlap of the blue crab isotopic niche
194 over that of the other species and vice versa.

195

196 *2.4. Estimates of trophic position*

197 The trophic position (TP) of blue crab and other local predators (octopus, fishes, and
198 crustaceans) was calculated according to the methodology and equation proposed by Vander
199 Zanden et al. (1997):

200

201 $$TP_{\delta 15N} = (\delta^{15}N_{Consumer} - \delta^{15}N_{Baseline}) / \Delta^{15}N + \lambda$$

202

203 Where $\delta^{15}N_{consumer}$ is the nitrogen isotopic signature of the consumer species, $\delta^{15}N_{Baseline}$ is the
204 mean isotopic signature of available filter feeding bivalves (*A. tuberculata, D. trunculus*, and *M.*
205 *stultorum*) which are well-known primary consumers (primary producers= trophic level 1,

206 primary consumers= trophic level 2, and so on). $\Delta^{15}N$ is the 3.4‰ $\delta^{15}N$ isotopic enrichment
207 considered across trophic levels (Post 2002), and λ the trophic level of the baseline indicator,
208 respectively. Bivalves, and particularly the Mediterranean mussel, have been commonly used as
209 the food web baseline in previous stable isotope studies (Mancinelli et al. 2013, 2016, 2017b;
210 Carrozzo et al. 2014; Aslan and Polito 2021; Prado et al. 2021) and therefore provide a robust
211 baseline for comparative purposes with other locations, and with previous studies. In the present
212 study, the mean of the 3 collected bivalve species was used for calculations since there were no
213 significant differences in $\delta^{15}N$ among them.
214
215 *2.5. Isotope mixing models*
216 To assess dietary contributions to female blue crab diets (N= 25; N≥ 10 is recommended to
217 prevent large bias and high dispersion; see Brett 2014) in the open sea of the Ebro Delta, the
218 MixSIAR Bayesian mixing model (V.3.1) was implemented as an open-source R package (Stock et
219 al. 2018), using a generalist prior distribution. The model was first outlined by Moore and
220 Semmens (2008) and modified later to improve the robustness of results. Prior to the
221 development of mixing models, an isospace plot was produced to visually inspect that consumer
222 $\delta^{15}N$ and $\delta^{13}C$ values fell within the prey polygon in isospace (Stock and Semmens 2016). MixSIAR
223 uses stable isotope signatures with their standard error (SE), and tissue-diet discrimination
224 factors as input variables to estimate the probability distributions of each food item to a mixture
225 within 95% confidence intervals and accounts for uncertainty associated with multiple sources.
226 The estimated median contribution (i.e., the 50% percentile) represents the median source
227 contribution value for each source and is usually given along with standard deviation (SD) and/or
228 confidence intervals for comparative purposes.
229 Models were run considering two different possible fractionations. First, the 0.4‰ $\Delta^{13}C$ and
230 the 3.4‰ $\Delta^{15}N$ values derived from literature data on non-herbivorous aquatic consumers (Post
231 2002) that have been largely used in other stable isotope works in invaded Mediterranean

232 regions (Mancinelli et al. 2013, 2016, 2017b; Carrozzo et al. 2014; Aslan and Polito 2021; Prado
233 et al. 2021) and associated SD values (1.3‰ for $\delta^{13}C$ and 1‰ for $\delta^{15}N$; Post 2002). Second, the
234 0.6 ± 0.7‰ $\Delta^{13}C$ and 1.6 ± 0.6‰ $\Delta^{15}N$ values obtained for blue crab feeding with clams and black
235 sea bass obtained by McCann and Jensen (2018). Large octopus (1-2 kg) were not included in the
236 runs since this size was experimentally demonstrated to be a highly effective predator of blue
237 crabs.
238 The Markov Chain Monte Carlo (MCMM) in MixSiar, a method for obtaining information and
239 estimating posterior distributions in Bayesian inference, was set as follows: chain length 100,000;
240 burn-in 50,000; thin 50; and number of chains 3. With these settings, model selection was based
241 on the adequacy of default diagnostic tests in MixSIAR: for the Gelman–Rubin diagnostic
242 convergence conditions < 1.05 in all cases, and for the Geweke diagnostic, the number of
243 variables falling outside the ± 1.96 range in the first and last part of a Markov chain being < 5%
244 for the three chains.
245
246 *2.6. Experimental feeding of Octopus vulgaris with blue crab*
247 A total of N= 13 individuals of *O. vulgaris* were obtained alive in late March from the outer
248 sea of the Ebro Delta by local fishermen in La Ràpita (DG051201-331/2021) and transported to
249 IRTA facilities. Individuals were first weighted to the nearest 0.01 kg within a mesh bag using a
250 CH-KERN tension dynamometer and then transferred to a large, 3000 L tank, equipped with PVC
251 hides, and seawater at ca. 17-20 °C, ≥80 % O_2 saturation, and 36 ppt for 2 months (late March to
252 late May 2022). Each animal was first adapted to captivity with dead blue crab (48 h) and then
253 fed daily with 1-2 live individuals bought from fishermen at the lower Ebro River (Deltebre) and
254 remains removed to avoid decomposition.
255 By the end of May, animals were weighted again to assess biomass incorporation prior SIA.
256 Then, a small fragment of a tentacle from each octopus was dissected and animals returned to
257 the open sea. For blue crabs, samples (n= 12) were obtained throughout the 2-month feeding

