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The trophic position of the Atlantic blue crab *Callinectes sapidus* Rathbun 1896 in the food web of Parila Lagoon (South Eastern Adriatic, Croatia): a first assessment using stable isotopes

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Abstract

The Atlantic blue crab *Callinectes sapidus* is to date widely distributed in the Mediterranean Sea, where it is recognized as an Invasive Alien Species. Noticeably, while records of its occurrence are increasing, quantitative information on its functional role in the benthic food webs of invaded coastal habitats are scarce.

Here, carbon and nitrogen isotopic signatures were measured in *C. sapidus* and other representative taxa of the benthic flora and fauna sampled in summer in the Parila Lagoon, a coastal basin located in the Neretva River estuary area (Croatia). $\delta^{15}N$ values of individual blue crab specimens were used to calculate their trophic position (TP), using the filter feeder mussel *Mytilus galloprovincialis* as the isotopic baseline.

In general, the isotopic signature of *C. sapidus* was comparable with those characterizing invertebrate and fish predators (e.g. *Hexaplex trunculus*, *Gobius niger*). An average trophic position of 3.73 was estimated for the blue crab population. However, a considerable inter-individual variation was observed in TP values, significantly related with the size of the specimens. Interestingly, a quadratic polynomial resulted the best fitting model for the data, characterized for small-size individuals by a positive body size-TP relationship, leveling or even turning negative for larger adult crabs.

The interplay between environmental factors and ontogenetic diet shifts in determining the trophic position of the blue crab and, ultimately, its ecological impact on the benthic community of the Parila Lagoon is discussed.

Keywords: Invasive species, *Callinectes sapidus*, food web, trophic level, ontogenetic diet shift.

Introduction

In the last decades the Mediterranean Sea has experienced a dramatic increase in the frequency of introduction and rate of expansion of alien species (Galil, 2000; Rilov & Galil, 2009; Katsanevakis *et al.*, 2014). Arthropods epitomize this on-going invasion process: in 2008, the CIESM Atlas of Exotic Species listed 70 alien species; three years later, Galil (2011) listed 106 species, while Nunes *et al.* (2014) counted 242 species. According to this recent study, lessepsian migrants from the Red Sea through the Suez Canal provide a considerable contribution (90 species), whose importance is expected to increase due to climate warming (Raitsos *et al.*, 2010; Galil & Goren, 2014). Ballast waters and other shipping-related vectors, however, currently represent the main pathway of introduction (121 species in total), in particular in the North-Western Mediterranean and in the Adriatic Sea.

Among other non-indigenous arthropods, the blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda, Brachyura, Portunidae) is characterized by an almost ubiquitous distribution in Mediterranean waters (Galil, 2011). The species, native of western Atlantic coastal habitats, was introduced in Europe presumably by ballast waters and was recorded for the first time on the Atlantic coasts of France in 1900 (Bouvier, 1901). In the Mediterranean Sea the species appeared in 1949 (Giordani Soika, 1951, but probably as early as 1935, due to intentional release: Enzenroß *et al.*, 1997). Since then, the number of records have increased in the last years throughout the Mediterranean and Black Sea (Castejón and Guerao, 2013; Cilenti *et al.*, 2015; see also Kapiris *et al.*, 2014 for a record in a freshwater habitat) and to date the species is considered invasive (Streftaris & Zenetos, 2006).

Noticeably, scant information are available on the trophic role of *C. sapidus* in European invaded habitats.

The functional importance of the blue crab in coastal ecosystems of NW Atlantic and other native habitats has been repeatedly assessed (e.g. Baird & Ulanowicz, 1989; Dittel *et al.*, 2000, 2006; Hines, 2007); conversely, to date an assessment of the trophic position and potential ecological impact of the species on benthic food webs has been performed only in two coastal basins in SE Italy (Mancinelli *et al.*, 2013a; Carrozzo *et al.*, 2014).

In this study, we analysed the trophic position of *C. sapidus* in the Parila Lagoon, a coastal basin located in the estuary of the Neretva River (Croatia). Interestingly, in this area the occurrence of an established population of *C. sapidus* has been recorded in the last decade (Dulčić *et al.*, 2011 and literature cited), yet no efforts have been made to assess the trophic role of the species. The trophic position of the blue crab was estimated in summer using nitrogen stable isotopes. The analysis of nitrogen stable isotopes ($\delta^{15}N$) has become a popular method to quantitatively assess trophic levels, as they simultaneously capture complex trophic interactions, and integrate the spatial and temporal diet signature of a consumer (Post, 2002; see also Mancinelli & Vizzini, 2015). Here, trophic positions were estimated using filter feeder bivalves (i.e. the mussel *Mytilus galloprovincialis*) as the isotopic baseline; in addition, the carbon and nitrogen isotopic signatures of other representatives of the benthic fauna and flora were determined, in order to cast the isotopic signature of the blue crab within the general framework of the food web of the Parila Lagoon.

