

Complex patterns of habitat use by juvenile spider crabs *Maja brachydactyla* revealed by stable isotope analyses

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ABSTRACT

Patterns of habitat use by spider crab juveniles, *Maja brachydactyla*, from two geographic areas in the NW coast of the Iberian Peninsula, were analyzed through the analysis of Carbon and Nitrogen stable isotope relations in two tissues (muscle and hepatopancreas). The main organisms that constitute the diet of spider crab juveniles in rocky and sandy habitats and the different organic matter sources in coastal food webs were analysed too. The isotopic ratios of C and N did not show any difference between rocky and sandy habitats. The use of carapace colour and epibiosis as an indicator of habitat use was not supported by our data. These results suggest much more frequent movements between both types of habitats than those suggested by former studies (carried out using behavioural observations and sampling distributions) that hypothesized seasonal and ontogenetic changes. In the coastal food web, two main trophic compartments were identified according to the organic matter source: one based in plankton and seaweeds, that corresponds, mainly, with rocky habitats, and another based in sedimentary particulate organic matter that correspond with sandy bottoms. The relationships observed between the C and N isotopic ratios and the proportions of lipids and proteins in muscle and hepatopancreas seem to indicate a clear relation between the trophic origin of the food consumed by the spider crab juveniles and its energetic condition, and the individuals that spent more time feeding in sandy areas would show a better energetic condition. Juveniles of *Maja brachydactyla*, independently of the habitat of capture, would consume in average approximately two thirds of the preys in rocky habitats and a third in sedimentary habitats. The results obtained indicate that, in exposed environments, large

juveniles spend most of the time in sedimentary bottoms, where they find more refuge, but they move frequently (probably at night) to the nearby rocky substrates to feed.

INTRODUCTION

Juvenile spider crabs *Maja brachydactyla* (Decapoda, Brachyura) (the NE Atlantic species previously known as *M. squinado*, see Newman, 1998 for taxonomic status) inhabit shallow waters (down to 10-15 m deep), using both rocky and sandy substrates and performing slow ($<100 \text{ m d}^{-1}$), small scale non-directional movements (González-Gurriarán & Freire 1994, Hines et al. 1995). Recent evidence points to ontogenetic movements when crabs are one year old and approximately 60 mm carapace length (CL), from very shallow rocky substrates (approx. 5 m), where the postlarval settlement would take place, to deeper rocky or sandy bottoms (5-20 m). Within this depth range, juveniles would spend their second year, after which they attain sexual maturity (Freire et al. 2002, Corgos 2004). Some months later, adults migrate to deeper waters ($>50 \text{ m}$), where they mate (González-Gurriarán et al. 1998).

The patterns of habitat use (especially regarding substrate type) of juvenile spider crabs in shallow waters is still to be discussed (González-Gurriarán et al. 1994, Hines et al. 1995, Freire et al. 2002), regarding the geographical and small-scale differences observed in habitat selection. Geographical variability in selected substrate type might be related to the degree of exposure, with crabs mostly selecting rocky areas in protected sites and sandy areas in more exposed sites (Corgos 2004). On the other hand, this author also points out the occurrence of variability in habitat selection by juveniles within a certain site, which is still to be explained.

Furthermore, there is a high variability in body coloration and epibiosis within and among local populations, which was interpreted to be related to habitat use, taking into account that the species presents masking behaviour (Parapar et al. 1997, Fernández et al. 1998). Therefore, crabs inhabiting soft bottoms are mostly slightly coloured (known as “white” crabs) and show little epibiosis, while in rocky habitats bright red-coloured crabs with abundant epibiosis dominate. Corgos (2004) observed that an important number of “red” crabs with algal epibiosis, episodically appeared in sandy areas, proposing the hypothesis of ontogenetic habitat changes (shifting from rocky to sandy areas when juveniles attain one year).

Relation among different stable carbon ($^{12}\text{C}/^{13}\text{C}$, expressed as $\delta^{13}\text{C}$) and nitrogen ($^{14}\text{N}/^{15}\text{N}$, expressed as $\delta^{15}\text{N}$) isotopes, is widely used tool for the study of trophic relationships (Peterson & Fry 1987, Wada & Hattori 1991, Cabana & Rasmussen 1994, Michener & Schell 1994) and food webs (Newell et al. 1995, Ben-David & Schell 1997, Burns & Walter 2000, Fry & Smith 2002, Cocheret de la Moriniere et al. 2003). On the other hand, isotope analysis allows migration, movements and habitat use to be traced when they involve changes in feeding behaviour and diet composition (Hobson et al. 1994, 1995, Hansson et al. 1997, Hobson 1999).

The fractionation of C and N isotopes when they are transferred through a food web involves changes in the relative proportion of their stable isotopes. $\delta^{15}\text{N}$ in consumer tissues is enriched in about a 3‰ respect to those of its preys (Owens 1987), what allows to estimate the trophic level for each organism in the trophic chain. $\delta^{13}\text{C}$ values, on the other side, show a slow fractionation with an increase of 0-1‰ for each trophic level (DeNiro & Epstein 1978, Fry & Sherr 1984), but are closely related to organic matter origin (Fry & Sherr 1984, Peterson et al. 1985, Wainwright et al. 1993, Fry 2002). In particular, those organisms using plankton-dependant organic matter sources show different values for the C isotopes ratio than those depending on benthic primary producers or detritus (France 1995, Jennings et al. 1997, Pinnegar & Polunin 2000).

Isotopic enrichment also depends on the tissue turnover rates, and can vary among different tissues within an organism (Tieszen et al. 1983, Lee-Thorp et al. 1989). Therefore, tissues such as muscle, with low turnover rates, integrate diet isotope relations corresponding to long periods in the animal life, while tissues with higher turnover rates (gonad, digestive gland), reflect the diet of short time periods (Raikow & Hamilton 2001).

The present study analyzes the patterns in habitat use in juvenile spider crabs *Maja brachydactyla*, using stable isotope analysis. C and N isotope relations were analyzed in two tissues (muscle and hepatopancreas or digestive gland) in two coastal sites of the NW of the Iberian Peninsula, 150 km apart. (Fig. 1): the Ria de Arousa (where crabs select protected rocky areas) and the Golfo Ártabro, where they tend to occur in soft, exposed bottoms. The main organisms constituting the diet of the spider crab juveniles (following Bernardez et al. 2000, for rocky areas; non published data for soft bottoms; Table 1) and the main organic

matter sources for coastal food webs (suspended organic matter, plankton and seaweeds) were also analyzed. The objectives were to test the following hypotheses:

- (1) Habitat determines variations in diet, in both the type of preys (Table 1) and the origin of the organic matter. These variations result in changes in isotope relationships that could be used as habitat indicators.
- (2) Ontogenetic changes in habitat use take place, with juveniles performing movements from rocky shallow habitats to deeper areas with rocky substrates in protected locations and sandy substrates in exposed sites.
- (3) Carapace colour and epibiosis are habitat-dependant, and thus red and white crabs should show isotope relations typical of diets corresponding to rocky and sandy bottoms, respectively, with no relation to the habitat they were captured in.
- (4) Hepatopancreas has a higher turnover rate than muscle, and so both tissues will be diet (and consequently, habitat) indicators at different time scales.
- (5) As a consequence of the results obtained concerning the high inter-individual variability existing in isotope ratios among individuals, a new exploratory hypothesis was developed, proposing the existence of a relationship between diet (shown as isotope ratios) and energetic condition in an individual.