258 period (i.e., 1-2 individuals per week). Samples of muscle from octopus and blue crabs were
259 processed as indicated previously in section 2.1.
260
261 *2.7. Data analyses*
262 Differences in the composition of $\delta^{13}C$ (normalized) and $\delta^{15}N$ signatures, and TP of blue crabs
263 and potential resources were investigated with a one-way nested ANOVA (Species fixed factor)
264 and SNK post-hoc analysis. ANOVA assumptions of normality (Chi-square test) and homogeneity
265 of variances (Cochran's test) were not always achieved by transformation and in those instances
266 the significance level was fixed to $p< 0.01$ to minimize the risk of making a Type I error
267 (Underwood 1997). All ANOVAs were performed using Statistica v.12 software.
268 Comparisons of isotopic signatures between blue crabs from the lower Ebro River (used
269 during the feeding experiment to determine stable isotope fractionation) and those from the sea
270 (trophic guild evaluation) were assessed with a two-tailed t-test for two independent samples
271 with equal variance in order to establish possible salinity effects that could affect interpretation
272 of isotopic patterns of octopuses in captivity and under natural conditions.
273
274 **3. Results**
275 *3.1. Isotopic signatures, TP and diet composition of blue crabs in the open sea of the Ebro Delta*
276 Female blue crabs from the open sea featured mean isotopic signatures of -17.2 ± 0.15 for
277 $\delta^{13}C$ and 16.4 ± 0.2 for $\delta^{15}N$. This resulted in a considerably high TP of 4.40 ± 0.06 using $\delta^{15}N$ of
278 bivalves as baseline for primary consumers. In fact, the TP of blue crabs from the open sea was
279 also 1.4 and 1.6 times higher than that found in other habitats of the Ebro Delta, such as the
280 Alfacs Bay and the Tancada Lagoon, respectively (Table 1).
281 The MixSiar model showed good performance (no variables higher than 1.05 in the Gelman-
282 Rubin diagnostic and adequate chain convergence in the Geweke test) using 0.4 and 3.4‰ as

283 fractionation values for $\delta^{13}C$ and $\delta^{15}N$, respectively. Results evidenced that blue crab exerts a
284 relatively even consumption of available resources, although with slightly higher contribution of
285 bivalve species (5.9 to 6.4%) and *P. kerathurus* (6.3%) than from the fish community (2.8 to 5.9%).
286 Yet, the total contributions explained by the model (50% percentile) was 80.8%, suggesting the
287 possible contribution of other not considered resources (Table 2). For the second run based on
288 0.6 ± 0.7‰ $\Delta^{13}C$ and 1.6 ± 0.6‰ $\Delta^{15}N$ values, the model did not fit Geweke test and was
289 discarded.
290
291 *3.2. Comparison of blue crab isotopic niche and TP with those of other local marine species*
292 Patterns of $\delta^{13}C$ showed significant differences across the food web (df= 16, MS= 9.6, *F*= 25.5,
293 *p*< 0.0001), with higher values in predatory fishes and crustaceans (-18.13 to -16.4), followed by
294 bivalves (-19.3 to -18.6), and lowest values in the fish *M. cephalus* (-21.9 ± 0.4; see Fig. 2). There
295 were also significant differences in patterns of $\delta^{15}N$ among species (df= 16, MS= 75,4, *F*= 104.0,
296 *p*< 0.0001), with highest values in the fishes *L. mormyrus*, *P. lascaris*, and *P. eritrinus* (17.8 to
297 16.7), and in the crustaceans *L. depurator* (16.6 ± 0.2) and *C. sapidus* (16.4 ± 0.2). Intermediate
298 signatures were observed in the fishes *S. rhombus*, *M. cephalus*, *T. draco*, *B. podas*, *S. umbra*,
299 and *S. senegalensis* (15.3 to 12.5) and in octopus (14.8 ± 0.3). Significant lower groupings were
300 also observed for *P. kerathurus* (10.8 ± 0.3) and bivalves (8.4 to 7.9) (Fig. 2).
301 The standard ellipse area corrected for small sample size (SEA_c) and the Bayesian standard
302 ellipse area (SEA_b) providing an indication of isotopic niche width showed highest sizes for blue
303 crab, *S. aurata* and *S. rhombus*, intermediate for harbor crab, *P. lascaris*, *L. mormyrus*, and *O.*
304 *vulgaris*, and lowest for bivalves, which appear as a clearly distinctive trophic group (Fig. 3A-B;
305 Table 3). SEA_b credibility intervals for each species showed higher ranges for *S. aurata,* and *S.*
306 *rhombus* and lowest for bivalves (Fig 3B). Blue crab showed niche overlaps of 46.2% of its SEA_b
307 with harbor crab, 36.9% with *L. mormyrus*, 33.7% with *O. vulgaris*, 30.6% with *S. rhombus*, 29.2%
308 with *P. lascaris*, and 14.9% with *S. aurata*. No overlap was found with bivalve species (Fig. 3A).

