Consistent habitat segregation between sexes in the spider crabs *Maja brachydactyla* and *Maja squinado* (Brachyura), as revealed by stable isotopes

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Summary: Differences in the resource use patterns of males and females of the spider crab species *Maja brachydactyla* Balss, 1922 and *M. squinado* (Herbst, 1788) from several geographic areas (three in the Atlantic and two in the Mediterranean) were studied through the analysis of stable isotopes of carbon and nitrogen in the exoskeleton of post-pubertal (adult) specimens. Results confirmed that males and females from the same population usually did not differ in $\delta^{15}N$ values and hence foraged at the same trophic level. In contrast, females were usually enriched in ^{13}C as compared with males from the same population, thus suggesting that females use shallower habitats than males before the terminal moult. The results reported here also indicate that stable isotopes can be useful for the traceability of commercial *Maja* species, but only if species and sex are incorporated in the analysis.

Keywords: *Maja brachydactyla*; *Maja squinado*; stable isotopes; trophic level; sexual segregation; traceability.

Segregación sexual del hábitat en los centollos *Maja brachydactyla* y *Maja squinado* (Brachyura) revelada mediante isótopos estables

Resumen: Se estudiaron mediante el análisis de isótopos estables de carbono y nitrógeno en el exoesqueleto de ejemplares adultos las diferencias en los patrones de uso de recursos de los machos y las hembras de los cangrejos *Maja brachydactyla* Balss, 1922 y *M. squinado* (Herbst, 1788) procedentes de varias zonas geográficas (tres en el Atlántico y dos en el Mediterráneo). Los resultados confirmaron que los machos y las hembras de la misma población, por lo general, no difieren de los valores de $\delta^{15}N$ y por lo tanto se alimentaban en el mismo nivel trófico. En contraste, las hembras por lo general se hallan enriquecidas en ^{13}C en comparación con los machos de la misma población, lo que sugiere que las hembras ocupan hábitats menos profundos que los machos antes de la muda terminal. Los resultados aquí presentados indican también que los isótopos estables pueden ser útiles para la trazabilidad de las especies comerciales del género *Maja*, pero sólo si la identidad de la especie y el sexo del ejemplar se incorporan al análisis.

Palabras clave: *Maja brachydactyla*; *Maja squinado*; isótopos estables; nivel trófico; segregación sexual; trazabilidad.

INTRODUCTION

The spider crabs of the genus *Maja* Lamarck, 1801 (Majoidea: Majidae), with around 20 described species worldwide, are represented on European coasts by four species: *M. brachydactyla* (Balss, 1922), *M. crispata* (Risso, 1827), *M. goltziana* (D'Oliveira, 1888) and *M. squinado* (Herbst, 1788) (Neumann 1998, Ng et al. 2008, Sotelo et al. 2008, 2009). *M. brachydactyla* and *M. squinado* are the largest European species of the genus and are found from subtidal areas to about 90 m depth (De Kergariou 1984, Števčić 1973). *M.*

brachydactyla inhabits the eastern Atlantic from the British Isles to Senegal and has a high commercial value, while *M. squinado* inhabits the Mediterranean and is considered an endangered species (UNEP 1996, Freire et al. 2002, Martín et al. 2012, Sotelo et al. 2008, Guerao and Rotllant 2010, Guerao et al. 2011, Abelló et al. 2014), although it was abundant until the first half of the 20th century (Pons Muñoz 1994). Until recently, *M. brachydactyla* was considered a synonym of *M. squinado* (Zariquiey-Álvarez 1968, Neumann 1996, 1998, Ng et al. 2008), but molecular studies indicated that *M. squinado* is more closely related to *M. crispata* than to *M. brachydactyla* (Sotelo et al. 2009). Molecular analysis also revealed that the divergence between *M. brachydactyla* and *M. squinado-M. crispata* clade occurred in the Miocene (9.5 mya) and the divergence between *M. squinado* and *M. crispata*, occurred in the Pliocene (3.9 mya). Accordingly, the large morphological resemblance between *M. brachydactyla* and *M. squinado* could be also due to convergent evolution (see Neumann 1996, 1998, Sotelo et al. 2008, 2009 for taxonomic status). A diagnostic tool was developed to identify the four species of *Maja* in Europe with morphological and molecular methods (Guerao et al., 2011). Adults of *M. brachydactyla* and *M. squinado* are commercially exploited species and a simple relationship between carapace length (CL) and antorbital spine length could be used to differentiate the two species.

