

## Patterns of juvenile habitat use by the spider crab *Maja brachydactyla* as revealed by stable isotope analyses

JUAN FREIRE<sup>1</sup>, SIRKA CARABEL<sup>1</sup>, PATRICIA VERÍSIMO<sup>1</sup>,  
CRISTINA BERNÁRDEZ<sup>1,2</sup> and LUIS FERNÁNDEZ<sup>1</sup>

<sup>1</sup>Departamento de Biología Animal, Biología Vegetal e Ecología, Universidade da Coruña, Campus da Zapateira s/n, E-15071, A Coruña, Spain. E-mail: jfreire@udc.es

<sup>2</sup>Current address: Centro de Ecología Costera, Centro Universitario de la Costa Sur, Universidad de Guadalajara, V. Gómez Farías, 82. San Patricio-Melaque, Jalisco, 48980, México.

**SUMMARY:** Patterns of habitat use by spider crab juveniles, *Maja brachydactyla*, from two geographic areas on the NW coast of the Iberian Peninsula were studied through the analysis of carbon and nitrogen stable isotope relations in muscle and hepatopancreas. Main potential preys of spider crab juveniles in rocky and sandy habitats and different organic matter sources in coastal food webs were also analysed. Isotopic ratios showed no 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 that movements between the two habitats are much more frequent than suggested in previous studies. In the coastal food web, two main trophic compartments were identified according to their organic matter source: one based on plankton and seaweeds (rocky habitats), and one based on sedimentary particulate organic matter (sandy bottoms). By means of the model of Phillips and Gregg (2003), it was found that juveniles of *Maja brachydactyla* from both habitats consumed approximately two thirds of the preys in rocky habitats and one third in sedimentary habitats. The results indicate that in exposed environments large juveniles spend most of the time on sedimentary bottoms, where they find more refuge, moving frequently to nearby rocky substrates to feed.

**Keywords:** coastal ecosystems, food webs, feeding, life history, marine crustaceans, stable isotopes, trophic level, energetic condition.

**RESUMEN:** PATRONES DE USO DEL HÁBITAT DE LA CENTOLLA *MAJA BRACHYDACTYLA* MEDIANTE EL USO DE ISÓTOPOS ESTABLES. – El patrón de uso del hábitat de los juveniles de centolla, *Maja brachydactyla*, de dos áreas geográficas en la costa NO de la Península Ibérica, se estudió a través del análisis de las relaciones de los isótopos estables del Carbono y del Nitrógeno en músculo y hepatopáncreas. Se analizaron las presas potenciales de los juveniles de centolla en hábitats rocosos y arenosos y las diferentes fuentes de materia orgánica de las redes tróficas costeras. Las relaciones isotópicas no mostraron ninguna diferencia entre hábitats arenosos y rocosos. El uso del color del caparazón y la epibiosis como indicadores del hábitat no se vio apoyado por nuestros datos. Los resultados sugieren que los movimientos entre hábitats son más frecuentes que los sugeridos en estudios previos. En la red trófica costera se identificaron dos compartimentos tróficos principales en base a la fuente de materia orgánica: uno basado en el plancton y macroalgas (hábitats rocosos) y otro basado en la materia orgánica particulada sedimentada (fondos arenosos). En base al modelo de Phillips y Gregg (2003) se estimó que los juveniles de *Maja brachydactyla* de ambos tipos de hábitat consumirían, aproximadamente, dos tercios de sus presas de hábitats rocosos y un tercio de hábitats sedimentarios. Los resultados obtenidos indican que, en ambientes expuestos, los juveniles pasan la mayor parte del tiempo en los fondos arenosos, donde encuentran refugio, moviéndose frecuentemente a zonas rocosas próximas para alimentarse.

**Palabras clave:** ecosistemas costeros, redes tróficas, alimentación, estrategia vital, crustáceos marinos, isótopos estables, nivel trófico, condición energética.