309 The TP of consumers showed significant differences across the food-web (df= 13, MS= 1.57,
310 *F*= 19.77, *p*< 0.0001) with values ranging from 4.67 ± 0.11 in *L. mormyrus* to 2.73 ± 0.08 in *P.*
311 *kerathurus* (Table 1). The harbor crab, *L. depurator* (4.46 ± 0.06) and other well-known benthic
312 fish predators such as *P. erythrinus* (4.48 ± 0.05) and *P. lascaris* (4.59 ± 0.05) showed TPs very
313 similar to that of blue crab. In contrast, the TP of octopus was slightly but significantly lower
314 (3.93 ± 0.09) (Table 1).
315
316 *3.3. Experimental feeding of Octopus vulgaris with blue crab*
317 Octopus easily captured and eagerly consumed blue crab individuals, provided in daily rations
318 (Fig. 4A-C). The average weight increment of octopus during the captivity period was 816.3 ± 121
319 g WW, from a mean initial size of 2243.3 ± 114.3 to a final weight of 3059.6 ± 129.2 g WW.
320 Individuals of *C. sapidus* from the lower Ebro River used for feeding octopus had a $\delta^{13}C$ of -
321 21.2 ± 0.1, which is 3.8‰ lower than those from the open sea (df= 35, *t*= -17.27; *p*<0.0001).
322 Similarly, values for $\delta^{15}N$ (13.3 ± 0.3) were also 3.1‰ lower compared to individuals from the sea
323 (df= 35, *t*= -9.14; *p*<0.0001). Feeding in these crabs from the Ebro River for two months resulted
324 on octopus' signatures of -19.4 ± 0.2 for $\delta^{13}C$, and 12.2 ± 0.1 for $\delta^{15}N$ (Fig. 2). Hence, experimental
325 results suggest 1.8‰ enrichment for $\delta^{13}C$ and negative values for $\delta^{15}N$ (by -1.1‰), which is
326 coherent with the ca. 1.5‰ higher $\delta^{15}N$ for *C. sapidus* observed in the field, whereas for $\delta^{13}C$ the
327 values were only 0.2‰ higher.
328
329 **4. Discussion**
330 Our results evince a high blue crab trophic position (TP= 4.4) in open waters of the Ebro Delta
331 (coherent with an average total animal contribution of >80%), very similar to that found for other
332 potential resource competitors of similar size. This situation contrasts with considerably lower
333 TPs found in Ebro Delta Bays and the Tancada lagoon (TP= 2.8), coherent with an average total
334 animal contribution of ca. 57% (Prado et al. 2022); and even more reduced values suggested for

other habitats with sparser animal resources such as drainage channels, the Encanyissada lagoon, and upriver sites. All values observed in the Ebro Delta are, however, within the range reported across invaded Mediterranean locations (Aslan and Polito 2021; Mancinelli et al. 2016, 2017b). The trophic position of organisms is greatly dependent on environmental factors. For instance, blue crabs are active and feeding during warm summer months while most are buried in cold winter months (Hines 2007). Reduced food intake and starvation could be a source of enrichment in $\delta^{15}N$ as indicated animals are using their own reserves during the fasting period (Oelbermann and Scheu 2002; Haubert et al. 2005; Prado et al. 2021). Alternatively, enhanced consumption of readily available plant material (not available in our study site), and a parallel decrease in cannibalism have also been proposed to account for more reduced trophic levels of blue crabs in winter than in summer, although results were not consistent across sampling locations (Mancinelli et al. 2017b). Given the opportunistic behavior of the blue crab, differences in resource availability across habitat types are, however, expected to be the main source of variability in stable isotopes signatures (Mancinelli et al. 2016, 2017b). In the open sea of the Ebro Delta the high availability of preferred animal items such as mollusks, crustaceans, and fish (Laughlin 1982; Rosas et al. 1994), might enhance TP compared to other estuarine habitats where vegetal material and/or detritus are abundant (Prado et al. 2022).

Trophic position arising from variation in consumer diet has been associated to consumer fitness and linked to evolution via natural selection (Moosmann et al. 2021). Blue crab individuals exclusively fed with an animal diet have shown lower mortality, and enhanced lipid storage and reproductive efforts compared to individuals fed vegetal diets (Belgrad and Griffen 2016a). In turn, population fitness has been indicated a linear relationship with $\log_{10}$ population size, starting at minimum sizes of at least 2000 individuals –to provide adequate levels of buffer against extinction resulting from environmental stochasticity–, and with no apparent maximum asymptote (Reed 2005). Hence, more optimal nutritional conditions in certain areas –particularly those concentrating ovigerous females–, could potentially support enhanced recruitment

361 success (Phillips 2002) and allow for the development of a large local population capable of
362 sustaining a high-pressure target fishery (López and Rodon 2018; López 2020). Considering the
363 pervasive occupation of all Ebro Delta habitats by blue crab (Prado et al. 2020; Clavero et al.
364 2022), local measures in the open sea area (e.g., increased local capture of gravid females) could
365 benefit population control at the large-scale.