Marine brachyuran crabs are usually opportunistic consumers (Števčić 1967, Choy 1986, Abelló and Cartes 1987, Abelló 1989) and this is also true for *M. brachydactyla* according to studies based on stomach contents (De Kergariou 1974, Bernárdez et al. 2000): *M. brachydactyla* feed on a large variety of preys, consuming 50% of algae and a mixture of animals (mollusks, crustaceans, echinoderms, etc.). De Kergariou (1974) and Bernárdez et al. (2000) found differences in the diet between males and females and between juveniles and adults which they attributed to an uneven bathymetric distribution, a pattern typical of many large brachyuran crabs (e.g. Bennett and Brown 1983, Hines et al. 1987, Spivak et al. 1994). However, recent research using stable isotopes has failed to find any differences between sexes in diet or habitat use patterns either in adults (Bodin et al. 2007) or juveniles (Bodin et al. 2007, Freire et al. 2009).

Stable isotope ratios of carbon and nitrogen are widely used in ecology for the study of trophic relationships and habitat use patterns, because the stable isotope ratios in a consumer's tissues reflect those in the diet, which in turn depends on the relative availability of the light and heavy isotopes of each element for primary producers (Hobson 1999, Koch 2008, Graham et al. 2010). Marine macrophytes are typically enriched in ^{13}C compared with phytoplankton, but macrophytes occur only in shallow waters and hence the stable isotope ratio of carbon consumers typically decreases with depth in coastal areas (e.g. Cardona et al. 2007, Drago et al. 2009, Freire et al. 2009, Pinela et al. 2010). Accordingly, the stable isotope ratio of carbon can be used to explore intraspecific differences in bathymetric distribution by sex or age, although tissue selection is critical because stable isotope turnover rates are tissue-dependent (Martínez del Río et al. 2009).

Previous studies on *M. brachydactyla* using stable isotope ratios concluded that no sexual difference existed in diet or habitat use (Freire et al. 2009, Bodin et al. 2007), in contrast with earlier studies based on stomach contents (De Kergariou 1974, Bernárdez et al. 2000). This incongruence may be due to the fact that they analysed tissues with a relatively high turnover rate sampled from specimens collected from the same habitat. This experimental design was useful to assess the existence of dietary differences between groups in the same habitat, but likely failed to detect any previous habitat segregation.

Tissues that remain inert after synthesis (e.g. feathers, hair, nails and arthropod exoskeleton) are a good choice for the study of habitat use patterns, as they record the unmodified stable isotope ratios of the habitat where they were formed (Hirons et al. 2001, Cherel and Hobson 2005). The organic matter in the exoskeleton, a mixture of chitin and proteins, offers a good alternative, as the stable isotope ratios of arthropod exoskeleton remain stable after synthesis (Hobson et al. 1999) and spider crabs have a terminal moult, i.e. they do not moult after the moult of maturity. The last intermoult period is the longest representing several months (González-Gurriarán et al. 1995, Corgos et al. 2007). During the moulting process, energy reserves are used to form the exoskeleton of these crabs (Vernet and Charmantier 1994), so the imprint of feeding during the last intermoult will be recorded in the carapace of the crabs. Thus, stable isotope ratios in the carapace are expected to represent a long-term signal of habitat use, compared to muscle and hepatopancreas tissues.

The aim of this study was to test the hypothesis that, within every population of *M. brachydactyla* and *M. squinado*, males and females forage at the same trophic level but differ in bathymetric distribution, and that this pattern is consistent across populations and species.