## INTRODUCTION

Juveniles approaching maturity of spider crabs *Maja brachydactyla* (Decapoda, Brachyura) inhabit shallow waters (down to 10–15 m deep), using both rocky and sandy substrates and performing slow (<100 m d<sup>-1</sup>), small-scale, non-directional movements (González-Gurriarán and Freire, 1994; Hines *et al.*, 1995). Recent evidence (Corgos, 2004) points to ontogenetic movements when crabs are one year old and have a carapace length (CL) of approximately 60 mm from very shallow rocky substrates (approx. 5 m), where the postlarval settlement takes place, to deeper rocky or sandy bottoms (5–15 m). Within this depth range, juveniles 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 m), where they mate (González-Gurriarán *et al.*, 1998).

The patterns of habitat use (especially regarding substrate type) of juvenile spider crabs (>1 year old) in shallow waters (González-Gurriarán and Freire, 1994; Hines *et al.*, 1995; Freire *et al.*, 2002) remain to be discussed, regarding the large and small-scale geographical differences observed in habitat selection. Geographical variability in selected substrate type might be related to the degree of wave exposure, with crabs mostly selecting rocky bottoms at sheltered sites (always with oceanic characteristics) and sandy bottoms at more exposed sites (Corgos, 2004). Corgos 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 has been interpreted to be related to habitat use, taking into account that the species shows 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 they are mostly bright red-coloured with abundant epibiosis. Corgos (2004) observed that a large number of “red” crabs with algal epibiosis episodically appeared on sandy bottoms, and put forward the hypothesis of ontogenetic habitat changes (shifting from rocky to sandy habitats when juveniles attain one year). However, a clear seasonal pattern was not found, so the presence of red crabs in sandy areas would be due to frequent movements between sandy and rocky bottoms. Data from mark-re-

capture experiments (Corgos, pers. comm.) showed that crabs from rocky habitats lose algal epibiosis progressively, and that in 2.5 months almost all of the crabs had lost it. The modification of the red coloration is a slower process.

The relationship among different stable carbon (<sup>12</sup>C/<sup>13</sup>C, expressed as δ<sup>13</sup>C) and nitrogen (<sup>14</sup>N/<sup>15</sup>N, expressed as δ<sup>15</sup>N) isotopes is a widely used tool for the study of trophic relationships (Peterson and Fry, 1987; Wada and Hattori, 1991, Cabana and Rasmussen, 1994, Michener and Schell, 1994) and food webs (Newell *et al.*, 1995; Burns and Walter, 2000; Fry and Smith, 2002; Cocheret de la Moriniere *et al.*, 2003). Isotope analysis also 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. δ<sup>15</sup>N in consumer tissues is enriched in about 3.4‰ (1 SD = 1‰) in comparison with those of its preys (Post, 2002), which allows the trophic level to be estimated for each organism in the trophic chain. δ<sup>13</sup>C values, on the other hand, show a slower fractionation with an increase of 0.4‰ (1 SD = 1.3‰) per trophic level (Post, 2002), but are closely related to organic matter origin (Fry and Sherr, 1984; Peterson *et al.*, 1985; Wainright *et al.*, 1993; Fry, 2002). In particular, those organisms using plankton-dependent organic matter sources show different values for the C isotopes ratio to those depending on benthic primary producers or detritus (France, 1995; Jennings *et al.*, 1997; Pinnegar and 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 and Hamilton, 2001).

The present study analyses the habitat use patterns of juveniles approaching maturity of the spider crab *Maja brachydactyla*, using stable isotope analysis. C and N isotope relations were analysed in two tissues (muscle and hepatopancreas or digestive gland) in two coastal sites of the NW Iberian Peninsula, 150 km apart: the Ria de Arousa, where

crabs select sheltered rocky areas, and the Golfo Artabro, where they tend to occur on soft, exposed bottoms. The main organisms constituting the diet of the spider crab juveniles and the main organic matter sources for coastal food webs (suspended organic matter, plankton and seaweeds) were also analysed. Variations in diet, in both the type of prey and the origin of the organic matter, result in changes in isotope relationships that could be used as habitat indicators. The objectives of the present study were to test the following hypotheses:

(1) Ontogenetic changes in habitat use take place, with juveniles performing movements from rocky shallow habitats to deeper areas with rocky substrates at sheltered locations and sandy substrates at exposed locations.

(2) Carapace colour and epibiosis are habitat-dependent, so 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.