366 Blue crab showed a large overlap of nearly half its isotopic niche with the harbor crab, *L.*
367 *depurator*, and lower but also important overlap occurred with octopus, and 4 species of benthic
368 fish (*L. mormyrus*, *S. rhombus*, *P. lascaris*, and *S. aurata*), pointing that they partly share trophic
369 resources. This overlap concurred with very similar values of TP between blue crab (4.4) and
370 harbor crab (4.46), octopus (3.93) as well as with three of the previous fish species (*L. mormyrus*,
371 *S. rhombus* and *P. lascaris*: 4.06 to 4.67), suggesting that, for the size of organisms investigated,
372 they feature a similar trophic role (see also Mancinelli et al. 2016). Food competition or eating
373 native species of concern has been indicated as a mechanism promoting the success of invasive
374 crustaceans (Weis 2009), and might partly account for the reported vulnerability to the spread
375 of blue crab by other decapods such as *Carcinus aestuarii* (Taybi and Mabrouki 2020; Clavero et
376 al. 2022). Our results suggest that it might also be the case of the harbor crab, a species that is
377 typically a scavenger and a carnivore (Abelló and Cartes 1987) and features a more similar
378 pattern of locomotor activity to blue crab than fish, which can further influence feeding (Moller
379 and Naylor 1980). For benthic fish, very few studies have addressed the impacts of the spread of
380 blue crabs at the population level, but species decreases reported in Ebro Delta estuarine
381 habitats (eel, sandsmelt, toothcarp, and grey mullet) are more arguably driven by predation than
382 by resource competition (Clavero et al. 2022). Conversely, the relatively large isotopic niche
383 overlap between blue crab and octopus –presumably capable of preying on large crabs– requires
384 further investigation, since the assessment of TP assumes the same patterns of isotopic
385 fractionation.

386 Results of the MixSiar model suggests that the blue crab is preying on all the available benthic
387 resources, including other predatory fish species and crustaceans which constituted the bulk of
388 the local community. This is consistent with pervasive impacts reported at multiple trophic levels
389 (Zenetos et al. 2005; Nehring 2011; Mancinelli et al. 2017a), with possible outcomes depending
390 on predator-prey size variability (Weitz and Levin 2006), the relative abundance of populations,
391 and/ or the predator hunting mode and prey behavior (Belgrad and Griffen 2016b). In the
392 particular case of the harbor crab, the species attains a maximum size of only about 4 cm
393 (caparace width) but can be found at much lower depths of up to ca. 400 m (Abelló et al. 1990),
394 being this partly segregated distribution a possible key aspect for the local persistence of both
395 species. Besides, given their similar isotopic signature, the possible effect of juvenile cannibalism
396 cannot be discarded (up to ca. 5% of the diet), since it has been pointed to as an important factor
397 contributing to population dynamics (Bromilow and Lipcius 2017).
398 For bivalves, although they are expected to be a preferred food source for the blue crab
399 (Laughlin 1982) a multifactorial decline on the Catalan Coast has been proposed (Baeta et al.
400 2018) that might account for the fact that they were only consumed at a slightly higher rate than
401 other resources (ca. 5.9 to 6.4% vs. 2.8 to 6.3%). Also, the abundance of other infauna, which
402 are usually a common prey of benthic predatory fish and crabs, such as polychaetes (Laughlin
403 1982; Whitehouse et al. 2017) was very low and could not be incorporated in the model,
404 although occasional predation might account for some part of the unexplained variation.
405 The TP of blue crab was very close to other investigated species and results do not show any
406 clear evidence of possible predation control. Unexpectedly, *O. vulgaris*, showed a TP below that
407 of blue crab (3.93 vs. 4.40). Additional fractionation experiments drew a similar outcome: higher
408 $\delta^{15}N$ in blue crab (13.3 ‰) than in octopus (12.2 ‰), resulting in a negative enrichment of -
409 1.13‰. Since animals had significantly increased in weight by an average of 816 g WW during
410 the 2-month captivity period, it was arbitrarily considered that they had reached the stage of
411 isotopic equilibrium (see for instance Miller 2006; Prado et al. 2012 for similar experimental

412 periods). However, $\Delta^{13}C$ was much higher than that observed in the field (1.8 vs. 0.2‰),
413 suggesting that the use of riverine crabs (lower Ebro River) with lower $\delta^{13}C$ signatures (Prado et
414 al. 2014, 2022) could have slowed the process. In contrast, $\Delta^{15}N$, was similar to the -1.6‰
415 difference observed in the open sea suggesting that predation of blue crab is possible both in
416 captivity and in the natural media (Fiorito et al. 1990; Grisley et al. 1999; this study). Junqueira
417 de Souza Dantas et al. (2020), used a $\Delta^{15}N$ of 3.3‰ to assess the diet of *O. insularis* with a mixing
418 model, and results pointed to a single dietary resource, the limpet, *Lottia noronhensis*, whereas
419 digestive contents and midden piles observations clearly evidenced crustaceans as the main diet
420 item (by ca. 90-100%). Yet, *Sepia officinalis* fed with a shrimp diet has shown $\Delta^{15}N$ of ca. 3.3‰
421 (Hobson and Cherel 2006), suggesting large intragroup variability. Particularly low values of $\Delta^{15}N$
422 were reported for mollusks and crustaceans featuring ammonia excretion (Vanderklift and
423 Ponsard 2003). Most mollusks species appear to have limited capacities for interconversion and
424 biosynthesis of amino acids (Bishop 1983) that could have implications for fractionation rates.
425 Additionally, amino acid oxidases of cephalopods, appear to differ from others found in
426 vertebrates (Dixon and Kenworthy, 1967).
427 Reduced $\Delta^{15}N$ has been reported for consumers feeding on high-quality food or on diets with
428 a high nitrogen content (Oelbermann and Scheu 2002; Haubert et al. 2005; Prado et al. 2012).
429 Extreme $\Delta^{15}N$ patterns, ranging from +2.85 to -24.96‰ have been found in laboratory-reared
430 black fly larvae (*Simulium vittatum* IS-7) fed isotopically distinct diets (Overmyer et al. 2008).
431 Among factors potentially accounting for negative fractionation, authors found that molts and
432 feces were enriched in $\delta^{15}N$ compared to the larvae (by 1.2 to 7.1‰ and up to 143‰,
433 respectively), although other effects such as high metabolism and growth rate, and/ or
434 differential assimilation of nutrients could not be discarded. Further, these patterns might also
435 point to possible homeostatic efforts of consumers, as indicated for nauplii of *Acartia tonsa*
436 (Aberle and Malzahn 2007).
437