Materials and Methods

Study site

The study was carried out in the Parila Lagoon, located in the estuary of the Neretva River, SE Adriatic Sea (Fig. 1). The estuary covers an area of 156 km^2, and extends for approximately 20 km. In the estuary, the river branches into several channels; additionally, the area is characterized by a number of springs, constituting a unique hydrologic system and one of the most important Mediterranean coastal marsh environments (Margeta, 2000; Zovko *et al.*, 2013).

The Parila Lagoon is located northward of the river mouth (Fig. 1); the basin is a shallow (1.5 m maximum depth) water body with a surface area and a perimeter of approximately 2 km^2 and 10 km, respectively. Both water temperature and salinity are characterized by strong seasonal variations, ranging between 7.2° C and 33.4° C, and 11.4 to 34.6 PSU (Dulčić *et al.*, 2011). The lagoon is characterized by muddy and sandy bottoms with extended stands of *Cymodocea nodosa*; hard natural and artificial substrata are located throughout the basin (Glamuzina *et al.*, 2014).

Sampling procedures

Traps of the type described in Carrozzo *et al.* (2014) were used to collect crab specimens. Each trap (60×60×60cm) was made of vinyl-coated 2×2 cm mesh wire with an upper and lower chamber ("kitchen" and "parlour" chamber, respectively; Sturdivant and Clark, 2011). The kitchen had a 15 cm-wide entrance on each of the four sides, and a conical bait well in the centre. The two chambers were separated by a wire-mesh panel, raised in the middle to form an inverted V. There were two openings along the apex of the V leading into the parlour.

In late June 2015, a sampling station was located in the Parila Lagoon (Fig. 1). Four crab traps were submerged at a distance of approximately 100 m from each other and baited with crushed mussels and fish carcasses. Trap deployment was carried out between 9.00 and 10.00 a.m. and they were retrieved the subsequent day after 24 h. After retrieval, crabs were harvested from the traps, stored on ice and transferred to the laboratory in refrigerated containers.

At each sampling occasion, before trap retrieval bottom water temperature, salinity, and oxygen concentration were measured in triplicate with an YSI 556 multiprobe (YSI Inc., Yellow Springs, OH). Representative submerged vegetation was collected by hand after trap deployment. For each taxon, 3 replicates (plants) were sampled at random. Triplicate samples of benthic algae were collected by carefully removing the upper layer of visible mats on rocks and other submerged surfaces. The leaf litter of submerged and riparian plant species was collected from natural accumulations. Contemporaneously, benthic primary consumers, represented by crustacean and gastropod grazers/detritivores/omnivores, and filter-feeder bivalves, were sampled using a hand net and by inspecting submerged rocks or litter accumulations. In addition, representative benthic fish taxa were captured using a seine net operated by two men for one hour within the lagoon.

After collection, floral and faunal samples were placed in separate sterile falcon tubes or plastic bags, labelled and stored on ice.

Laboratory procedures

In the laboratory, crabs were identified to the species level, enumerated, and sexed after examination of the shape of the abdomen. For each specimen the carapace maximum width (CW) was measured as the distance (in mm) between the two outermost lateral spine tips. Specimens with a CW < 94 mm were classified as juveniles (Ju *et al.*, 2001; Mancinelli *et al.*, 2013b). After measurements, crabs were euthanized by thermal shock (-20° C for 10 min).

Floral samples were identified to the lowest taxonomic level possible and inspected to remove contaminants (e.g. shell fragments, bryozoa, periphyton). Similarly, macroinvertebrates were identified to the lowest