(3) Hepatopancreas has a higher turnover rate than muscle, so both tissues will be diet (and consequently, habitat) indicators at different time scales.

(4) There is a relationship between diet (shown as isotope ratios) and energetic condition in an individual. This is an exploratory hypothesis arising

from the results obtained concerning the high inter-individual variability in isotope ratios among individuals.

## MATERIAL AND METHODS

The Ria de Arousa is a long bay that penetrates the coast of Galicia in a SW-NE direction. Connection with the ocean is delimited by the Salvora Island, the northern mouth being very shallow (10 m). Therefore, almost all the exchange with the shelf takes place through the southern mouth (70 m deep) (Rosón *et al.*, 1995). A total of 39 juvenile crabs were caught in May 1999 in the Ria de Arousa for isotope analysis. Sampling was carried out in a sheltered, rocky-bottom area located at the mouth of the ria (Fig. 1), where juveniles (having a carapace length of <120-140 mm) are found predominantly (González-Gurriarán and Freire, 1994). The Golfo Artabro is located on the NW side of the Galician coast, and includes the three largest Galician Rias Altas (Ferrol, Ares-Betanzos and A Coruña) (Bode and Varela, 1998). For the Golfo Artabro, another 46 crabs were obtained between April and July 1999 in two exposed areas (Bastiaqueiro and Canabal) located in the inner part of the gulf. Captures in the inner

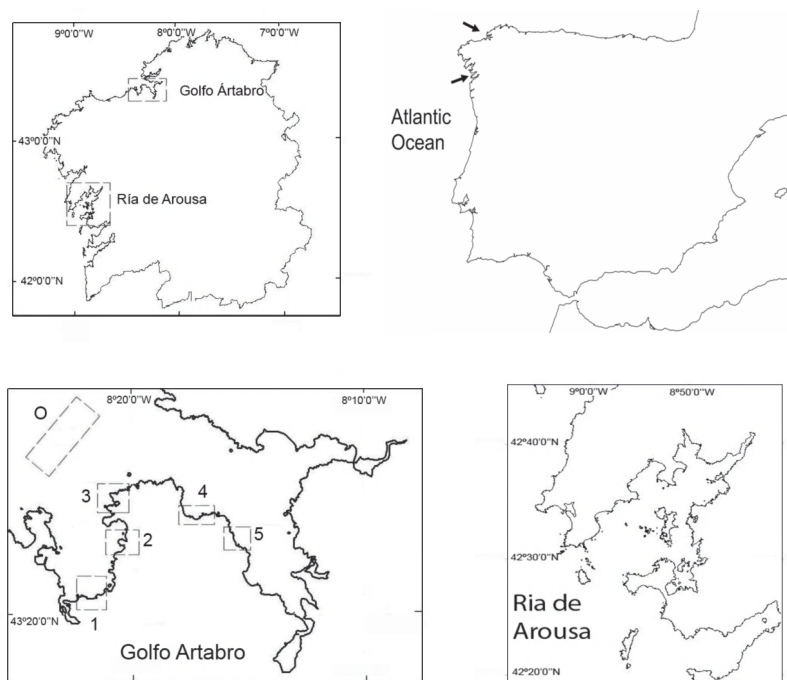


FIG. 1. – Location of sampling areas in the Golfo Artabro and Ria de Arousa; 1 to 5, coastal embayments. O: Oceanic station; 1: Bastiaqueiro; 2: Canide; 3: Canabal; 4: Lorbé; 5: Carnoedo. *Maja brachydactyla* were obtained from the Ria de Arousa and station 1 and 3 in Golfo Artabro. Food web samples were obtained at all locations from the Golfo Artabro.

TABLE 1. – Frequency of appearance of food components in the gut contents of *Maja brachydactyla* (percentages of the total number of guts containing food are shown) in rocky (N=160, data from Bernárdez *et al.*, 2000) and sandy habitats (N=92, data obtained by the authors).