5. Conclusions

Blue crab in open waters displayed a very high TP, comparable to that found in the harbor crab, *L. depurator*, and several predatory fish species. This contrast with TPs found in other habitats of the Ebro Delta where animal prey has become rare (Prado et al. 2022), but agrees with findings pointing to important rates of isotopic niche overlap with native predators, and with dietary modelling suggesting generalist predation. Functional trophic similarity with native species could potentially cause changes in the biodiversity and the functioning of the entire ecosystem (Finke and Denno 2005). In turn, high nutritional status might have positive effects in population fitness (Phillips 2002) and help to maintain local fishing rates. Besides humans, octopus may be one of the few species capable of exerting an effective predation on adult crabs (Fiorito et al., 1990; Grisley et al., 1999; this study). However, preliminary results for $\Delta^{15}N$ point to unusual metabolic pathways causing a slight depletion of the heavy isotope. Further research is necessary to clarify the metabolic routes involved in octopus $\delta^{15}N$ fractionation.

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Fig. 1. Map of the Ebro Delta showing the position of the study area (in blue from ca. 40°39'21.23"N; 0°47'51.58"E to 40°37'38.38"N; 0°44'41.01"E) at ca. 12-16 km south from the Ebro River mouth, and the lower section of the Ebro River where crabs were bought to local fishermen for the enrichment experiment.