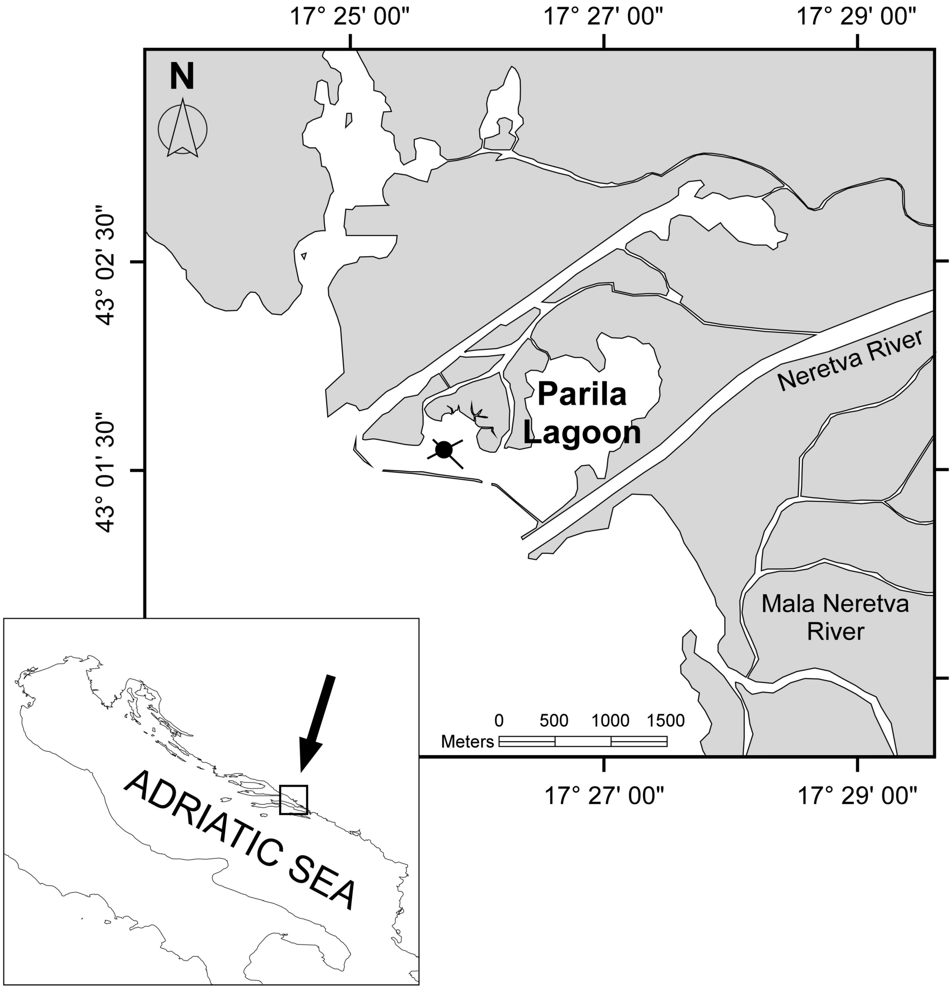

Fig. 1*:* Location of the Parila Lagoon in the Neretva estuary (Croatia). The station where vegetal and animal samples were collected in summer for stable isotope analysis is indicated (full circle).

possible taxonomic level, euthanized and inspected for contaminants; shells were removed from mollusc samples. A sample of dorsal muscle tissue was removed from each fish specimen after identification, while *C. sapidus* and other autochthonous brachyurans had portions of the right claw muscle removed with a ceramic scalpel. All samples were stored in falcon tubes, frozen (-20° C, 48h), freeze-dried for 48 h, and subsequently ground to a fine powder using a mortar. Crabs and large bivalves were ground individually; smaller invertebrates (e.g. amphipods) were powdered in composite samples obtained by combining 2-5 conspecifics of similar size.

Stable isotope analysis and trophic position estimation

Subsamples were pressed into Ultra-Pure tin capsules (Costech Analytical Technologies) and analyzed using an Isotope Ratio Mass Spectrometer (Thermo Scientific Delta Plus XP) connected to an Elemental Analyser (Thermo Scientific Flash EA 1112). Isotopic signatures were expressed in conventional ä notation (as parts per mil) in relation to international standards (PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen). Analytical precision based on the standard deviation of replicates of internal standards (International Atomic Energy Agency IAEA-NO-3 for δ^{15}N and IAEA-CH-6 for δ^{13}C) was 0.2 ‰ for both δ^{13}C and δ^{15}N.

The trophic position (TP hereafter) of brachyuran species and, only for comparative purposes, of individuals of fish species (see results) was calculated according to the equation:

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

The formula is a generalization of that presented in Jepsen and Winemiller (2002), where ä^{15}N$_{\mathrm{Consumer}}$ is the nitrogen isotopic signature of the blue crab, Δ^{15}N is the trophic level fractionation of ä^{15}N, while ä^{15}N$_{\mathrm{Baseline}}$ and λ are the nitrogen isotopic signature and the trophic level of the baseline indicator, respectively. ä^{15}N$_{\mathrm{Baseline}}$ and the associated standard deviation SD$_{\mathrm{Baseline}}$ was calculated using the mean isotopic signature of the filter feeder bivalve *Mytilus galloprovincialis*, expected to be one of the blue crab preferred prey (e.g., Hines, 2007). Accordingly, a λ= 2 was adopted (assuming basal resources = trophic level 1, primary consumers = trophic level 2, etc.).