MATERIAL AND METHODS

A total of 39 juvenile crabs were caught in May 1999 in the Ria de Arousa for isotope analysis. Sampling was carried on in a protected area with dominating rocky bottoms at the mouth of the bay (Fig. 1). For the Golfo Ártabro, another 46 crabs were obtained between April and July 1999 in an exposed area (Bastiagueiro) located in the inner part of the Golfo. Captures in the inner part of the bay took place both in rocky habitats and in unvegetated sandy areas (where most of the local population of juveniles live, Corgos 2004).

In both areas, the crabs were caught using experimental traps. They were all over 60 mm CL and in intermoult. Carapace length, colour (red or white, according to degree of pigmentation) and algal epibiosis (Parapar et al. 1997, Fernández et al.1998) were noted. Taking into account that colour and degree of epibiosis are highly related, just colour will be mentioned from now.

In the lab, muscle from the fourth pereopod and hepatopancreas were extracted and tissues were freeze-dried and ground with a mortar. Tissues with high lipidic content usually show

more depleted $\delta^{13}\text{C}$ values, due to positive ^{12}C discrimination during lipid synthesis (DeNiro & Epstein 1977). To determine the effect of high lipid content in the hepatopancreas on isotope recordings, and to be able to compare results with other tissues (such as muscle, with lower lipid content), a complementary analysis was performed in ten hepatopancreas samples after a lipid extraction (Bligh & Dyer 1959). For this complementary analysis the samples were selected to cover the observed range of hepatopancreas lipid content estimated through C content (see below).

Representative preys were selected according to gut content analysis by Bernárdez et al. (2000) for rocky habitat and the authors (unpublished data) for sandy bottoms (Table 1). Samples of potential organic matter sources for the foodweb involving the spider crab and its preys were collected in 2002 in five locations in coastal bays and one oceanic location (O) (Fig. 1 and Table 2) in the Golfo Ártabro using the following methodology.

Three replicates per location of sedimentary organic matter of detrital origin (SOM) were collected and later resuspended in seawater and filtered with GF/F precombusted filters. Suspended particulate organic matter (SPOM) was taken using a Niskin bottle at a depth of 2 m. Three 1.5 l water replicates were collected in each station and were filtered using GF/F precombusted filters. Plankton was sampled towing a 200 μm mesh net (diameter, 0.4 m), obtaining 6 replicates in the oceanic location. Each sample was divided in four size fractions: 100-200 μm , 200-300 μm , 300-500 μm and 500-750 μm .

Seaweeds and macrofauna (bivalves, gastropods, polychaetes, echinoderms and crustaceans) were captured in the intertidal and subtidal areas of the coastal locations (Fig. 1). At the lab, epiphytes were removed from seaweeds (*Laminaria ochroleuca*, *Cystoseira baccata*, *Ulva rigida*), and the gut and exoskeleton from sea urchins (*Paracentrotus lividus*). Soft tissues were extracted from mussels (*Mytilus edulis*) and large individuals of the gastropod *Hinia reticulata*. The whole animal was processed in the case of small individuals of this species and all the *Bittium* sp., polychaetes (*Nereis diversicolor*, *Eulalia viridis* and a composite sample of different less-abundant errant polychaete species), sea cucumbers (*Aslia lefevrei* e *Holothuria forskali*) and decapod crustaceans (*Liocarcinus arcuatus*, *Xantho incisus* and Paguridae).

Processing of samples previous to isotope analysis followed the protocols proposed by Carabel et al. (submitted). Plankton samples, large *Hinia reticulata*, polychaetes, sea cucumbers and crustaceans were divided into two subsamples, one for C analysis (that was acidified with HCl 1 M during 3 hours) and another for N analysis, which was not acidified. Samples of small *H. reticulata* and the other gastropods were analyzed for both N and C after acidification, due to the small amount of tissue available. For the mussels and sea urchins, where soft tissues were extracted, and for seaweeds, no acidification was performed. SOM samples were not acidified, but the C isotope results were corrected taking into consideration previous results (Carabel et al. submitted, unpublished data) that demonstrated a decrease of 2‰ in $\delta^{13}\text{C}$ after acidification. Every sample was freeze-dried and ground in an agate mortar until a fine dust was obtained, except for the filters, which were kept intact.

Isotope analyses were performed in the Servicios Xerais de Apoio á Investigación (SXAIN, Universidade da Coruña). C and N contents and isotope analysis were determined using an elemental analyzer FlashEA1112 by ThermoFinnigan connected to an isotope ratio mass spectrometer DELTA^{plus} by Finnigan MAT, using a ConFlo II interface.

Relative proportions of isotopes are estimated following:

$$\delta^{15}\text{N} \text{ ‰} = \left[\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right] \cdot 1000$$

$$\delta^{13}\text{C} \text{ ‰} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \cdot 1000$$

Atmospheric N and VPDB (Pee Dee Belemnites) were used as standards for isotope analysis of N and C, respectively.

In addition, elemental composition of spider crab samples (percentage of C and N) was used as an indicator of biochemical composition of tissues (Anger & Dawirs 1982, Anger et al. 1983, Gnaiger & Bitterlich 1984). High N content in the muscle and hepatopancreas would reflect a high percentage of protein, while a high C percentage would be showing a high lipidic content in those tissues.

Differences in the spider crab isotope ratios between geographical areas, habitat of capture and carapace colour, as well as the effect of lipid extraction in hepatopancreas records were tested using ANOVA. Preliminary analyses (not shown here) indicated no differences in isotope ratios related to sex and body size. To test variability between tissues of the same

crab, a t-student test was used. To explore variability among individuals in terms of isotope ratio and elemental composition (C and N as indicators of lipid and protein content and energetic condition), we used principal component analysis (PCA) to explore the relationships between isotope variability (related to diet) and body condition (biochemical composition).

Stable isotope analyses are frequently used to determine the relative contribution of different food sources to an animal diet (Hobson 1999). Similarity between isotope ratios of the tissues of an organism and its food sources gives an idea of their relative importance in its diet. The relative importance of preys in the diet of the spider crab could be estimated using the isotope ratios of a single element (i.e. $\delta^{13}\text{C}$) (Kwak & Zedler 1997). The model of Phillips and Gregg (2003) has been used to estimate the relative contribution of each potential organic matter source (plankton, SOM, SPOM and seaweeds) to the diet of the spider crab. This model allows to expand the use of mixing models to complex systems, and the associated IsoSource computer program determinates ranges of source contributions when the number of sources is too large to allow unique solutions from simple stable isotope mixing models. The fitting algorithm uses increases of 1% in the relative contribution of each source (from 0 to 100%), using a tolerance of 0.1‰. To apply this model, $\delta^{15}\text{N}$ values of the sources were increased by 4.5‰, taking into account the omnivory of the spider crab and assuming a 3‰ increases in the isotopic ratio of N for each trophic level.