MATERIALS AND METHODS

Sampled individuals

In 2007-2009, 98 adult specimens of *M. brachydactila* and *M. squinado* (CL=141±20 mm) were collected by commercial fishery vessels in the Atlantic and Mediterranean Sea (Table 1, Fig. 1). Males and females were collected during the same sampling trials from all localities, except from Morocco, where only males were available.
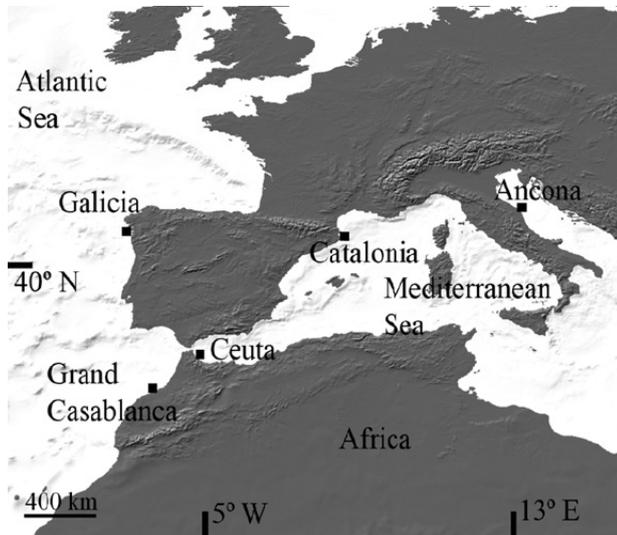
Stable isotope analysis

Exoskeleton samples were collected from the propodus and dactylus segments without debris areas of the pereiopods of individuals from each population and sex. Exoskeletons were chosen to have at least a one-year signature integrating fed intake in a broad area of distribution where specimens lived and fed.

Samples were stored frozen at –20°C, thawed, and dried at 60°C for 48-72 h. Then, each sample was sub-

Table 1. – Sampling locations for isotope studies of *Maja brachydactyla* and *Maja quinado*.

Species	Collection sites	n	Coordinates	year
M. brachydcatyla	E Atlantic (Galicia, Spain)	19	42°16'00.22"N; 9°01'04.99"W	2007-08
	E Atlantic (Grand Casablanca, Morocco)	31	33°49'43.58"N; 7°25'40.17"W	2009
	SW Mediterranean (Ceuta, Spain)	10	35°40'19.19"N; 5°34'46.82"W	2009
M. squinado	W Mediterranean (Catalonia, Spain)	15	42°08'22.75"N; 3°29'40.01"E	2007-09
	Central Mediterranean (Adriatic Sea, Italy)	23	43°40'59.56"N; 13°46'02.53"E	2009

Fig. 1. – Map of sampling locations for isotope studies of *Maja brachydactyla* and *Maja quinado*.

divided in two sub-samples. Lipids were removed from one of them by rinsing the powdered samples several times with a 2:1 chloroform:methanol solution (Bligh and Dyer 1959). The same sub-samples were then treated for decarbonation with a 0.5 M hydrochloric acid (HCl) solution (e.g. Drago et al. 2009, Eder et al. 2012). As lipid extraction and decarbonation with HCl may affect the $\delta^{15}N$ values (Bunn et al. 1995, Yokoyama et al. 2005), the untreated exoskeleton samples were used to measure $\delta^{15}N$. One milligram of sample was used for $\delta^{15}N$ determination and 0.5-0.7 mg of sample was used for $\delta^{13}C$ determination. The samples were weighed in tin cups (3.3-5 mm) and combusted in a continuous-flow isotope-ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA, Thermo Finnigan, Bremen, Germany). The samples were processed at the Serveis Científics i Tecnològics of the University of Barcelona.