To consider the effect of inter-individual variability in the nitrogen signatures of prey taxa, a parametric bootstrap procedure was used (Vander Zanden and Fetzer, 2007; see also Mancinelli *et al.*, 2013c). In brief, for each crab and fish specimen, a value was randomly drawn from a distribution of mean $\delta^{15}N_{Baseline}$ and standard deviation $SD_{Baseline}$, and used to calculated a TP value. This procedure was repeated 999 times, and the generated data were averaged to estimate the tropic level of each crab specimen.

TP estimations were performed adopting a mean trophic fractionation $\Delta^{15}N$ of 3.4‰ derived from the literature data on non-herbivorous aquatic consumers (Post, 2002).

Statistical analyses

Values in the text are expressed as mean ± 1 SE if not otherwise specified. For ANOVA and other univariate analyses, data were tested for conformity to assumptions of variance homogeneity (Cochran's C test) and log- or square root-transformed if required. Normality was tested using the Kolmogorov-Smirnov test.When significant effects were detected, multiple comparisons of means were performed using the Tukey HSD test.

For invertebrate and fish taxa characterized by a number of isotopic measurements ≥ 2, inter-specific differences were tested with 1-way permutational multivariate analysis of variance (PERMANOVA) based on a Euclidean distance resemblance matrix constructed using ä^{13}C and ä^{15}N untransformed data. Due to uneven sample sizes, PERMANOVA analyses were performed using a Type III partial sums of squares with 9999 permutations; permutations of residuals were run under a reduced model to maximize the accuracy of Type I error estimates (Anderson *et al.*, 2008). Taxa were considered isotopically indistinct when pairwise tests were characterized by an α level [P(perm): Anderson, 2001] > 0.05; for tests with <10 unique permutations, Monte Carlo p-values were interpreted (Anderson and Robinson, 2003).

In addition, following Mancinelli (2010), Longo & Mancinelli (2014), and Mancinelli & Pasquali (2016), individual TP values of blue crabs were regressed against the respective carapace widths (expressed as log-transformed mm CW) by least squares using a simple linear, or, alternatively, a quadratic polynomial model. Model comparisons were carried out adopting a parsimonious procedure (Burnham & Anderson, 2002) based on the second-order Akaike Information Criterion AICc (Sugiura, 1978, after Akaike, 1974).

In general, all statistical analyses were performed in the R environment (R Development Core Team, 2016). PERMANOVA analyses were performed using the *adonis* and *betadisper* functions of the *vegan* package (Oksanen *et al.*, 2015); the comparison of regression models was performed using the iterative procedure implemented in the *MuMIn* R package (Bartoń, 2016).

Results

During trap deployment operations, the mean water temperature, salinity, and dissolved oxygen concentration measured at the sampling station was 22.3 ± 0.2° C, 31.5 ± 0.5PSU, and 10.7 ± 0.7 mg L^{-1} (mean ± SE).

In total, 14 specimens of *Callinectes sapidus* were sampled; other brachyuran species collected in crap traps were *Pachygrapsus marmoratus* (5 specimens), and *Carcinus aestuarii* (2). Additionally, two specimens of the gastropod *Hexaplex trunculus* were captured.

In general, the majority of the crabs were males, with an overall sex ratio of 0.5:1 (female: male); for *C. sapidus* the sexes were approximately balanced (0.75:1), while in *P. marmoratus* and *C. aestuarii* males dominated (0.25:1 and 0:1, respectively).

The carapace width of individual blue crabs was characterized by an almost three-fold variation (Table 1, max/min = 2.6), with negligible differences between sexes (t-test for separate variances, t = -0.17, P = 0.86, 10.1 d.f.). Specifically, four specimens were characterized by a CW considerably lower than 94 mm (min = 73.4 mm, max = 91.4 mm) and consequently were classified as juveniles; two additional specimens with CWs, measuring 113.2 and 114.2 mm, showed clear juvenile characters. Inter-individual variations in the size of both *P. marmoratus* and *C. aestuarii*, conversely, was considerably lower (Table 1; max/min = 1.6 and 1.4, respectively), even though these differences are not conclusive due to the high variability in sample number across the three crab species (Table 1).