RESULTS

Geographical variability

Isotope ratios of muscle and hepatopancreas of juvenile spider crabs from rocky bottoms in the Ria de Arousa and Golfo Ártabro were compared, showing only significative differences in hepatopancreas with enriched values in the crabs from the northern location for both isotopes (Table 3, Fig. 2, ANOVA, $F_{1,47} = 10.63$, $p < 0.01$ for $\delta^{15}\text{N}$; $F_{1,47} = 12.45$, $p < 0.01$ for $\delta^{13}\text{C}$). In the muscle, differences in isotope ratios between crabs from both areas, were not significant (ANOVA, $F_{1,48} = 0.06$, $p = 0.81$; $F_{1,48} = 0.23$ $p = 0.64$, for C and N, respectively).

Variability between habitats and body colour

For the study of changes in diet in relation to habitat and the use of body colour as an indicator of habitat type (Table 3), we used crabs just from the Golfo Ártabro. No significant

differences were observed in the isotopic relationships of both muscle and hepatopancreas between different carapace colours or substrates (Table 4).

Variability between tissues

Muscle showed more enriched values of both isotope ratios than hepatopancreas in the Golfo Ártabro ($\delta^{15}\text{N}$: $t_{41} = 19.85$, $p < 0.01$; $\delta^{13}\text{C}$: $t_{41} = 18.34$, $p < 0.01$). Lipid removal from the hepatopancreas caused a significant increase of $\delta^{13}\text{C}$ (ANOVA, $F_{1,18} = 16.93$, $p < 0.01$), on average 1.74 ‰ (SE = 0.10, range = 1.09 - 2.18). No significant changes were found in $\delta^{15}\text{N}$ after lipid removal ($F_{1,18} = 0.09$, $p = 0.76$). Variation in C isotope ratio was independent ($r = -0.30$, $p = 0.39$) of C percentage in the hepatopancreas (an indicator of lipid levels in that tissue), thus indicating no bias in the results presented depending of the biochemical composition of the tissues. After correcting C isotope values for the hepatopancreas (adding 1.74 ‰ to each crab value), significant differences remained when comparing both tissues ($t_{41} = 9.24$, $p < 0.01$).

Inter-individual variability

Variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values observed among crabs both for the hepatopancreas and the muscle was much higher than that related to geographical location, habitat and body colour (Fig. 2, Table 3). For the N, the variation range observed in both tissues (3.03 and 3.15‰ for muscle and hepatopancreas, respectively), which is equivalent to a complete trophic level, indicates the occurrence of important differences in diet composition among crabs caught in the same habitat and with similar biological characteristics. For the C, this range (4.01 ‰ hepatopancreas and 2.36 ‰ muscle) shows that spider crabs base their diet on different sources of organic matter. On the other hand, the opposite behaviour is shown for elemental composition (percentage of C and N) of muscle and hepatopancreas (Fig. 3), with a negative correlation between N and C in hepatopancreas ($r = -0.65$; $p < 0.001$), while in the muscle both variables show a positive correlation ($r = 0.82$; $p < 0.001$).

In the PCA for isotope ratios and elemental composition (Fig. 4), factor 1 explains 44.2 % of the variance, and mostly represents variability among individuals in elemental composition. Thus, both C and N percentages in muscle and C percentage in hepatopancreas, show a strong negative correlation with axis 1, while N percentage in hepatopancreas shows a positive correlation. This axis would distribute crabs according to their energetic condition,

distinguishing those individuals in good condition (high levels of C and N in the muscle, related to high protein content, and high C content in the hepatopancreas, showing high lipid content) from those in bad condition (with lower protein and lipid content in muscle and hepatopancreas, respectively). Isotope ratios for N showed a low correlation with axis 1, suggesting that energetic condition is not dependant on trophic level, but on the contrary $\delta^{13}\text{C}$ in the muscle is associated to crabs in good body condition (showing more enriched $\delta^{13}\text{C}$), while $\delta^{13}\text{C}$ in hepatopancreas is related to crabs in bad condition. Factor 2 explains 24.2 % of the variance and establishes a positive relation between $\delta^{15}\text{N}$ values in hepatopancreas and muscle, and can be considered associated with trophic level.

Coastal food web and spider crab diet

A wide range of values for $\delta^{13}\text{C}$ was observed in the different compartments of the food web, with minimum values (-20.7 ‰) for the SPOM and maximum for SOM (-9.6 ‰) (Fig. 5). For plankton and seaweeds, values were closer to those of SPOM ($\delta^{13}\text{C} = -19.8$ ‰ and -18.5 ‰ respectively). Assuming a fractionation of 0-1 ‰ for the C, $\delta^{13}\text{C}$ values for the spider crab are very similar to the values for the rest of the decapods analyzed (Fig 5), both from rocky and sandy bottoms, with values in between the pelagic and benthic compartments.

The average values for $\delta^{15}\text{N}$ in the different components of the food web for the Golfo Ártabro, ranged from 5.39 ‰ (SPOM) to 11.72 ‰ (*Eulalia viridis*) (Fig 5). Assuming a 3 ‰ enrichment in each trophic level, the range comprised slightly more than two trophic levels. Trophic level based in $\delta^{15}\text{N}$ for the spider crab in the Golfo Ártabro is similar to other carnivores of this food web, and higher than omnivorous crustaceans and typical herbivore invertebrates (Fig. 6).

Distributions of feasible diet proportions obtained using the model of Phillips and Gregg (2003) show that SOM and seaweeds appeared to constitute the main organic matter sources (1-99th percentiles: 40-45% and 35-58%, respectively), whereas SPOM (0-15%) and plankton (0-22%) have smaller dietary contributions (Fig 7). It has to be taken into consideration the information provided by isotopic analyses carried out with preys to assess the results of the mixing model. In this sense, $\delta^{15}\text{N}$ values for *Paracentrotus lividus* are lower than those found for the organic matter sources, indicating it could be feeding on some seaweed species different than those we analyzed. On the other hand, $\delta^{13}\text{C}$ for *Nereis*

diversicolor and *Liocarcinus arcuatus* differ about a 2 ‰ from plankton values, being much closer to macroalgae and minimum values of SOM, suggesting that drift algae in soft bottoms could be an important part of their diet. Something similar would happen to pagurids that inhabit sandy bottoms. $\delta^{13}\text{C}$ values for rocky bottom crustaceans (Paguridae and *Xantho incisus*) are more difficult to explain, due to its distance from both seaweeds and herbivore values. Due to their mobility, it is possible that they would move to sandy areas where they would be feeding on organisms from soft bottoms, but another possible cause would be to assume much enriched C values for *Ulva rigida*, such as the observed by other authors (-14.5‰, Sauriau & Kang 2000, -11.8 and -10.8‰ Page & Lastra 2003), putting ratios for this primary producer much closer to the decapods included in the present study.