Isotopic composition was expressed in the standard δ notation in parts per thousand (‰) relative to predefined international standards, V-PDB (Vienna Pee Dee Belemnite) calcium carbonate for $\delta^{13}C$ and atmospheric N_2 (air) for $\delta^{15}N$, according to:

$$\delta X = [(R_{sample} / R_{standard}) - 1]\, 10^3$$

where X is ^{13}C or ^{15}N, R_{sample} is the heavy-light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and $R_{standard}$ is the heavy-light isotope ratio in the reference standards. The conventional use of these internationally accepted standards with relatively low and high levels of ^{13}C and ^{15}N, respectively, results in negative carbon and positive nitrogen isotope values. The international stable isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the International Atomic Energy Agency (IAEA, Vienna, Austria), namely polyethylene (IAEA CH_7, $\delta^{13}C=-31.8‰$), L-glutamic acid (IAEA $USGS_{40}$, $\delta^{13}C=-26.3‰$) and sucrose (IAEA CH_6, $\delta^{13}C=-10.4‰$), were used for calibration at a precision of 0.2‰. For nitrogen, international stable isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N1, $\delta^{15}N=+0.4‰$ and IAEA N2, $\delta^{15}N=+20.3‰$), L-glutamic acid ($\delta^{15}N=-4.5‰$), and caffeine (IAEA 600 $\delta^{15}N=+.0‰$), were used to a precision of 0.3‰.

Data analysis

Two-way analysis of variance (ANOVA) (sex x population) was used to test whether males and females differed in $\delta^{13}C$ and $\delta^{15}N$ values consistently across populations and species. As only males were available from Morocco, one-way ANOVA was used to analyse the effect of population on the stable isotope ratios of males.

We estimated the isotopic niche width using the convex hull area in the isotopic space defined by $\delta^{13}C$ and $\delta^{15}N$ (Layman et al. 2007). However, the use of Euclidean methods, such as convex hulls to define the isotopic niche space of a species in a community (Layman et al. 2007), is subject to sampling biases and sensitive to sample size (Jackson et al. 2011). The standard ellipse areas estimated by Bayesian inference can incorporate uncertainties such as sampling biases and small sample sizes into niche metrics (Jackson et al. 2011). Then we estimated the width of the isotopic niche at the population level using the Bayesian standard ellipse area by the function SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011) of the library SIAR (Stable Isotope Analysis in R; Parnell et al. 2010) in the free software R (R Core Team 2013). Furthermore, we calculated the magnitude of the isotopic overlap between sexes in each population based on 100000 posterior draws of the standard ellipse areas corrected for small sample size parameters (Jackson et al. 2011). This approach, based on Markov-chain Monte Carlo simulation, assigns measures of uncertainty to construct parameters of ellipses in a similar way to a bootstrap.

Discriminant Analysis was applied to $\delta^{13}C$ and $\delta^{15}N$ to test their reliability when used for individual assignation to the population of origin.

RESULTS

Figure 2 shows the $\delta^{13}C$ and $\delta^{15}N$ values of males and females of *M. brachydactyla* and *M. squinado*
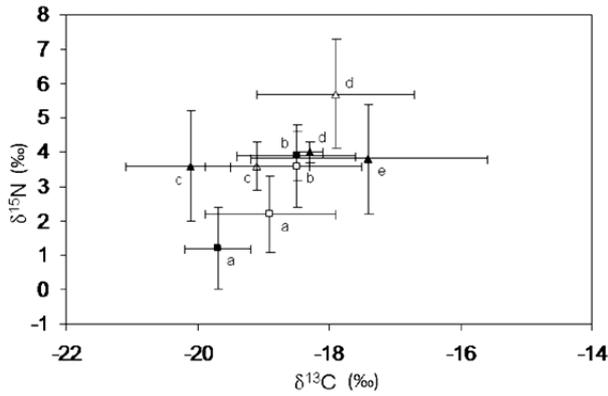
Fig. 2. – Distribution of $\delta^{13}C$ and $\delta^{15}N$ values (mean±SD) among population and sex of *Maja brachydactyla* (triangles) and *M. sqinado* (squares). Sex is denoted by colour (males, black; females, white) and population by letter: a, Ancona; b, Catalonia; c, Ceuta; d, Galicia; e, Morocco.