Three taxa of living primary producers - i.e. the macroalgae *Cladophora* sp. and *Ulva* sp., and the vascular plant *Cymodocea nodosa* - and two leaf litters - i.e. *Zostera noltii* and the riparian *Phragmites australis* were collected. On average, the $\delta^{15}N$ and $\delta^{13}C$ signatures of basal resources were 4.81 ± 0.41‰ and -20.18 ± 2.77‰, respectively (mean ± SE, n = 5). Nitrogen isotopic signatures were characterized by a lower variability compared to carbon (CV = 18.9% and 30.7%, respectively; Fig. 1). Extreme values were shown by leaf litter for both $\delta^{15}N$ (3.67‰ and 5.98 for *P. australis* and *Z. noltii*, respectively) and $\delta^{13}C$ (-27.56‰ and -13.27‰ for *P. australis* and *Z. noltii*, respectively).

Eleven taxa of invertebrate primary consumers and five fish species were sampled (Table 1). Among invertebrates, bivalves (i.e. *Cerastoderma glaucum*, *Chamelea gallina*, *Mytilus galloprovincialis*, and *Ruditapes decussatus*), *Patella caerulae* and undetermined Gammaridea were characterized by the lowest $\delta^{15}N$ values (Fig. 1); however, the $\delta^{13}C$ varied considerably, suggesting that different carbon sources contributed to their diet. Indeed, a PERMANOVA analysis followed by pairwise comparisons indicated that *M. galloprovincialis* was significantly different from both *C. glaucum*, *R. decussatus*, and *C. gallina* [max P(perm) = 0.018 for *R. decussatus*]; the latter three species were characterized by negligible differ-

Table 1. List of invertebrate and fish taxa sampled in the Parila Lagoon in summer 2015 and analysed for carbon and nitrogen stable isotopes. The number of samples (N) is included. For brachyurans collected in crab traps, the average carapace width CW (mm; SE in parentheses) and the CW range (mm) are reported.

Origin	Taxon	N	CW	Range (min – max)
Crab trap	*Callinectes sapidus*	14	131.9 (10.5)	73.3 – 190.3
	Carcinus aestuarii	2	42.6 (6.4)	36.2 – 49.1
	Hexaplex trunculus	2		
	Pachygrapsus marmoratus	5	31.1 (2.3)	22.9 – 37.2
Hand net	*Cerastoderma glaucum*	4		
	Cerithium vulgatum	2		
	Chamelea gallina	3		
	Diogenes pugilator	1		
	Gammaridea undetermined	2		
	Mytilus galloprovincialis	3		
	Palaemon adspersus	3		
	Palaemon serratus	2		
	Patella caerulae	2		
	Ruditapes decussatus	3		
	Turritella sp.	1		
Seine net	*Gobius niger*	1		
	Lithognathus mormyrus	1		
	Liza saliens	1		
	Syngnathus typhle	2		
	Zosterisessor ophiocephalus	1		

ences [min P(perm) = 0.19 for the comparison *C. glaucum vs. C. gallina*]. In addition, while no differences were observed between *P. caerulae* and Gammaridea [P(perm) = 0.62], both taxa were characterized by significantly different isotopic signatures compared to bivalves [max P(perm) = 0.016 for the comparison Gammaridea *vs. C. glaucum*]. The gastropod *Turritella* sp. showed an intermediate position, while the hermit crab *Diogenes pugilator* was characterized by an isotopic position close to that of Gammaridea; in both cases the availability of only one specimen prevented inclusion in statistical analyses.

Conversely, the mollusks *Cerithium vulgatum* and *H. trunculus* showed the highest δ^{15}N, and, accordingly, the highest TP values, followed by *C. sapidus* and *C. aestuarii* (Fig. 1, Table 2). PERMANOVA results indicated that, while significant differences occurred between mollusks and brachyurans [max P(perm) = 0.02 for *C. sapidus vs. Cerithium*)], within each taxonomic group negligible differences occurred [min P(perm) = 0.119 for *Hexaplex vs. Cerithium*]. Noticeably, the trophic position of both crab species were comparable to those determined for individuals of predatory fish species (i.e. *Gobius* sp., *Lithognathus mormyrus*, and *Zosterisessor ophiocephalus*: Table 2). The third brachyuran included in this study, *P. marmoratus*, was characterized by an isotopic signature similar to *Palaemon serratus* and *P. adspersus* [min P(perm) = 0.076 for *P. serratus*] and significantly different from the other two crab species [max P(perm) = 0.011 for *C. aestuarii*], with a TP value (2.96) lower by approximately 20% than that of the other brachyurans, and comparable with *Liza saliens* and *Syngnathus typhle* (Table 2).

Noticeably, while no significant pattern occurred between individual *C. sapidus* CWs and δ^{13}C, a significant relationship was observed with δ^{15}N values (Fig. 2). The parsimonious procedure based on Akaike Information Criterion indicated that, compared to a simple linear

Table 2. Trophic positions of *Callinectes sapidus* and other brachyurans captured in crab traps in summer in the Parila Lagoon. SE in brackets; if SE is not reported, the trophic position was calculated for a single specimen. For the sake of comparison, the trophic position of benthivorous fish species sampled by seine net are included.