DISCUSSION

Geographical differences in C and N isotope ratios were very small for the muscle of *Maja brachydactyla*, but reach significant values for the hepatopancreas. Muscle values, due to its lower turnover rate, provide an integrated signal of the animal diet in the medium term (months), while hepatopancreas reflects diet in the shorter term (days or weeks) (Raikow & Hamilton 2001, Lorrain et al. 2002). Differences found in hepatopancreas values between areas could be showing differences in diet during the days previous to the sampling, but muscle values indicate that in the long term, the diet would have similar isotopic signatures in both locations.

C and N isotope ratios did not show differences between habitats (rocky vs. sandy) in the Golfo Ártabro, both for muscle and hepatopancreas. These results suggest that movements between both habitats are much more frequent than the suggested in previous studies (González-Gurriarán & Freire 1994, Hines et al. 1995, Corgos 2004, Bernárdez et al., unpublished data). It has to be taken into account that previous studies used telemetry to obtain daily locations, but restricted to daylight time, and trap sampling to map distributions. The use of body colour as an indicator of habitat preference was found to be inconsistent.

DeNiro & Epstein (1978) showed that fractionation patterns exhibited in different tissues can be dependant on the relative distribution of individual fractions (i.e. lipids, proteins, carbohydrates). Pinnegar & Polunin (1999) and Lorrain et al. (2002) observed that the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the muscle and hepatic tissue of an organism can be strongly related to relative abundance of lipids, known that these tissues show great differences in

lipidic content. The observed increase in $\delta^{13}\text{C}$ after lipid removal in the hepatopancreas of selected crabs of the Golfo Ártabro (1.74 ‰) was much higher than the fractionation of this isotope between a predator and its prey (0-1‰), what could result in a wrong interpretation of the organic matter sources. Differences between hepatopancreas and muscle before lipid removal were reduced after the process, but were still significant. Thus, it seems that they indicate actual differences in short and long term diet, and that these are not due to differences in lipid content between tissues.

The negative correlation between $\delta^{13}\text{C}$ in hepatopancreas and muscle could be reflecting diet variations in the short and medium term, and suggests very frequent movements between both habitats used by juvenile spider crabs. For example, crabs with enriched $\delta^{13}\text{C}$ muscle values that would be associated in the medium term to a diet based on preys that feed in SOM-dependant trophic routes (most enriched $\delta^{13}\text{C}$ values), have depleted hepatopancreas values of $\delta^{13}\text{C}$ (associated with rocky habitats). The relationships between C and N isotopic ratios in muscle and hepatopancreas for the crabs sampled in the Golfo Ártabro and the relative proportions of lipids and proteins in these tissues, seem to indicate a strong relation between the trophic origin of preys consumed by the spider crab and its energetic condition (that would be independent from the capture habitat and colour). So, the relation between $\delta^{13}\text{C}$ and the percentage of C and N in both tissues would indicate that the crabs that spent more time feeding in soft bottoms would be in a better energetic condition.

In the foodweb studied here for the Golfo Ártabro, two main trophic compartments according to their organic matter source were identified (Fig. 6): one based on plankton ($\delta^{13}\text{C} = -19.4$) and macroalgae ($\delta^{13}\text{C} = -18.5$) mostly corresponding to rocky habitats, and the other based on sedimentary particulated organic matter (SOM) ($\delta^{13}\text{C} = -9.62$), corresponding to sandy bottoms. Seaweeds constituted from 34 to 60%, whereas SOM represented approx. a 40% of the diet. This suggests that juvenile spider crabs ($\delta^{13}\text{C} = -15.7$) consume approximately two thirds of its preys in rocky bottoms (with a variable contribution from plankton and SPOM depending of the contribution of seaweeds) and one third in sandy areas, although this last proportion could be subestimated due to the large range of variability of SOM. This result does not seem to fit previous hypotheses about habitat use in the studied area (Corgos 2004), given that captures are much more abundant in sandy areas than in nearby rocky substrates. Juvenile crabs might be spending most of their time in soft bottoms, where they find refuge in

this highly exposed area, but move frequently to rocky areas, where they feed. Given the fact that previous telemetry studies were based in daylight observations (González-Gurriarán & Freire 1994, Hines et al. 1995, Bernárdez et al., unpublished data) and no short-term habitat changes were detected, our results suggest that probably spider crabs would make nightly movements among habitats to feed.

Our results demonstrate two patterns in the behaviour of spider crab juveniles: 1) in average feeding is carried out mainly in rocky bottoms (approx. 2/3 of diet is obtained in this habitat); 2) analyzing inter-individual variability in food sources, body condition appears to be better in crabs increasing the proportion of food obtained in sandy habitats respect to the average. Spider crab juveniles remain most of the time in sandy areas because in these habitats they probably find refuge (constituting aggregations and burying to decrease predation risk and physical stress). In rocky areas, when juveniles attain certain body size, physical stress is higher and refuges in crevices are scarce (Corgos 2004). However probably food from sandy areas is of higher quality but risk of predation on foraging crabs is higher in sandy than in rocky substrates (reverse to the relative risks of predation and physical disturbance of inactive crabs in both substrates).

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Table 1. Frequency of appearance of food components in the gut contents of *Maja brachydactyla*. (Percentages respect to the total number of guts containing food are shown) in rocky (N=160, data from Bernárdez et al. 2000) and sandy habitats (N=92, unpublished data).

Food components	Sandy	Rocky
<i>Corallina</i> spp.	---	34.4
<i>Cystoseira</i> spp.	---	8.8
<i>Laminaria</i> spp.	20.7	33.1
Other seaweeds	---	11.3
Sponges	2.2	17.5
Polychaetes	40.2	5.0
<i>Acanthochitona crinitus</i>	---	9.4
<i>Bittium</i> spp.	1.1	32.5
Trochiidae	7.6	58.1
Other gastropods	10.9	25.7
<i>Mytilus edulis</i>	---	28.8
Other bivalves	40.2	18.8
Balanida	---	8.8
Peracarid and decapod crustaceans	25.0	16.9
<i>Aslia lefevrei</i>	---	37.6
<i>Paracentrotus lividus</i>	3.3	16.9
Irregular sea urchins	25.0	---
Ophiuroids	1.1	1.3
Solitary ascidians	---	21.3
<i>Didumnum</i> sp.	---	3.1
Fish	3.3	3.1

Table 2. Food web compartments (organic matter sources and organisms) in the Golfo Artabro sampled for stable isotope analysis. Nominal trophic level, habitat type in which samples were taken (S= sandy substrates, R= rocky substrates, P= pelagic), sampling locations (see Fig. 1) and body size or fresh weight of organisms are shown.