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>Acanthochiton</i> <i>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>Didemnum</i> sp.	---	3.1
Fish	3.3	3.1

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 men-

tioned 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  $^{12}\text{C}$  discrimination during lipid synthesis (DeNiro and 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 and 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 their frequency of appearance in the gut contents of spider crabs and their abundance in the study area. Gut content data came from two different sources: Bernárdez *et al.* (2000) for rocky habitats, and data obtained by the authors 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 at five locations in coastal bays and one oceanic location (O) (Fig. 1 and Table 2) in the Golfo Artabro. Three sampling areas were chosen along the east coast of the Ria de A Coruña, from the inner part (with greater continental influence) to the outer part (with oceanic characteristics) (Cabanas *et al.*, 1987). The other two areas, with influence of riverine deposition (Sánchez-Mata *et al.*, 1999), are

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	Herbivore	S	R	R			
<i>Mytilus edulis</i>	Total length	Herbivore		R		R	R	
<i>Paracentrotus lividus</i>	Test diameter	Herbivore	R	R	R	R	R	
Terebellidae	0.12-0.16 g	Deposit and suspension feeders						R
<i>Nereis diversicolor</i>	0.3-2.2 g	Deposit feeder			S			
<i>Aslia lefevrei</i>	Total length	Omnivore		R	R	R		
<i>Liocarcinus arcuatus</i>	Carapace width	Omnivore	S			S		
Paguridae	Carapace length	Omnivore		S,R	R		S	
<i>Xantho incisus</i>	Carapace width	Omnivore	R		R	R	R	
<i>Eulalia viridis</i>	0.1-0.3 g	Carnivore	R	R	R	R	R	
<i>Hinia reticulata</i>	Total length	Carnivore	S	S,R		S,R	S,R	
<i>Holothuria forskali</i>	57-94 g	Carnivore		R				



located in the more protected western coast of the Ria de Ares-Betanzos.

For prey samplings, 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 at each station and were filtered using GF/F precombusted filters. Plankton was sampled by towing a 200 µm mesh net (diameter, 0.4 m), and 6 replicates were obtained at the oceanic location. Each sample was divided into four size fractions: 100-200, 200-300, 300-500 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). In 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 species of the family Terebellidae), sea cucumbers (*Aslia lefevrei* and *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.* (2006). Plankton samples, large *H. reticulata*, polychaetes, sea cucumbers and crustaceans were divided into two subsamples, one for C analysis (which was acidified with HCl 1 M for 3 hours) and one for N analysis, which was not acidified. Samples of small *H. reticulata* and the other gastropods were analysed for both N and C after acidification, due to the small amount of tissue available. For the mussels and sea urchins, in which 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.*, 2006) that demonstrated a decrease of 2‰ in δ<sup>13</sup>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 a FlashEA1112 elemental analyser by ThermoFinnigan connected to an isotope ratio mass spectrometer DELTA plus by Finnigan MAT, using a ConFlo II interface.

Relative proportions of isotopes were estimated following:

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

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

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 and Dawirs, 1982; Anger *et al.*, 1983; Gnaiger and Bitterlich, 1984). A high N content in the muscle and hepatopancreas would show a high percentage of protein, while a high C content would show a high percentage of lipids.

To study differences in the spider crab isotope ratios between geographical areas, both muscle and hepatopancreas from specimens collected in rocky bottoms of the Ria de Arousa and Golfo Artabro were used. To determine changes due to habitat of capture, carapace colour and the effect of lipid extraction in hepatopancreas, only crabs from Golfo Artabro were used. Differences 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 Student's *t*-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).

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. δ<sup>13</sup>C) (Kwak and Zedler, 1997). The model of Phillips and

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						
				Mean	$\delta^{15}\text{N}$ Min	$\delta^{15}\text{N}$ Max	Mean	$\delta^{13}\text{C}$ Min	$\delta^{13}\text{C}$ Max	Mean	$\delta^{15}\text{N}$ Min	$\delta^{15}\text{N}$ Max	Mean	$\delta^{13}\text{C}$ Min	$\delta^{13}\text{C}$ 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

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 mixing models to be extended 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%), with 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 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 Artabro were compared, showing only significant 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 the two 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

In the study of changes in diet in relation to habitat and the value of body colour as an indicator of habitat type (Table 3), no significant differences