Group	Species	Trophic position
Brachyurans	*Callinectes sapidus*	3.73 (0.06)
	Carcinus aestuarii	3.63 (0.11)
	Pachygrapsus marmoratus	2.96 (0.05)
Fishes	*Gobius niger*	3.79
	Lithognathus mormyrus	3.87
	Liza saliens	3.19
	Syngnathus typhle	3.25 (0.01)
	Zosterisessor ophiocephalus	3.97

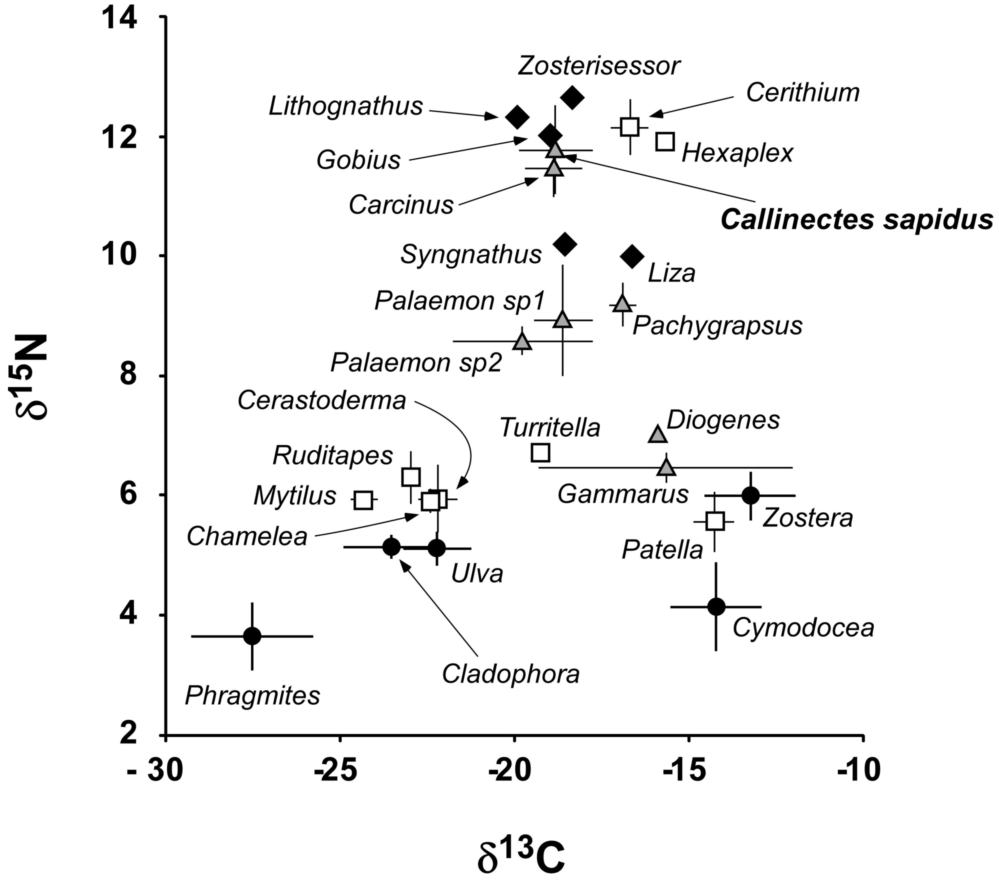

Fig. 2: Bi-plot of $\delta^{13}C$ and $\delta^{15}N$ signatures of basal resources (circles), molluscs (squares), crustaceans (triangles), and fish (diamonds) sampled in the Parila Lagoon in summer. Bars = 1SE; if SE is not reported, isotopic signatures were calculated for a single specimen. *Palaemon* sp1 = *P. adspersus*; *Palaemon* sp2 = *P. serratus*.

model, a quadratic polynomial was characterized by the best fit (Fig. 2; ΔAICc linear *vs*. polynomial = 15.8, $P > 99.3\%$ for the polynomial model). Accordingly, an unimodal pattern was observed for the trophic positions of specimens, increasing from values of 3.34 to 3.58 for juveniles and becoming almost constant around a TP value of 3.78 or even decreasing to TPs of between 3.76 and 3.64 for large-sized adults with a CW > 161 mm.