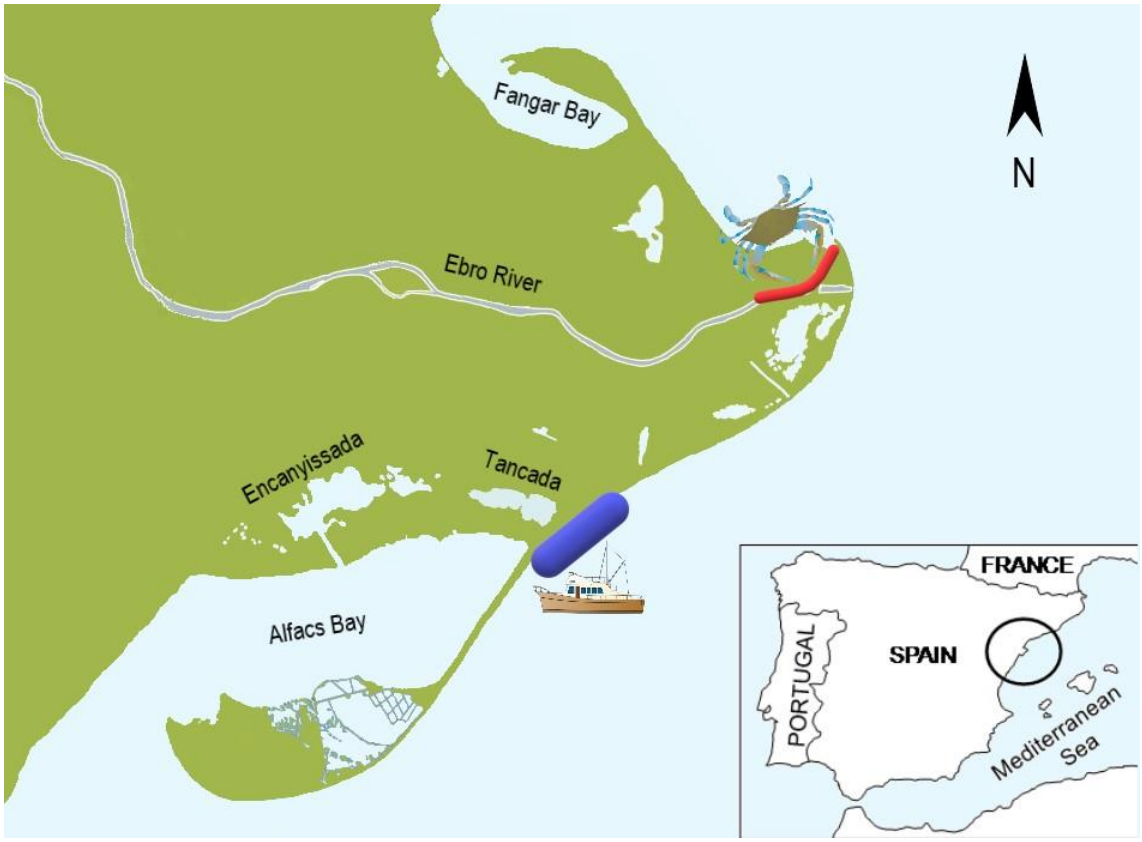


Fig. 2. Mean values of $\delta^{13}C$ and $\delta^{15}N$ of blue crab (triangles) and octopus (squares) from the Ebro Delta open sea (grey) and the vivarium experiment (white). Values for these items are: blue crab field -17.2 ± 0.1 $\delta^{13}C$ and 16.4 ± 0.2 $\delta^{15}N$ (N= 25); octopuses' field -17 ± 0.2 $\delta^{13}C$ and 14.9 ± 0.3 $\delta^{15}N$ (N= 5); blue crab vivarium -21.2 ± 0.1 $\delta^{13}C$ and 13.3 ± 0.3 $\delta^{15}N$ (N= 12); and octopuses' vivarium -19.2 ± 0.2 $\delta^{13}C$ and 12.2 ± 0.1 $\delta^{15}N$ (N= 13). Other species are indicated with letters of genus and species names: *Liocarcinus depurator* (LD, N= 10), *Penaeus kerathurus* (PK, N= 3), *Pegusa lascaris* (PL, N= 5), *Lithognathus mormyrus* (LM, N= 5), *Trachinus draco* (TD, N= 4), *Pagellus erythrinus* (PE, N= 4), *Scophthalmus rhombus* (SR, N= 6), *Solea senegalensis* (SS, N= 3), *Mugil cephalus* (MC, N= 4), *Sparus aurata* (SA, N= 5), *Bothus podas* (BP, N= 3), *Sciaena umbra* (SU, N= 3), *Donax trunculus* (DT, N= 7), *Mactra stultorum* (MS, N= 3), *Acanthocardia tuberculata* (AT, N= 14). Error bars are SE.

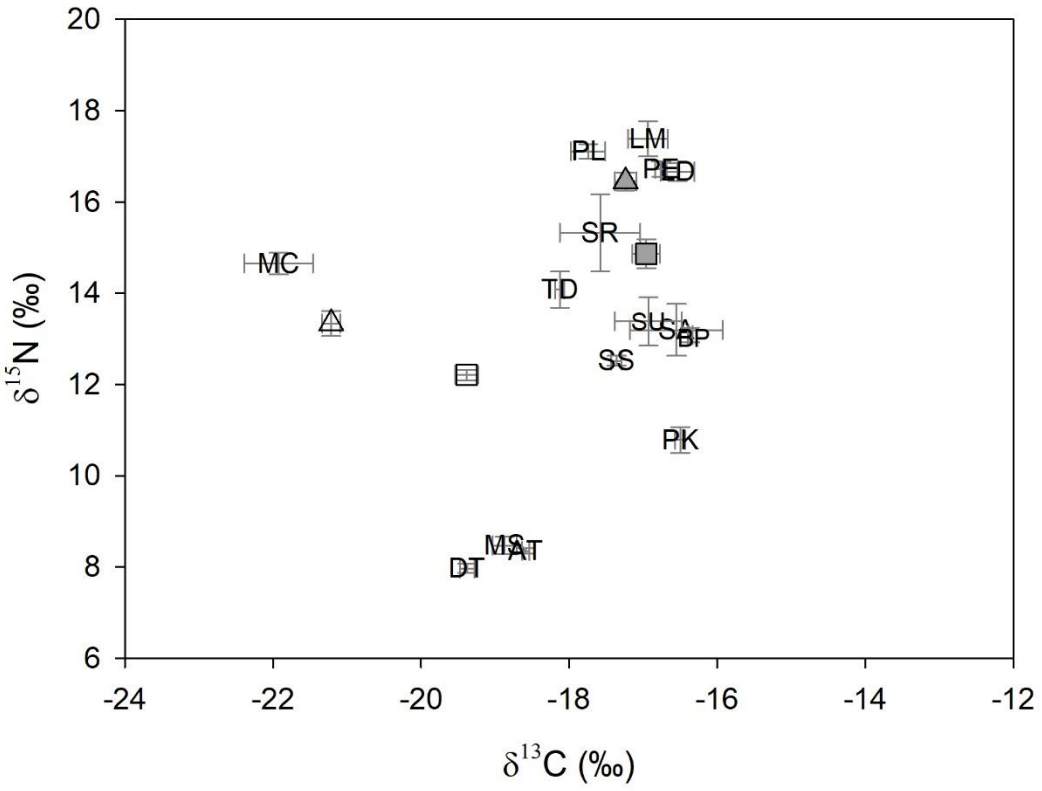


Fig 3. Isotopic niches of *Callinectes sapidus* (CS), and other investigated species (LD: *L. depurator* PL: *P. lascaris*, LM: *L. mormyrus*, SR: *S. rhombus*, SA: *Sparus aurata*, DT: *D. trunculus*, AT: *A. tuberculata*). A: Points are individual $\delta^{13}C$ and $\delta^{15}N$ meassurements, and colored solid lines the standard ellipse areas corrected for small sample size (SEA_C). B: Boxplots of the SIBER Bayesian ellipse areas (SEA_b in ‰^{2}) for the different species. 50, 75 and 95% credivility intervals are indicated, and dots represent the modes of the Bayesian distributions. The cross indicates the standard ellipse areas computed using the frequentist algorithm for small samples sizes (SEA_c).