Table 2. – Results of the Tukey post-hoc tests. Values are the regional averages.

Design	Location	Homogenous clusters		
		Group 1	Group 2	Group 3
^{13}C; both sexes	Ceuta	−19.6		
	Ancona	−19.3	−19.3	
	Catalonia		−18.5	−18.5
	Galicia			−18.1
^{15}N; both sexes	Ancona	1.7		
	Ceuta		3.6	
	Catalonia		3.8	3.8
	Galicia			5.0
^{13}C; males	Ceuta	−20.1		
	Ancona	−19.6	−19.6	
	Catalonia		−18.5	
	Galicia		−18.3	−18.3
	Morocco			−17.1
^{15}N; males	Ancona	1.2		
	Ceuta		3.6	
	Catalonia		3.9	
	Galicia		4.0	
	Morocco		4.5	

from the five considered populations. Males were consistently depleted in ^{13}C when compared with females from the same population (ANOVA, model: $F_{7.64} < 0.001$, sex: $F_{1.64} < 0.023$, population, $F_{3.64} < 0.001$). Furthermore, differences among populations were unrelated to species identity, as a Tukey post-hoc test revealed differences between the $\delta^{13}C$ of *M. brachydactyla* from Ceuta and Galicia, but no differences between the $\delta^{13}C$ of *M. brachydactyla* from Ceuta and that of *M. squinado* from Ancona or between the $\delta^{13}C$ of *M. squinado* from Catalonia and the $\delta^{13}C$ of *M. brachydactyla* from Galicia (Table 2). That geographic pattern did not change when the males from Morocco were incorporated into the analysis and all the females were removed (ANOVA, model: $F_{4.42} < 0.001$), as a Tukey post-hoc test revealed differences between the $\delta^{13}C$ of *M. brachydactyla* from Ceuta and those from Galicia and Morocco but no differences between the $\delta^{13}C$ of *M. squinado* from Catalonia and the $\delta^{13}C$ of *M. brachydactyla* from Galicia and those from Morocco.

Statistically significant differences were observed among populations for $\delta^{15}N$ values, but differences between sexes were not consistent across populations, although results were on the verge of similarity (ANOVA, model: $F_{7.64} < 0.001$, sex: $F_{1.64} < 0.083$, population, $F_{3.64} < 0.001$). Differences among populations were also unrelated to species identity, as a Tukey post-hoc test revealed differences between the $\delta^{15}N$ of *M. brachydactyla* from Ceuta and Galicia but no differences between the $\delta^{15}N$ of *M. squinado* from Catalonia and the $\delta^{15}N$ of *M. brachydactyla* from Galicia (Table 2). The same was true when the females were removed from the analysis and the males from Morocco were incorporated, as only the males of *M. squinado* from Ancona were statistically different from the males of *M. squinado* from Catalonia and those of *M. brachydactyla* from Ceuta, Morocco and Galicia (ANOVA, model: $F_{4.42} < 0.001$: Table 2).