Discussion

The present investigation provided original data on the isotopic signature of the blue crab *Callinectes sapidus* and other representatives of the benthic flora and fauna of the Parila Lagoon in the Neretva Estuary. The preliminary nature of the study is apparent, as well as its inadequacy in resolving the complexity of the benthic food web of the study area, e.g. regarding the fish community, due to the low number of sampled species and replicates per species. Nonetheless, it presented robust evidence on the trophic position of *C. sapidus* in relation to other native crab and fish species, and highlighted a number of ecological issues that may be further investigated in future, more extended studies. For example, the significant differences in the isotopic signature observed between *Mytilus galloprovincialis* and other bivalve species suggest that they may rely on different carbon and nitrogen sources, e.g. phytoplankton *vs*. sediment organic matter (Riera *et al*., 1999; Herman *et al*., 2000). Future analyses including a wider set of basal resources will clarify this aspect, and verify whether, notwithstanding the low depth of the lagoon, different energy routes originate from the water column and the benthic environment.

The average trophic position calculated here for the blue crab - 3.73 - identified it as a fully carnivorous predator. The trophic position and, in general, the isotopic signatures were comparable with those characterizing *Gobius niger*, *Lithognathus mormyrus,* and *Zosterisessor ophiocephalus*, suggesting that in the Parila Lagoon the blue crab may share the same set of trophic resources with these benthivorous fish species (Stergiou & Karpouzi, 2002).

The TP estimation is consistent with that of other investigations performed using stable isotopes, ranging from 2.8 to 4.3 (Moncreiff & Sullivan, 2001; Abreu *et al*., 2006; Rooker *et al*., 2006; Rodriguez-Graña *et al*., 2008; Winemiller *et al*., 2007; Carrozzo *et al*., 2014). Most importantly, this study indicated that a considerable size-related variation occurs in the trophic position of *C. sapidus*, increasing from values as low as 3.34 for juveniles to values in the 3.7 – 4.11 range for the largest individuals. Stable isotope studies indicate, in general, a positive shift in the trophic position of juvenile blue crabs with increasing sizes in their native habitats (Fantle *et al.*, 1999; Dittel *et al.*, 2000, 2006). The present study confirms these results, and further suggests that, once the adult stage is reached, *C. sapidus* may actually level or even decrease its trophic position, as suggested by the quadratic polynomial resulting the best-fitting model of the δ^{15}N-CW relationship (Fig. 3). The observed variation in TP values may be explained by the progressive exclusion from the diet of plant material, together with the increase in the trophic contribution of invertebrates, including other brachyuran species and conspecifics, as well as fish, living or in the form of carcasses (Hines, 2007). From these findings, it is apparent that the blue crab is characterized by a potentially high ecological impact, being able during ontogeny to interact - by competition or predation - with a wide spectrum of native crab and fish species. Indeed, juveniles have trophic positions comparable with those characterizing the marbled crab *Pachygrapsus marmoratus*, as well as the benthivorous fish species *Liza saliens* and *Syngnathus typhle* (Stergiou and Karpouzi, 2002; Cardona, 2016). The marbled crab *P. marmoratus*, specifically, is an opportunistic omnivore, including, as for the blue crab, both plant and animal prey in its diet (Cannicci *et al.*, 2002; Chartosia *et al.*, 2010). It is worth noting that previous estimations of the trophic position using stable isotopes indicated for the marbled crab a TP value of 2.12 (Carrozzo *et al.*, 2014), fully consistent with a trophic habit including mainly macrophytes. Conversely, in the present study the estimated TP was 2.96 (Table 2), identifying *P. marmoratus* as a predator. In addition, the mean δ^{15}N signature of primary producers was on average 4.4‰ lower than that characterizing the marbled crab (Fig. 2), a value incompatible with the δ^{15}N fractionation generally observed for brachyurans (Carrozzo *et al.*, 2014 and literature cited).

Limpets and mussels are common prey items of *P. marmoratus* in Mediterranean coastal habitats (Cannicci *et al.*, 2002), and both items are extremely abundant on hard substrata in the Parila Lagoon (Carrozzo, personal observation). It can be hypothesized that, given the high availability of these prey items and their similar ^{15}N signatures (5.56 ± 0.51‰ and 5.95 ± 0.13‰ for *P. caerulae* and *M. galloprovincialis*, respectively), *P. marmoratus* opportunistically turned to an almost exclusively carnivorous diet, its trophic niche approaching that characterizing juvenile blue crabs and thus increasing the potential for competition or intra-guild predation within the brachyuran assemblage. This hypothesis is supported by the δ^{15}N values of *Palaemon adspersus* and *P. serratus*. Both shrimp species are benthivorous predators (Guerao, 1993; Guerao & Ribera, 1996), and the nitrogen isotope signatures, substantially identical to those of the marbled crab, indicate a similar carnivorous diet.