Trophic compartment	Body size	Trophic level	Sampling area					
			1	2	3	4	5	O
SOM	-	Organic matter	S	S		S	S	
SPOM	-	Organic matter	P	P	P	P		P
Plankton	-							P
<i>Cystoseira baccata</i>	-	Primary producer	R		R	R	R	
<i>Laminaria ochroleuca</i>	-	Primary producer	R		R	R	R	
<i>Ulva rigida</i>	-	Primary producer	R		R	R	R	
<i>Bittium</i> spp.	Total length	3.9-4.8 mm	S	R	R			
<i>Mytilus edulis</i>	Total length	50-80 mm		R		R	R	
<i>Paracentrotus lividus</i>	Test diameter	46-73 mm	R	R	R	R	R	
<i>Aslia lefevrei</i>	Total length	47-82 mm		R	R	R		
<i>Liocarcinus arcuatus</i>	Carapace width	28-33 mm	S			S		
Paguridae	Carapace length	15-23 mm		S,R	R		S	
Errant polychaetes		0.05-0.09 g	R	R	R	R	R	
<i>Xantho incisus</i>	Carapace width	31-39 mm	R		R	R	R	
<i>Eulalia viridis</i>		0.1-0.3 g	R	R	R	R	R	
<i>Hinia reticulata</i>	Total length	26-33 mm	S	S,R		S,R	S, R	
<i>Holothuria forskali</i>		57-94 g		R				
<i>Nereis diversicolor</i>		0.3-2.2 g			S			

Table 3. Average (SE in parentheses) and minimum and maximum values for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in hepatopancreas and muscle of spider crabs *Maja brachydactyla* from the Ria de Arousa and Golfo Ártabro, in relation to habitat (substrate) and body colour. N=number of individuals.

Area	Habitat	Colour	N	Muscle						Hepatopancreas						
				$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			
				Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
Golfo Ártabro	Sandy	White	16	10,84 (0,10)	10,17	11,65	-15,57 (0,15)	-16,62	-14,64	16	8,93 (0,09)	8,38	9,59	-19,17 (0,23)	-21,24	-18,12
Golfo Ártabro	Sandy	Red	15	11,08 (0,19)	10,03	12,56	-16,06 (0,18)	-17,00	-14,74	15	8,63 (0,22)	7,55	10,70	-19,25 (0,30)	-21,85	-17,84
Golfo Ártabro	Rocky	White	6	10,66 (0,28)	10,12	11,76	-15,82 (0,24)	-16,60	-15,19	6	9,24 (0,16)	8,71	9,72	-19,44 (0,40)	-20,56	-18,03
Golfo Ártabro	Rocky	Red	5	10,61 (0,23)	9,99	11,36	-15,53 (0,13)	-15,88	-15,15	5	9,10 (0,20)	8,82	9,55	-19,44 (0,45)	-20,75	-18,44
Golfo Ártabro	All	All	42	10,80 (0,09)	9,99	12,56	-15,75 (0,10)	-17,00	-14,64	42	8,97 (0,09)	7,55	10,70	-19,32 (0,16)	-21,85	-17,84
Arousa	Rocky	Red	39	10,71 (0,07)	9,53	11,56	-15,64 (0,10)	-16,89	-14,36	38	8,72 (0,07)	7,84	9,52	-20,3 (0,10)	-21,53	-18,12
Total			81	10,80 (0,06)	9,53	12,56	-15,71 (0,07)	-17,00	-14,36	80	8,81 (0,06)	7,55	10,70	-19,78 (0,11)	-21,85	-17,84

Table 4. Results of ANOVAs (F-statistic and probability level in parenthesis) used to test differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the muscle and hepatopancreas of *Maja brachydactyla* in the Golfo Ártabro, between habitats and body colours (df= 1,38).

	Muscle				Hepatopancreas			
	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
Habitat	2.44	(0.127)	0.44	(0.510)	3.30	(0.077)	0.24	(0.627)
Colour	0.22	(0.641)	0.25	(0.621)	0.98	(0.328)	0.06	(0.813)
Interaction	0.51	(0.481)	3.39	(0.074)	0.13	(0.717)	0.06	(0.808)

Figure Legends

Figure 1. Location of sampling areas in the Golfo Ártabro and Ria de Arousa; 1 to 5, coastal embayments, O: oceanic station. *Maja brachydactyla* were obtained from the Ria de Arousa and station 1 in Golfo Artabro. Food web samples were obtained in all locations from the Golfo Artabro.

Figure 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios for hepatopancreas and muscle of juvenile spider crabs *Maja brachydactyla* in the Ria de Arousa and Golfo Ártabro. Substrate type of capture area and body colour are indicated.

Figure 3. Elemental composition of muscle and hepatopancreas of the juvenile spider crabs *Maja brachydactyla* caught in the Golfo Ártabro. Relationships between N and C percentages are shown for both tissues.

Figure 4. Correlation of elemental composition (shown as C and N percentages) and isotope ratios ($\delta^{13}\text{C}$ e $\delta^{15}\text{N}$) for hepatopancreas (hep) and muscle (mus) with the two main factors extracted from PCA.

Figure 5. Isotope ratios of the main compartments of the coastal food web in the Golfo Ártabro. Mean values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for thophic components from rocky (■) and sandy (△) benthic habitats and pelagic habitats (○) are shown. Confidence intervals represent standard error. $\delta^{13}\text{C}$ values for SOM are corrected by a factor of -2‰ (see text for the explanation).

Figure 6. Representation of the main trophic flows in the food web related to *Maja brachydactyla* in the Golfo Ártabro, from N and C stable isotope analysis. (See Figure 5 for data). $\delta^{13}\text{C}$ values for SOM are corrected by a factor of -2‰ (see text for the explanation).

Figure 7. Mixing polygon for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the potential organic matter sources for *Maja brachydactyla* in the Golfo Ártabro (after correcting for trophic fractionation). Histograms show the distribution of feasible contributions from each source to *M. brachydactyla* diet (percent frequency is represented in Y axis and source proportion in X axis).