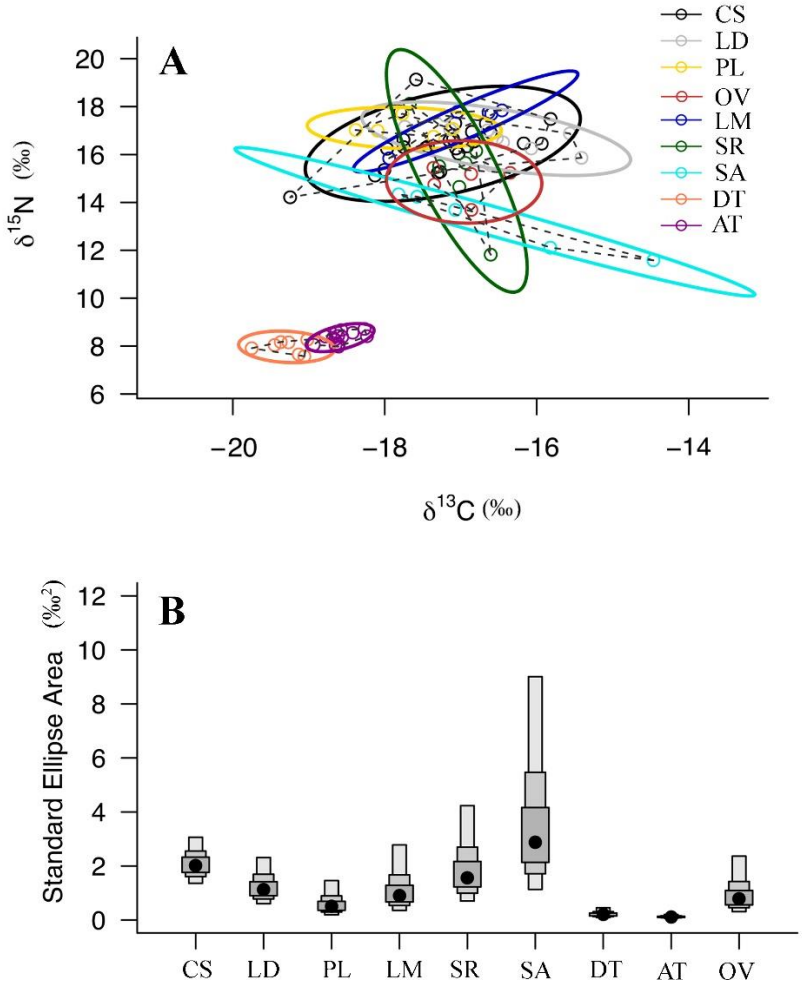
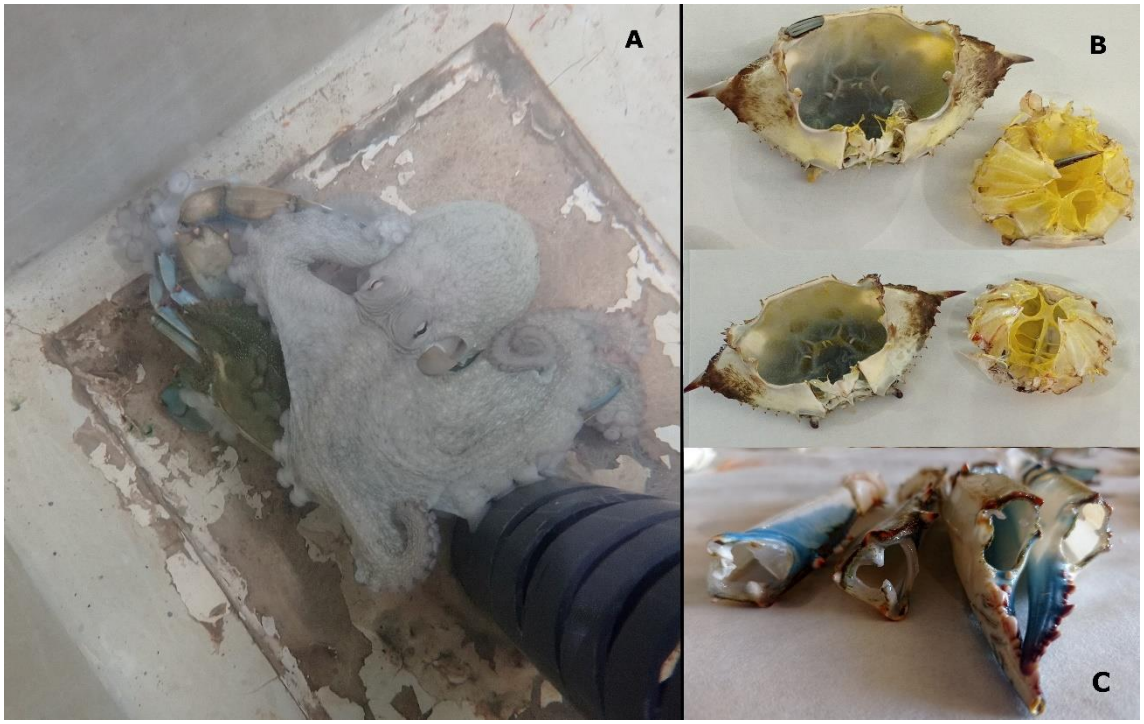