The Bayesian ellipses of females were larger than those of males in the population of *M. brachydactyla* from Galicia and in the two populations of *M. squinado* from Catalonia and Ancona, whereas the opposite was true for the population of *M. brachydactyla* from Ceuta
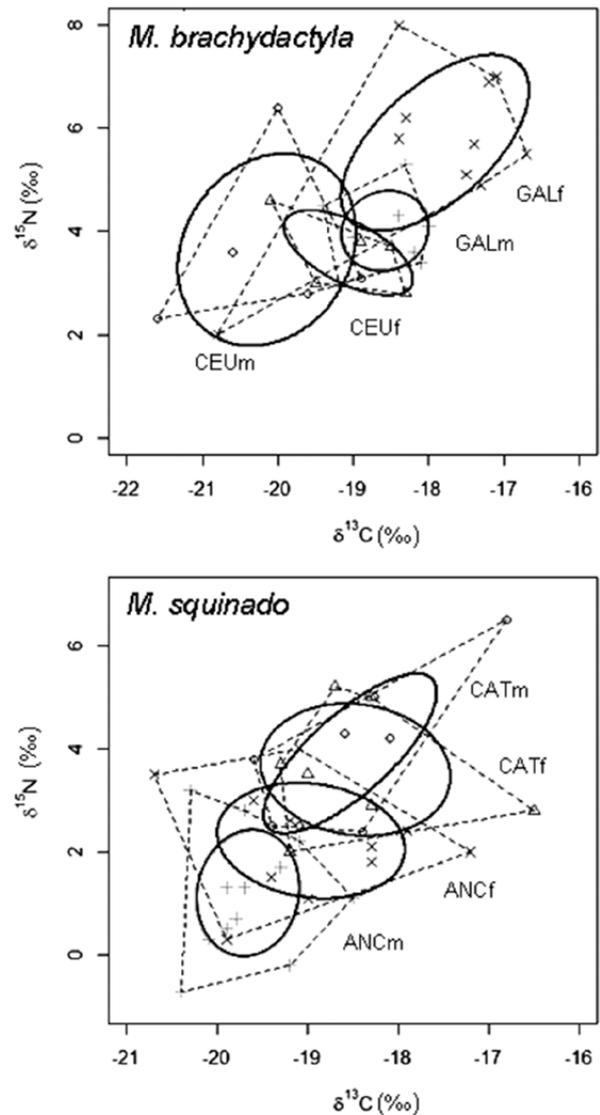

Fig. 3. – Bayesian standard ellipse areas (solid lines) and their respective convex hulls (dashed lines) for *Maja brachydactyla* (top panel) and *Maja squinado* (bottom panel), calculated by SIBER using the bivariate isotopic values (individual symbols) of each species, population and sex.
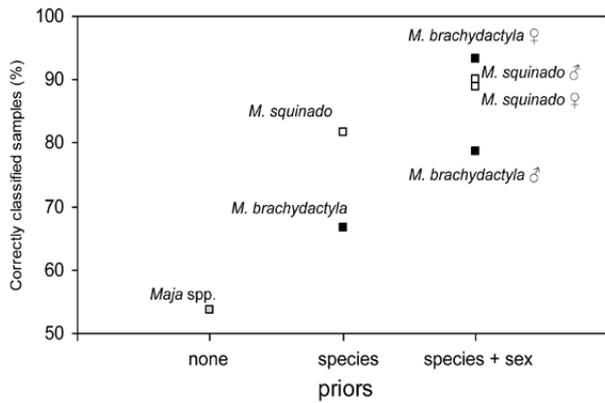
Fig. 4. – Performance of discriminant analysis based on $\delta^{13}C$ and $\delta^{15}N$ without any prior information, incorporating species identity, and incorporating species and sex.

(Fig. 3). Everywhere, the overlap of the Bayesian ellipses of both sexes was highly asymmetrical. In the population of *M. brachydactyla* from Galicia and in the two populations of *M. squinado* from Catalonia and Ancona, the overlap area represented a larger fraction of the surface of the male ellipses than that of the female ellipses (42.1% vs. 11.3%, 74.3% vs. 52. 9% and 32.6% vs. 19.7% respectively), whereas the opposite was true for the population of *M. brachydactyla* from Ceuta (males, 13.8%; females, 53.5%).

Discriminant analysis ignoring species and sex classified correctly only 53.7% of the samples (Fig. 4), which is significantly higher than expected by chance but too low for traceability. Individual assignation improved when discriminant analysis was run independently for each species, as 81.6% of the samples of *M. squinado* and 66. 7% of those of *M. brachydactyla* were classified correctly. The highest accuracy was achieved when the discriminant analysis was run independently for the males and females of each species (Fig. 4). Figure 4 shows the accuracy of individual assignments for each species, sex and locality. The lowest accuracy was achieved for males of *M. brachydactyla* from Galicia (70%) and the highest for the females of the same species from Ceuta (100%) and males of *M. squinado* from Ancona (100%).