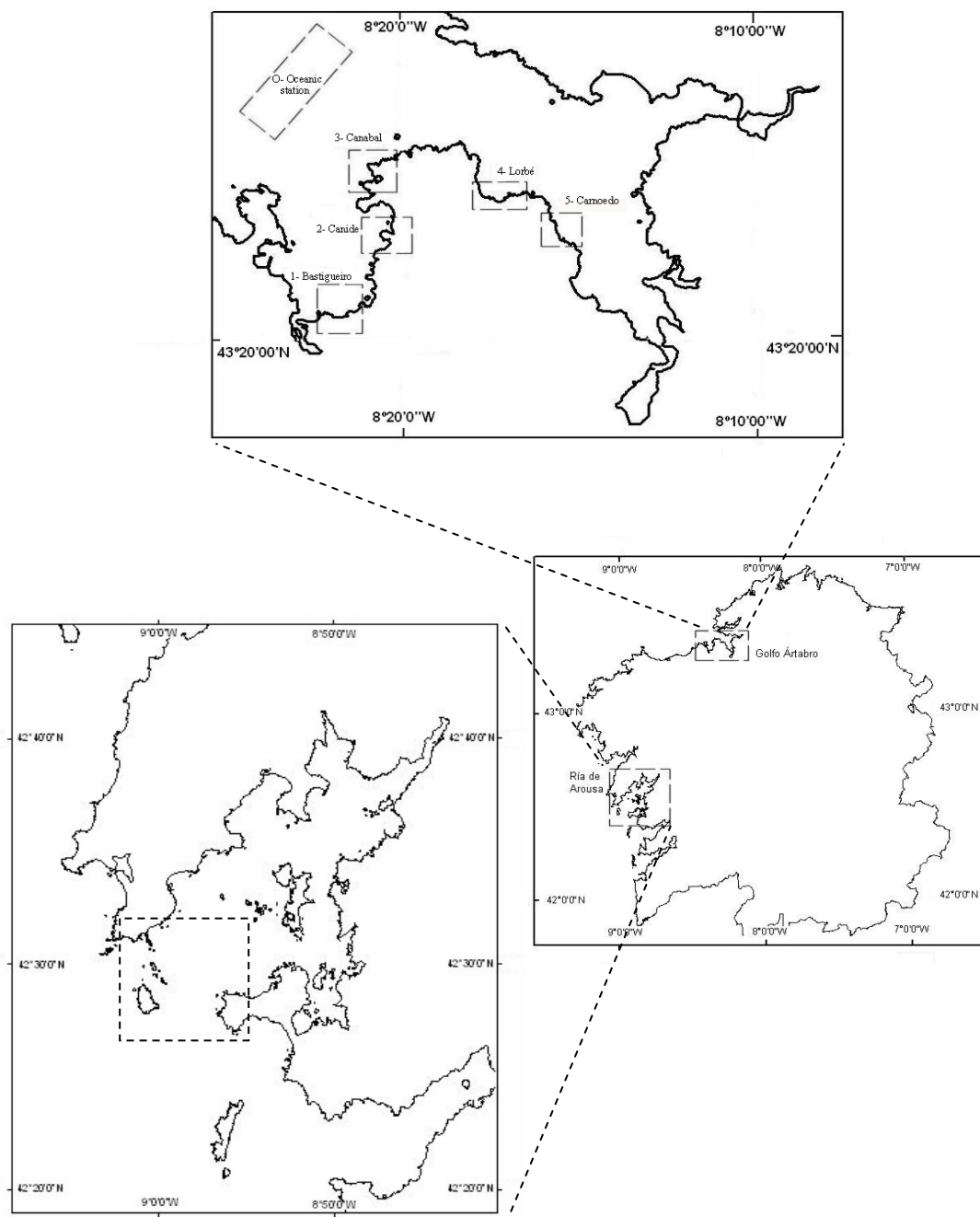


Fig. 1. Freire et al. Habitat use and stable isotopes in *Maja*

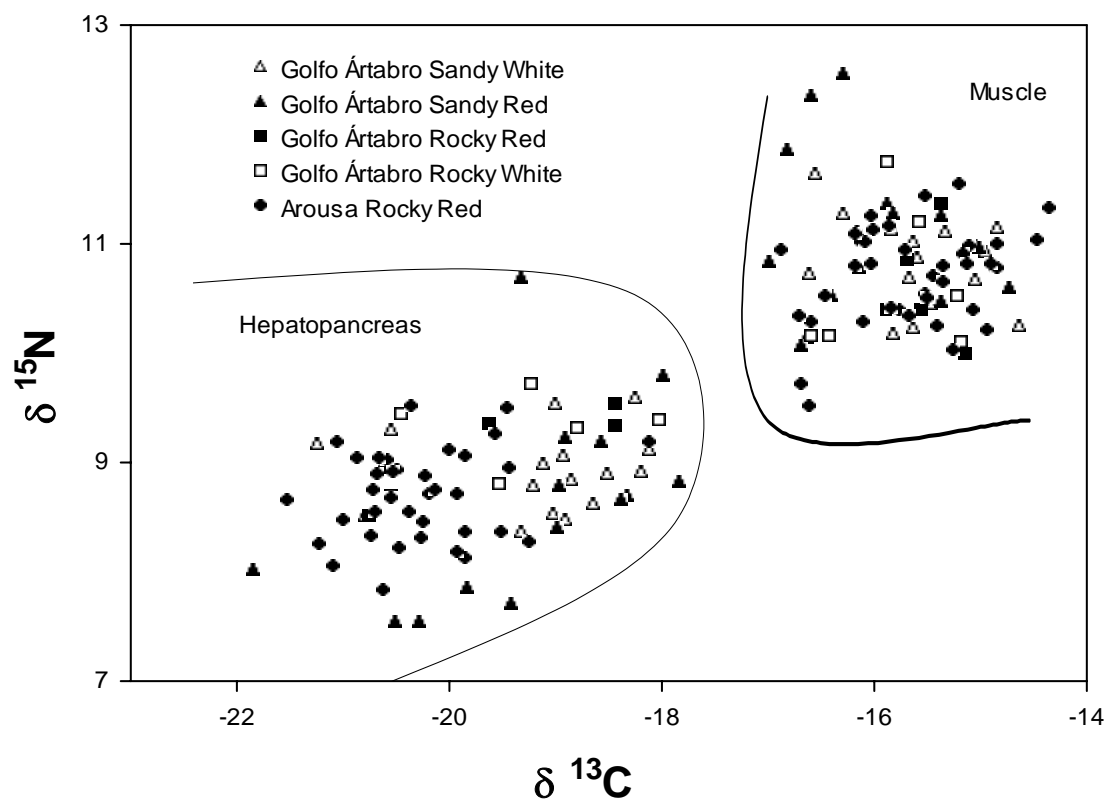


Fig. 2. Freire et al. Habitat use and stable isotopes in *Maja*

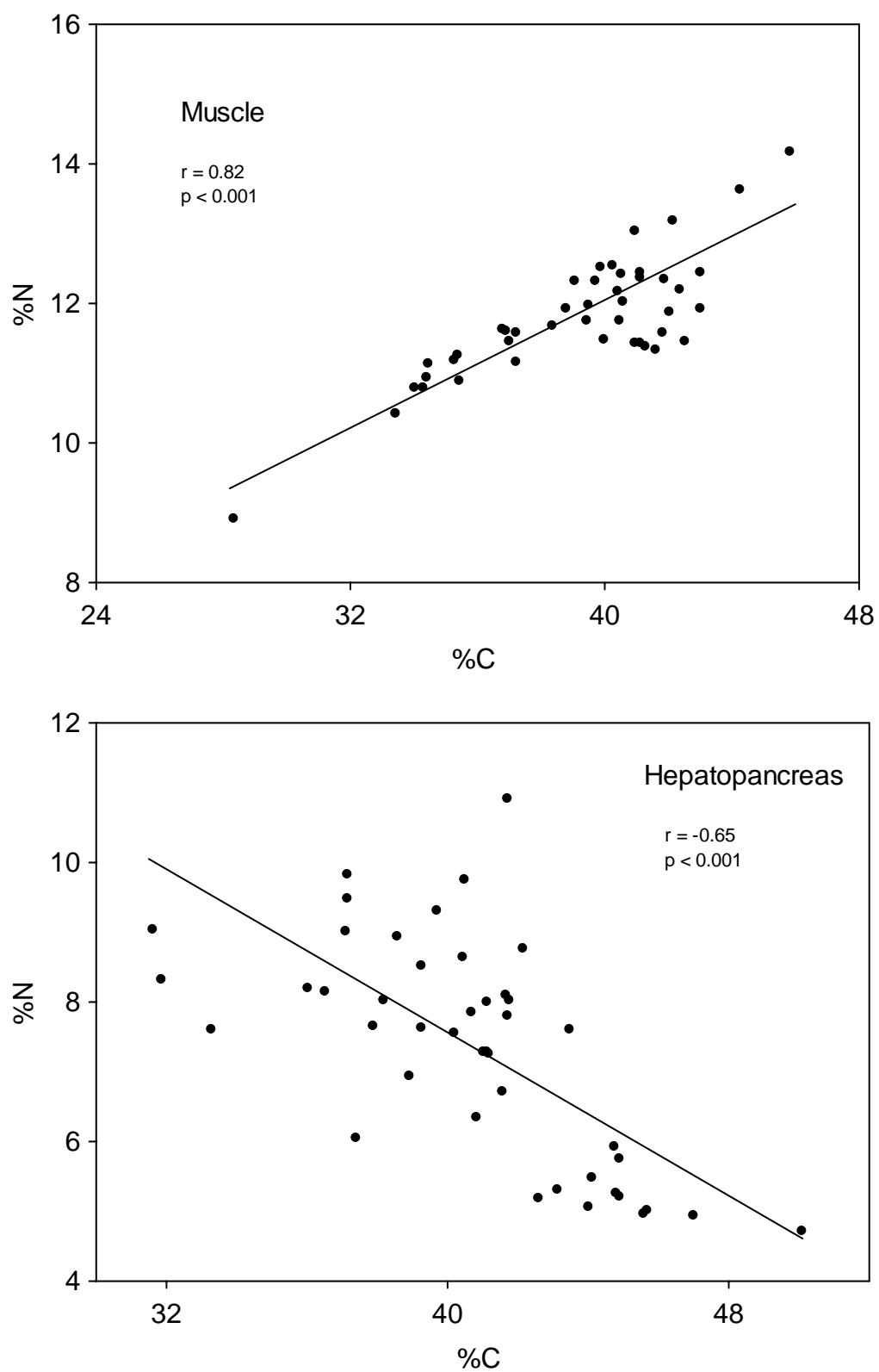