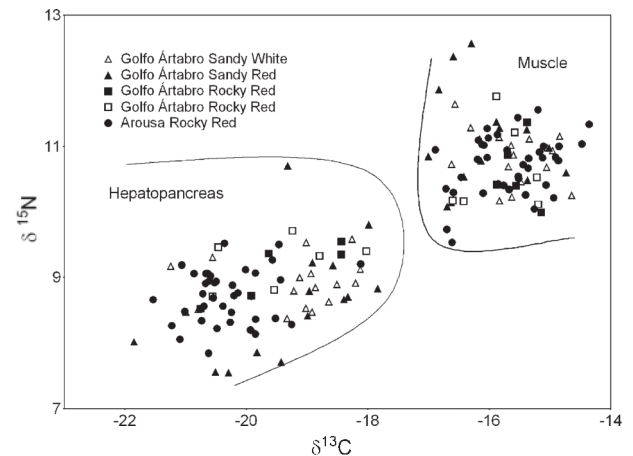


FIG. 2. –  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios for hepatopancreas and muscle of juvenile spider crabs *Maja brachydactyla* in the Ria de Arousa and Golfo Artabro. Substrate type of capture area and body colour are indicated.

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)

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

### Variability between tissues

Muscle showed more enriched values of both isotope ratios than hepatopancreas in the Golfo Artabro ( $\delta^{15}\text{N}$ :  $t_{41} = 19.85$ ,  $p < 0.01$ ;  $\delta^{13}\text{C}$ :  $t_{41} = 18.34$ ,  $p < 0.01$ ). Lipid removal from the hepatopancreas caused a significant increase in  $\delta^{13}\text{C}$  (ANOVA,  $F_{1,18} = 16.93$ ,

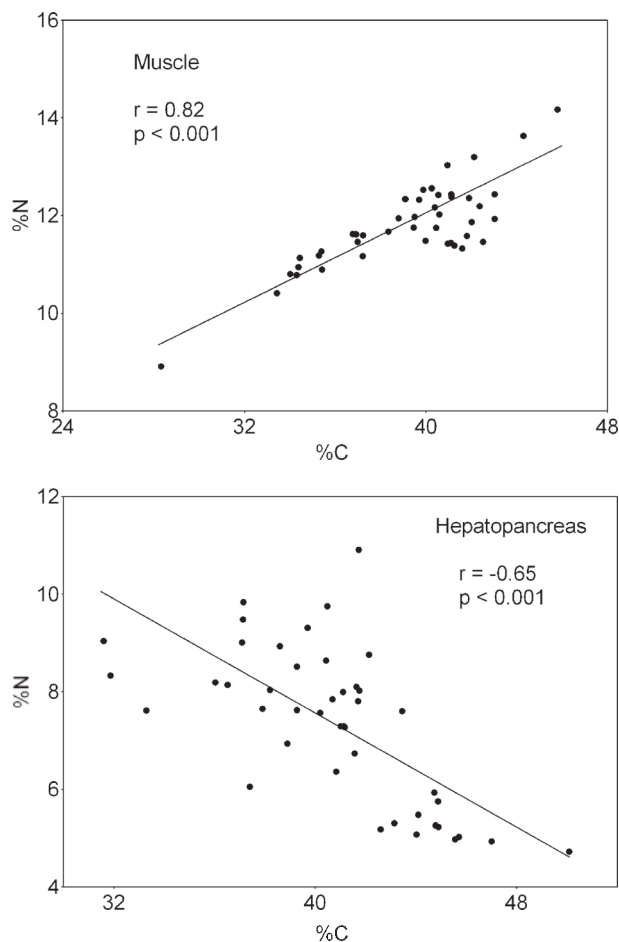


FIG. 3. – Elemental composition of muscle and hepatopancreas of the juvenile spider crabs *Maja brachydactyla* caught in the Golfo Artabro. Relationships between N and C percentages are shown for both tissues.