DISCUSSION

Maja brachydactyla is an opportunistic species that consumes any range of food items that is available (Števčić 1967, De Kergariou 1974, Bernárdez et al. 2000). Although there are no studies on the feeding habits of *M. squinado*, it seems quite possible that this species is also an opportunistic feeder. Both species have a complex biological cycle, with varying habitat requirements and changes in behaviour depending on the growth phase and the specific time period in the life history (Števčić 1973, González-Gurriarán and Freire 1994, Hines et al. 1995, González-Gurriarán et al. 2002). The juvenile stage lasts approximately two years until a terminal moult, at which time sexual maturity is achieved (González-Gurriarán et al. 1995, Hines et al. 1995, Sampedro et al. 1999). After the terminal moult, the adult individuals migrate to deeper waters (González-Gurriarán et al. 2002), where mating probably occurs. Only the adult females return to the shallower waters to spawn. In terms of carbon and nitrogen stable isotopic composition, tissues with high turnover rates (muscle and hepatopancreas) reflect the diet in the short term, and could be indicative of differences in diet during the days or weeks previous to their capture and analysis (Freire et al. 2009). However, the carapace provides an integrated signal of the animal assimilated diet on a long-term time scale (Hobson et al. 1999). In the present study, all animals were post-pubertal specimens and their exoskeleton was formed before the terminal moult (Sampedro et al. 1999). Therefore, isotope signatures may provide insight into the trophic preferences during the last intermoult period before the animals reached sexual maturity (<3 months; Corgos et al. 2007).

As expected, the stable isotope ratios of carbon and nitrogen in *M. brachydactyla* and *M. squinado* differed dramatically among populations, independently of species identity, hindering the capacity of stable isotopes for traceability unless species and sex are included in the analysis as priors. The relative abundance of ^{15}N in marine primary producers and their consumers depends on the balance of fixed and recycled nitrogen and is usually very low in oligotrophic regions (Graham et al. 2010, Somes et al. 2010), so it is lower in the Mediterranean than in the adjoining Atlantic (e.g. Borrell et al. 2006, Gómez-Díaz and González-Solís 2007, Giménez et al. 2013). Actually, the lowest $\delta^{15}N$ values were observed in the specimens of *M. squinado* from Ancona, likely to be captured in the northern Ionian Sea, a region characterized by very low $\delta^{15}N$ values in particulate organic matter (Pantoja et al. 2002). Moreover, the relative abundance of ^{13}C in marine primary producers and their consumers depends on the availability of inorganic carbon within their boundary layer, which in turn depends on primary productivity, water turbulence, and plant architecture (Post 2002). Interestingly, the highest values were observed in specimens from Morocco, Galicia and Catalonia, which are the most productive of the five regions considered (Longhurst 1998, Bosc et al. 2004). This result suggests that the highest interpopulation variability in the $\delta^{13}C$ values of the two species of crabs considered here depends on the intensity of the regional primary productivity, although other factors such as differences in the arrival of terrestrial allochthonous organic matter may also play a role.

The $\delta^{13}C$ values within an area are also influenced by depth, because macrophytes are typically enriched in ^{13}C when compared with phytoplankton in both the Atlantic (Freire et al. 2009) and the Mediterranean (Cardona et al. 2007), but macrophytes occur only in shallow waters. As a consequence, the $\delta^{13}C$ values typically decrease in deeper, off-shore habitats as compared with shallow, on-shore habitats where the relevance of macrophytes as a source of organic carbon is higher (e.g. Cardona et al. 2007). In this scenario, the consistent enrichment in ^{13}C of females across populations and species suggests that female spider crabs use

shallower habitats than males before the post-pubertal moult. Furthermore, Bayesian ellipses show that sexes differ dramatically in the breadth of their isotopic niches in all the populations studied and that females usually have a broader isotopic niche than males, except for *M. brachydactyla* in Ceuta.