Larger *C. sapidus* specimens, conversely, showed a trophic position and isotopic signatures similar to those characterizing the green crab *Carcinus aestuarii*. The green crab, similarly to C. *maenas*, is a voracious shore predator, preying especially on molluscs (Sanchez-Salazar *et al.*, 1987), and is an active scavenger (Moore & Howarth, 1996; Bergmann *et al.*, 2002). It is characterized by trophic habits similar to those shown by adult blue crabs (Hines, 2007); however, its size at maturity is much smaller than that of the blue crab (Berrill, 1982; Kennedy & Cronin, 2007), it is less aggressive and thus

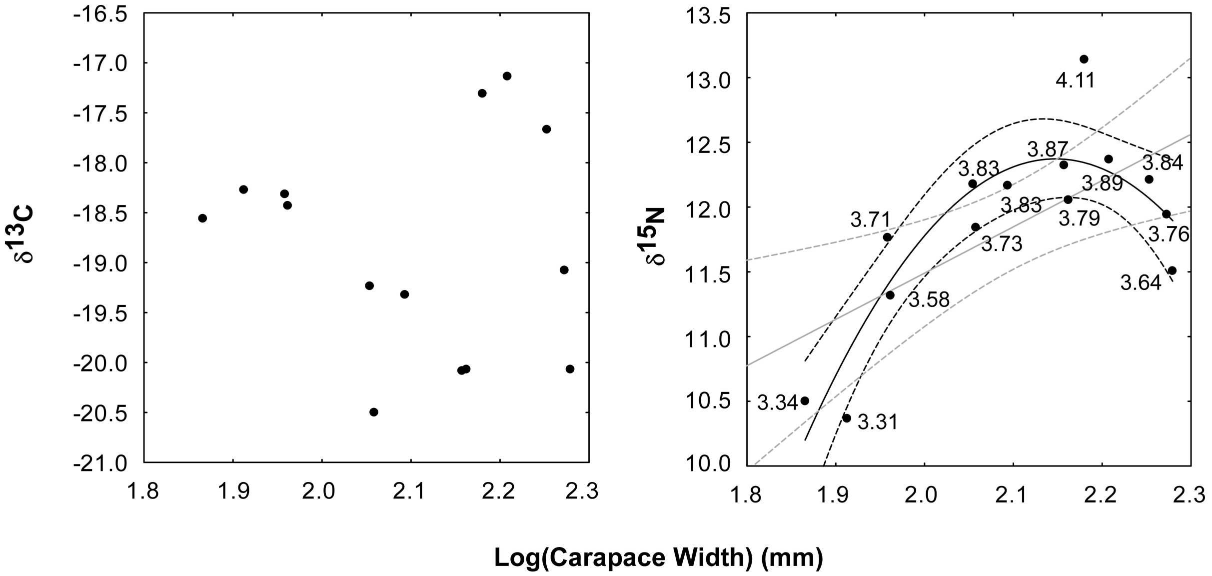

***Fig. 3**:* Relationship between the carapace width (mm) of individual *Callinectes sapidus* and the respective δ^{13}C (left) and δ^{15}N signatures (right). Numbers in the δ^{15}N diagram refer to trophic position estimations. Note that in both diagrams carapace width data in the abscissa have been log-transformed. Continuous lines refer to best-fitting linear (grey) or quadratic polynomial models (black); dashed lines show the respective ±95% confidence intervals.

more susceptible to negative competitive and predatory interactions (Rossong *et al.*, 2012). Indeed, *C. sapidus* is acknowledged as a major predator of *C. maenas* in North American coastal habitats, where the green crab is an invasive species, and at high enough densities can limit green crab populations (deRivera *et al.*, 2005 *et al.*, 2005; see also Johnson, 2015).

In conclusion, although previous investigations have focused on *C. sapidus* populations in the Neretva River estuary (Onofri *et al.*, 2008; Dulčić *et al.*, 2010, 2011), this study is the first using stable isotope analysis to present original and quantitative information on the trophic role of the blue crab, and to cast this invasive species within the trophic structure of the benthic food web. More studies are necessary in order to provide an advanced resolution of the functional role of *C. sapidus* in Croatian waters and assess its potential ecological impact on coastal benthic communities. In particular, future efforts need to verify whether seasonal variations occur in blue crab occurrence and trophic position. Given the low winter water temperatures of the area (Dulčić *et al.*, 2011), the species is expected to be characterized by a period of quiescence and inactivity. Indeed, information regarding captures of individuals in March 2016 report the occurrence of "muddy" individuals (Glamuzina, personal observation), suggesting that they may have recently emerged after overwintering buried in the sediments.

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