$p < 0.01$ ), on average  $1.74\text{‰}$  ( $SE = 0.10$ , 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 resulting from the biochemical composition of the tissues. After correcting C isotope values for the hepatopancreas (adding  $1.74\text{‰}$  to each crab value), significant differences remained between the two 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 for both the hepatopancreas and the muscle was much higher than that related to geographical location, habitat and body colour (Fig. 2; Table 3). For N, the variation range observed in both tissues ( $3.03$  and  $3.15\text{‰}$  for muscle and hepatopan-

creas, respectively), which is equivalent to a complete trophic level, indicates the occurrence of major differences in diet composition among crabs with similar biological characteristics caught in the same habitat. For C, this range ( $4.01\text{‰}$  hepatopancreas and  $2.36\text{‰}$  muscle) shows that spider crabs base their diet on different sources of organic matter. On the other hand, the behaviour shown for elemental composition (percentage of C and N) in muscle and hepatopancreas was different (Fig. 3), with a negative correlation between N and C in hepatopancreas ( $r = -0.65$ ;  $p < 0.001$ ), while in the muscle both variables showed 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

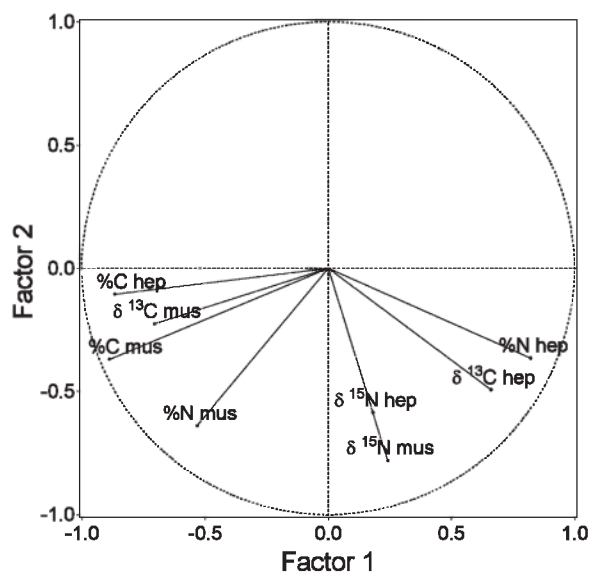


FIG. 4. – Correlation of elemental composition (shown as C and N percentages) and isotope ratios ( $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ ) for hepatopancreas (hep) and muscle (mus) with the two main factors extracted from PCA.

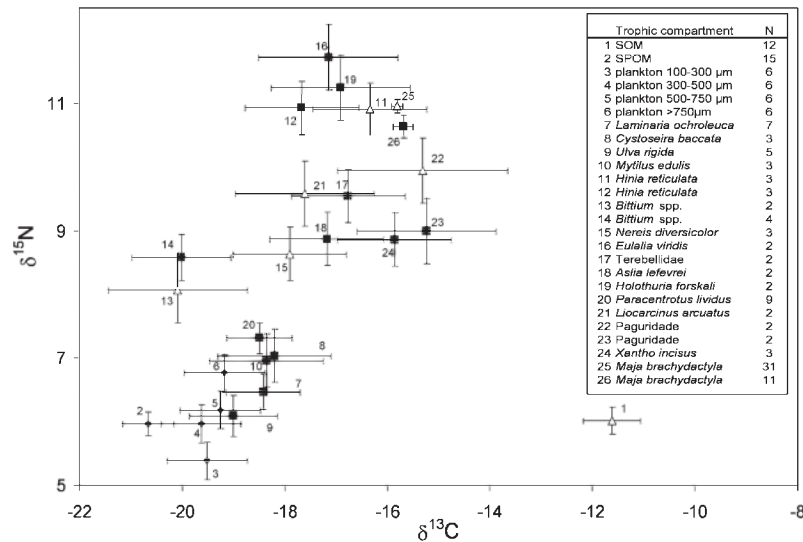


FIG. 5. – Isotope ratios of the main compartments of the coastal food web in the Golfo Artabro. Mean values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for trophic components from rocky (squares) and sandy (triangles) benthic habitats and pelagic habitats (circles) are shown. Confidence intervals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