Fig. 3. Freire et al. Habitat use and stable isotopes in *Maja*

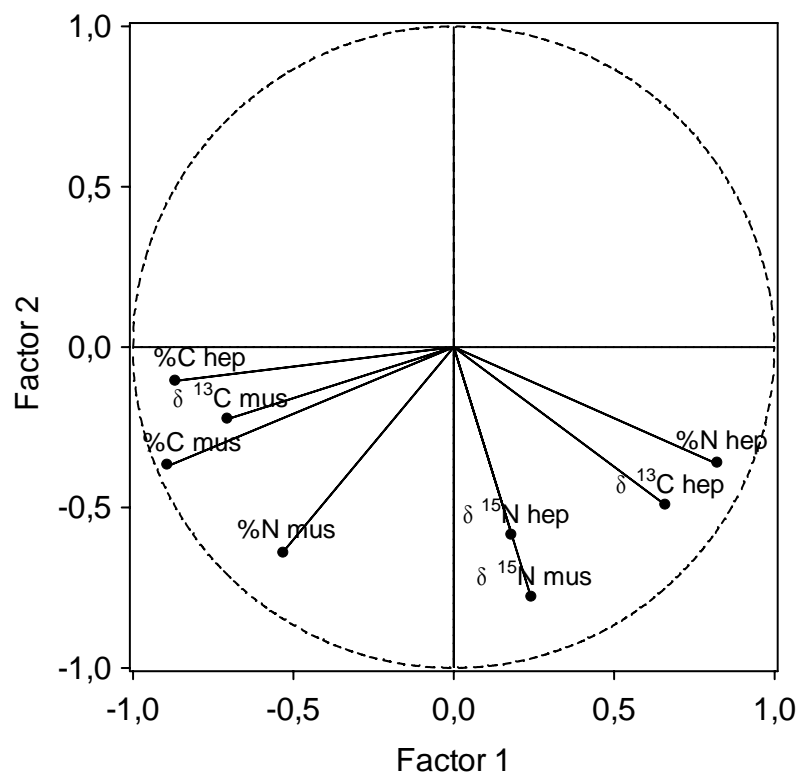


Fig. 4. Freire et al. Habitat use and stable isotopes in *Maja*

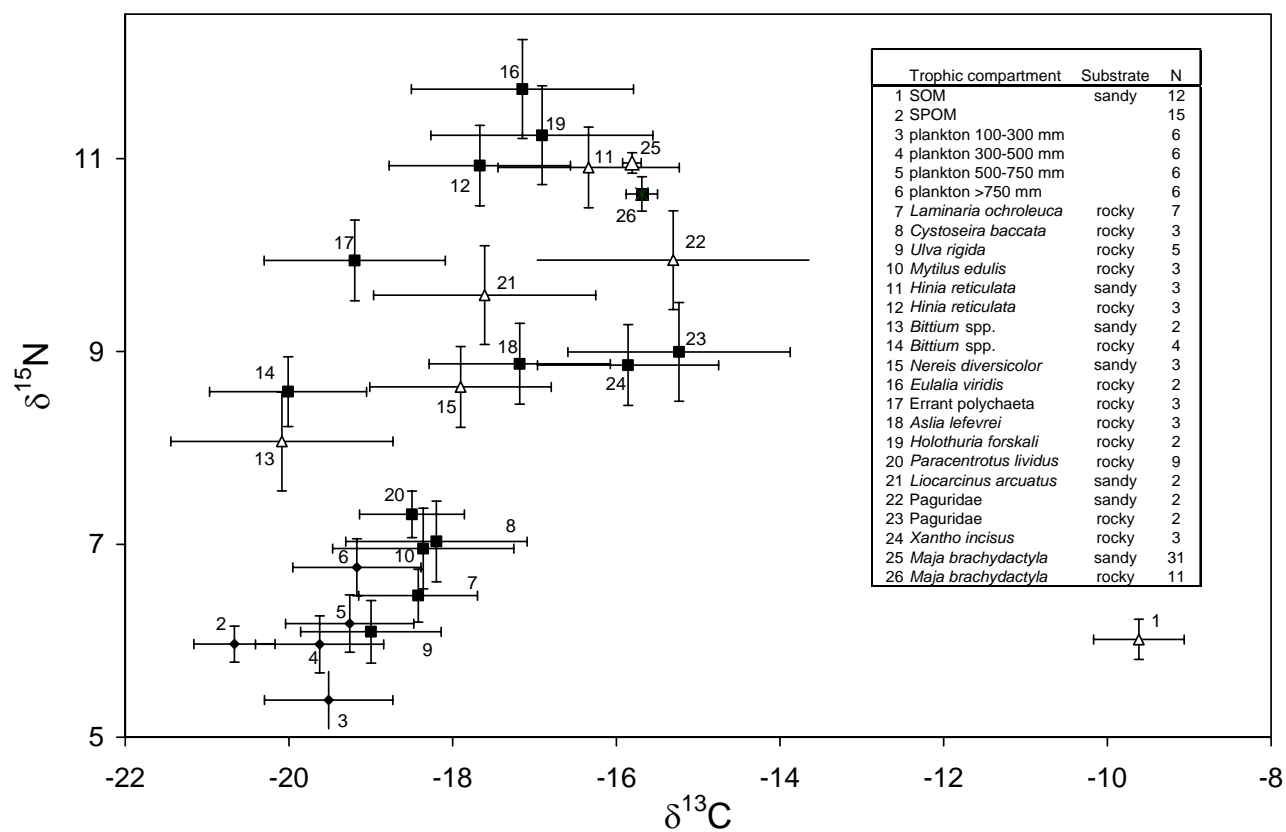