These results are in agreement with the strong evidence that spatial and seasonal segregation exists by sex between adults and reproductive stage in this group of species (De Kergariou 1971, 1984, Števčić 1973, Rodhouse 1984, González-Gurriarán and Freire 1994, González-Gurriarán et al. 2002, Corgos et al. 2007, Fahy and Carroll 2009). *M. brachydactyla* adults migrate to deep water in association with gonad maturation and the development of seminal receptacles in the females. They return to shallow inshore water to incubate eggs and release larvae. However, males migrate longer distances than females (Fahy and Carroll 2009). Our results suggested that males and females may be spatially segregated before the terminal (pubertal) moult, as indicated by $\delta^{13}C$ values in their exoskeletons, indicating that pre-pubertal males migrate to deeper waters than females, though females may remain in shallow waters and migrate to deep water mainly for coupling. However, $\delta^{13}C$ short-term isotopic signature values in the muscle of migrating males and females were similar in *M. brachydactyla* from western Brittany (Bodin et al. 2007), indicating that both sexes co-habited and preyed on similar food items during migratory periods.

The $\delta^{15}N$ values of individuals from the same species inhabiting the same area may also vary, depending on their trophic level (Post 2002). In the present study, the absence of statistically significant differences between sexes for $\delta^{15}N$ suggests that males and females did not differ consistently in their trophic level, although differences may exist in some populations. Experiments in captivity suggest that the exoskeleton of crustaceans has a negative ^{15}N fractionation, in contrast with the positive fractionation of muscle (Yokoyama et al. 2005). These differences explain why the $\delta^{15}N$ values reported here are much lower than those reported for the muscle of *M. brachydactyla* (Bodin et al. 2007, Freire et al. 2009). Considering an average diet-to-exoskeleton fractionation of –2.4‰ reported by Yokohama et al. (2005), the $\delta^{15}N$ values reported here for *M. brachydactyla* from Galicia suggest a diet with a $\delta^{15}N$ value of approximately 7.4‰, consistent with an omnivorous diet with a strong plant component according to the regional isospace (Freire et al. 2009). Likewise, the $\delta^{15}N$ values reported here for *M. squinado* from the Mediterranean (2.5‰) suggest a diet with a $\delta^{15}N$ value of approximately 4.9‰, also consistent with an omnivorous diet with a strong plant component according to the regional isospace (Cardona et al. 2007).

Stomach content studies have shown that macroalgae were food items of importance in the diet of these species (Števčić 1967, Bernárdez et al. 2000). Isotopic signals from the current study suggested that macroalgae seemed to be better digested and assimilated than other preys ingested (e.g. crustaceans, mollusks and echinoderms). This hypothesis is supported by the high level of α-amylase activities found in adult specimens held in captivity, which were one order of magnitude higher than total proteases (Rotllant et al. 2013). In the present study, differences between sexes for $\delta^{15}N$ values were significant for populations of *M. brachtylatyla* from Galicia and *M. squinado* from Ancona. The low $\delta^{15}N$ values observed in males indicate a higher capacity to digest organic material from vegetal origin, which is supported by their higher digestive enzyme capacities, in particular in α-amylase activity, observed in males compared to females caught in Galicia (Rotllant et al. 2013). In our study, higher $\delta^{15}N$ values were observed in Atlantic populations than in Mediterranean ones, as previously reported for other species (e.g. Borrell et al. 2006, Gómez-Díaz and González-Solís 2007, Giménez et al. 2013).

The results reported here are useful for product traceability. In the Spanish market, during the whole year, but particularly during the spider crab closed season (May to November approximately, annual variations depending on total fishing quotas), the fisheries market is supplied by *M. brachydactyla* specimens caught in Ireland, France, the United Kingdom and/or Morocco (Mercasa 2007), although crabs from Galicia fetch higher prices. In this study, stable isotope signatures for commercial *Maja* species allow their geographical origin of capture to be identified. Thus, this methodology could be used for fisheries management to identify the origin of the product, prevent fraud and guarantee its traceability along the commercial chain.

ACKNOWLEDGEMENTS

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