on trophic level. However,  $\delta^{13}\text{C}$  in the muscle is associated with 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 to be 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 ( $-20.7\text{‰}$ ) for SPOM and maximum values for SOM ( $-11.6\text{‰}$ ) (Fig. 5). For plankton and seaweeds, values were closer to those of SPOM ( $\delta^{13}\text{C} = -19.8\text{‰}$  and  $-18.5\text{‰}$  respectively). Assuming a fractionation of  $0\text{-}1\text{‰}$  for the C,  $\delta^{13}\text{C}$  values for the spider crab were very similar to the values for the rest of the decapods analysed (Fig. 5), both from rocky and sandy bottoms, with values 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 Artabro ranged from  $5.39\text{‰}$  (SPOM) to  $11.72\text{‰}$  (*Eulalia viridis*) (Fig. 5). Assuming a  $3\text{‰}$  enrichment in each trophic level, the range comprised slightly more than two trophic levels. The trophic level based on  $\delta^{15}\text{N}$  for the spider crab in the Golfo Artabro is similar to that of other carnivores of this food web, and higher than that of omnivorous crustaceans and typical herbivore invertebrates (Fig. 6).

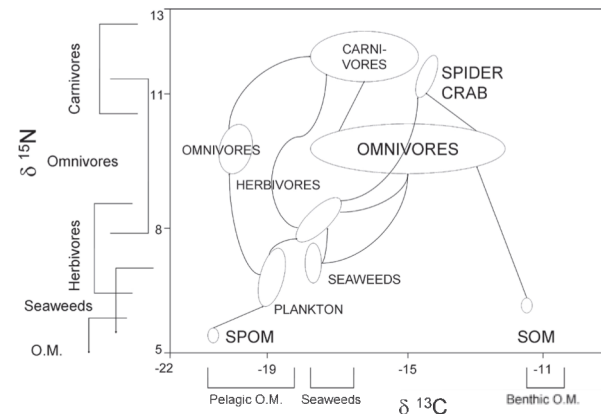


FIG. 6. – Representation of the main trophic flows in the food web related to *Maja brachydactyla* in the Golfo Artabro, from N and C stable isotope analysis. (See Fig. 5 for data).  $\delta^{13}\text{C}$  values for C are corrected by a factor of  $-2\text{‰}$  (see text for the explanation).

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%) made smaller dietary contributions (Fig. 7). The information provided by isotopic analyses carried out with preys to assess the results of the mixing model must be taken into consideration. *Paracentrotus lividus* showed lower  $\delta^{15}\text{N}$  values than those found for the organic matter sources, indicating that it could be feeding on some seaweed species different to those we analysed. On the other hand,  $\delta^{13}\text{C}$  for *Nereis diversicolor* and *Liocarcinus arcuatus* differ by about  $2\text{‰}$  from plankton



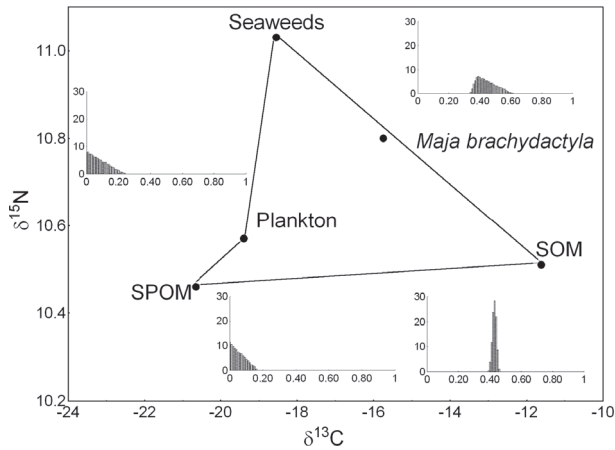


FIG. 7. — Mixing polygon for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of the potential organic matter sources for *Maja brachydactyla* in the Golfo Artabro (after correcting for trophic fractionation). Histograms show the distribution of feasible contributions from each source to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

values; they were much closer to macroalgae and minimum values of SOM, suggesting that drift algae on 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 their distance from both seaweed 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 highly enriched C values for *Ulva rigida*, such as those observed by other authors (-14.5‰ Sauriau and Kang, 2000; -11.8 and -10.8‰ Page and Lastra, 2003), which would place the ratios for this primary producer much closer to those of 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 reached significant values for the hepatopancreas. Due to their lower turnover rate, muscles provide an integrated signal of the animal diet in the medium term (months), while the hepatopancreas reflects the diet in the shorter term (days or weeks) (Raikow and Hamilton, 2001; Lorrain *et al.*, 2002). Differences found in hepatopancreas values between areas could indicate 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 at both locations.