Fig 5. Freire et al. Habitat use and stable isotopes in *Maja*

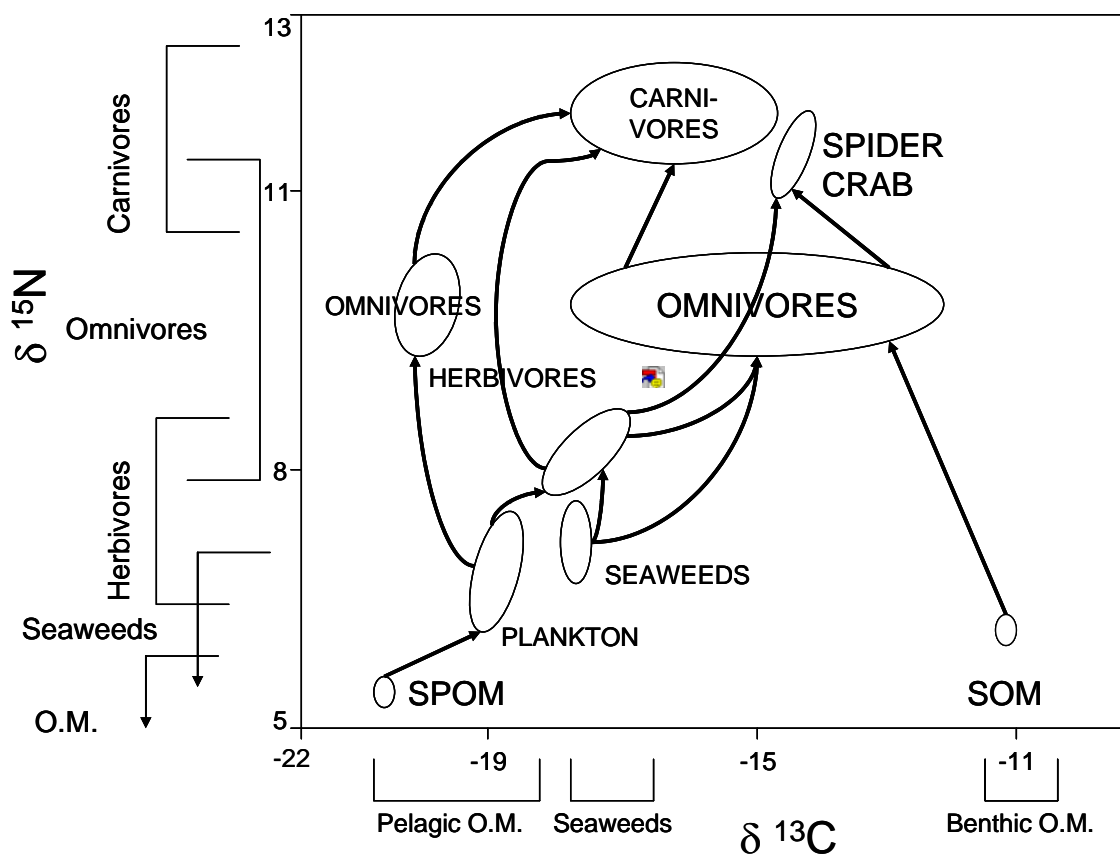


Fig. 6. Freire et al. Habitat use and stable isotopes in *Maja*

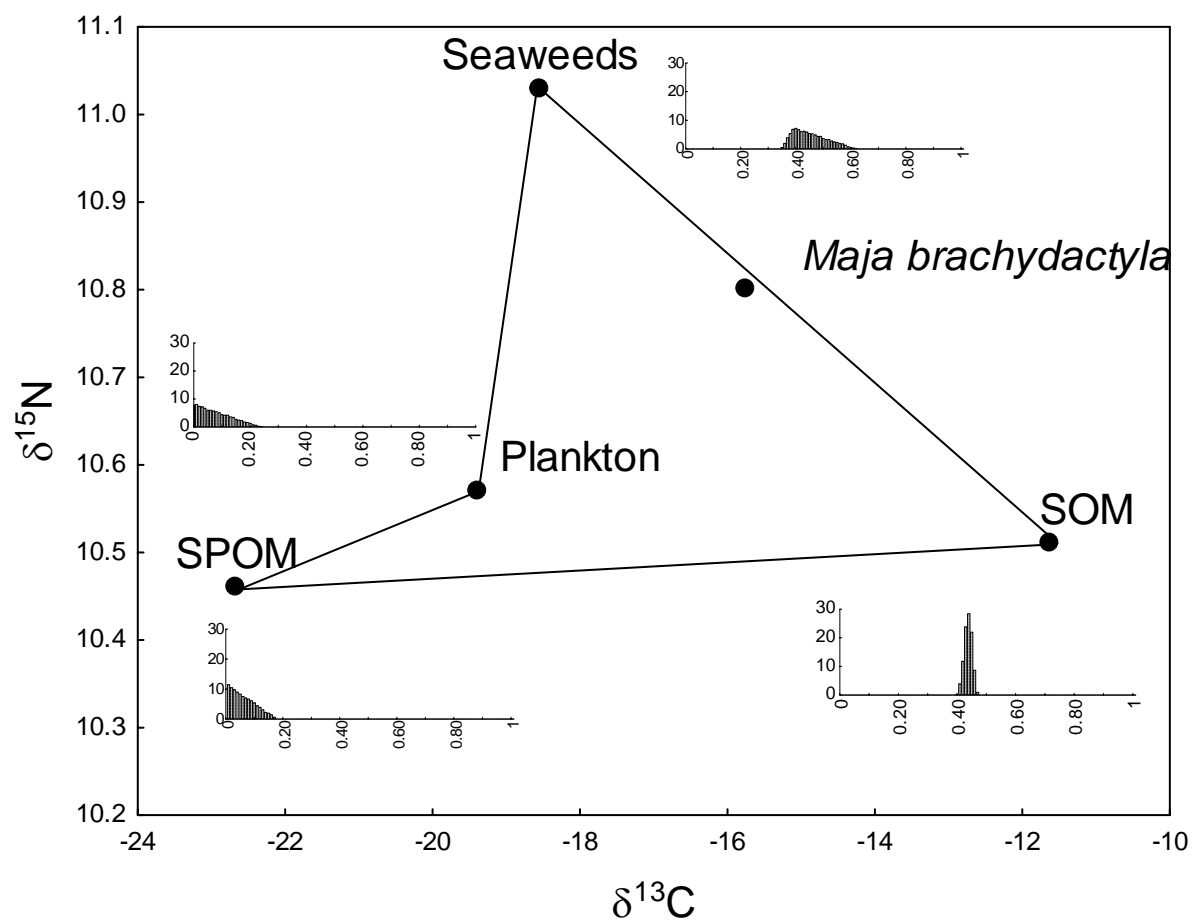


Fig 7. Freire et al. Habitat use and stable isotopes in *Maja*