Fig. 4. A: Octopus capturing one blue crab during the stabling period for the evaluation of isotopic enrichment. B: Empty blue crab shells after being consumed by *Octopus vulgaris*.



676 **Table 1.** Results of Trophic Position (TP) for the different predators investigated in this study.
677 For *C. sapidus*, TP values found in other habitats of the Ebro Delta are also shown ([1]Prado et al.
678 2021) for further comparison.

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Animal group	Species	TP
Cephalopoda	*Octopus vulgaris*	3.93 ± 0.09
Crustaceans	*Callinectes sapidus* (open sea)	4.40 ± 0.06
	C. sapidus (Alfacs Bay)[1]	3.06 ± 0.03
	C. sapidus (Tancada lagoon)[1]	2.64 ± 0.21
	Penaeus kerathurus	2.73 ± 0.08
	Liocarcinus depurator	4.46 ± 0.06
Fish	*Pagellus erythrinus*	4.48 ± 0.05
	Lithognathus mormyrus	4.67 ± 0.11
	Sparus aurata	3.44 ± 0.17
	Trachinus draco	3.70 ± 0.12
	Sciaena umbra	3.41 ± 0.05
	Mugil cephalus	3.87 ± 0.07
	Pegusa lascaris	4.59 ± 0.05
	Scophthalmus rhombus	4.06 ± 0.25
	Solea senegalensis	3.24 ± 0.03
	Bothus podas	3.50 ± 0.16

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Table 2. MixSiar results (mean, SD and 2.5, 5, 25, 50, 75, 95, and 97.5 percentiles) showing relative contributions of food resources to blue crab diet. Median values are indicated in **bold**. We used the $\Delta^{15}\delta N$ of 3.4 and a $\Delta^{13}\delta C$ of 0.4 given for non-herbivorous aquatic consumers (Post 2002), and previously used in blue crab (Mancinelli et al. 2016, 2017a, b; Prado et al. 2021).

		Mean	SD	Percentiles						
				2.5%	5%	25%	50%	75%	95%	97.5%
Bivalves	*Acanthocardia tuberculata*	0.073	0.056	0.002	0.005	0.028	**0.062**	0.108	0.180	0.205
	Mactra stultorum	0.072	0.056	0.002	0.004	0.026	**0.059**	0.107	0.180	0.202
	Donax trunculus	0.074	0.053	0.003	0.006	0.031	**0.064**	0.107	0.176	0.197
Crustaceans	*Penaeus kerathurus*	0.076	0.059	0.003	0.006	0.030	**0.063**	0.110	0.194	0.222
	Liocarcinus depurator	0.067	0.055	0.002	0.004	0.023	**0.053**	0.096	0.179	0.207
Fish	*Pagellus erythrinus*	0.065	0.052	0.003	0.005	0.024	**0.053**	0.095	0.169	0.197
	Lithognathus mormyrus	0.060	0.051	0.001	0.003	0.020	**0.048**	0.088	0.162	0.186
	Sparus aurata	0.072	0.059	0.003	0.005	0.026	**0.058**	0.104	0.187	0.218
	Trachinus draco	0.072	0.061	0.002	0.004	0.025	**0.055**	0.103	0.192	0.225
	Sciaena umbra	0.073	0.060	0.002	0.005	0.027	**0.058**	0.106	0.192	0.222
	Mugil cephalus	0.045	0.033	0.002	0.004	0.017	**0.037**	0.064	0.108	0.123
	Pegusa lascaris	0.064	0.051	0.002	0.005	0.023	**0.053**	0.091	0.164	0.190
	Scophthalmus rhombus	0.038	0.035	0.001	0.002	0.012	**0.028**	0.053	0.107	0.135
	Solea senegalensis	0.074	0.064	0.001	0.003	0.023	**0.058**	0.107	0.203	0.237
	Bothus podas	0.074	0.062	0.002	0.005	0.026	**0.059**	0.108	0.193	0.228

Table 3. Summary table of isotopic niche metrics provided by the SIBER package. TA: total area of the convex polygon; SEA_c: standard ellipse area corrected for small sample size; SEA_b: the estimated Bayesian modes of the standard ellipse area.

	CS	**LD**	**PL**	**LM**	**SR**	**SA**	**DT**	**AT**	**OV**
TA	7.78	2.79	0.61	0.53	1.88	1.15	0.27	0.29	0.99
SEA_c	2.14	1.32	0.77	0.77	1.85	1.50	0.25	0.12	1.23
SEA_b	1.97	1.11	0.47	0.84	1.60	3.15	0.19	0.10	0.79