The fact that masking behaviour of *M. brachydactyla* is more pronounced in juveniles (Fernández *et al.* 1998) supports the general belief that this behaviour corresponds to a protection mechanism against predators and not a system for camouflage to facilitate prey capture. Differences observed in the extent of covering on the different parts of the carapace are related to habitat. As slightly coloured crabs (known as “white” crabs) show little epibiosis, while bright red-coloured crabs usually have abundant epibiosis (Corgos, 2004), we expected to find a relation between body colour and habitat, but the use of body colour as an indicator of habitat preference was found to be inconsistent.

C and N isotope ratios, both for muscle and hepatopancreas, showed no differences between habitats (rocky vs sandy) in the Golfo Artabro, as happened with body colour. All these results suggest that movements between the two habitats are much more frequent than suggested in previous studies (González-Gurriarán and Freire, 1994; Hines *et al.*, 1995; Corgos, 2004). 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.

DeNiro and Epstein (1978) showed that fractionation patterns exhibited in different tissues can be dependent on the relative distribution of individual fractions (i.e. lipids, proteins, carbohydrates). Pinengar and 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, in view of the fact that these tissues show great differences in lipid content. Bodin *et al.* (2007) observed changes in the hepatopancreas C and N stable isotope ratios of *Maja brachydactyla* after lipid extraction. Variation observed for C by these authors (3‰) was higher than those obtained in this study. Nevertheless, the observed increase in  $\delta^{13}\text{C}$  after lipid removal in the hepatopancreas of selected crabs of the Golfo Artabro (1.74‰) was much higher than the fractionation of this isotope between a predator and its prey (0-1‰), which could result in an erroneous 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 reflect diet variations in the short and medium term, and suggests very frequent movements between the two habitats used by juvenile spider crabs. For example, crabs with enriched  $\delta^{13}\text{C}$  muscle values, which would be associated in the medium term with a diet based on preys feeding on 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 Artabro 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 (which would be independent from the capture habitat and colour). Thus, the relation between  $\delta^{13}\text{C}$  and the percentage of C and N in both tissues would indicate that the crabs that obtain most of their food on soft bottoms would have a better energetic condition.

In the foodweb studied here for the Golfo Artabro, two main trophic compartments were identified according to their organic matter source (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 one based on sedimentary particulate organic matter (SOM) ( $\delta^{13}\text{C} = -11.6$ ), corresponding to sandy bottoms. Seaweeds constituted 34 to 60%, whereas SOM constituted approximately 40% of the diet. This suggests that juvenile spider crabs ( $\delta^{13}\text{C} = -15.7$ ) consume approximately two thirds of their preys on rocky bottoms (with a variable contribution from plankton and SPOM depending on the contribution of seaweeds) and one third in sandy areas, although the latter 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 study area (Corgos, 2004), given that captures are much more abundant in sandy areas than on nearby rocky substrates. Juvenile crabs might be spending most of their time on soft bottoms, where they find refuge in this highly exposed area, but move frequently to rocky areas, where they feed. Because previous telemetry studies were based on daylight observations (González-Gurriarán and Freire, 1994; Hines *et al.*, 1995) and detected no short-term habitat changes, our results suggest that spider crabs probably make nightly movements among habitats to feed.

Our results demonstrate two patterns in the behaviour of spider crab juveniles: 1) on average feeding is carried out mainly on rocky bottoms, where they obtain approximately 2/3 of their diet; 2) inter-individual variability in food sources shows that body condition appears to be better in crabs that obtain a higher than average proportion of food in sandy habitats. Spider crab juveniles remain most of the time in sandy habitats because they probably find refuge there (constituting aggregations and burying to decrease predation risk and physical stress). In rocky habitats, when juveniles attain a certain body size, physical stress is higher and refuges in crevices are scarce (Corgos, 2004). Though food from sandy habitats is probably of higher quality, the risk of predation on foraging crabs is higher on sandy than on rocky substrates (whereas for inactive crabs it is higher on rocky substrates).

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