Effects of the invasive macroalga *Lophocladia lallemandii* on the diet and trophism of *Pinna nobilis* (Mollusca: Bivalvia) and its guests *Pontonia pinnophylax* and *Nepinnotheres pinnotheres* (Crustacea: Decapoda)

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SUMMARY: *Pinna nobilis* and its guests *Pontonia pinnophylax* and *Nepinnotheres pinnotheres* were sampled from *Posidonia oceanica* meadows invaded and non-invaded by the red alga *Lophocladia lallemandii*. Understanding the interactions among native and invasive species was the primary aim. Therefore, the effects of the invasive *L. lallemandii* on the percentage contribution of the food sources of *P. nobilis* and its guests and their trophic associations were investigated by applying mixing models to the δ^{13}C and δ^{15}N stable isotopes. Isotopic analyses revealed that the guests of *P. nobilis* occupied a higher trophic level than their host due to their capacity for food selection. The invasion of *L. lallemandii* altered the percentage contribution of the food sources to the consumers' diet. Whereas the percentage contribution of the food sources to the δ^{13}C signatures did not really change, *L. lallemandii* produced variations in the percentage contribution to the δ^{15}N signatures. This red macroalga represented one of the main food sources for *P. nobilis*, with a real contribution of 19.4% in the invaded meadows. Moreover, *L. lallemandii* slightly decreased the trophic level of the consumers. This study provides the first isotopic and trophic data for the pea crab *N. pinnotheres*.

Keywords: *Pinna nobilis*, *Pontonia pinnophylax*, *Nepinnotheres pinnotheres*, food sources, stable isotopes, mixing models, *Lophocladia lallemandii*, *Posidonia oceanica*.

RESUMEN: EFECTOS DE LA INVASIÓN DE LA MACROALGA INVASORA *LOPHOCLADIA LALLEMANDII* EN LA DIETA Y EL TROFISMO DE *PINNA NOBILIS* (MOLLUSCA: BIVALVIA) Y SUS HUÉSPEDES *PONTONIA PINNOPHYLAX* Y *NEPINNOTHERES PINNOTHERES* (CRUSTACEA: DECAPODA). – *Pinna nobilis* y sus huéspedes, *Pontonia pinnophylax* y *Nepinnotheres pinnotheres*, fueron muestreados en praderas de *Posidonia oceanica* invadidas y no invadidas por el alga roja *Lophocladia lallemandii*. Uno de los principales objetivos es entender las interacciones entre las especies nativas e invasoras. Para ello, se investigaron los efectos de la macroalga invasora *L. lallemandii* sobre el porcentaje de contribución de las fuentes de alimentación y sobre las asociaciones tróficas de *P. nobilis* y sus huéspedes, aplicando "mixing models" a los isótopos estables de δ^{13}C y δ^{15}N. Los análisis isotópicos revelaron que los huéspedes de *P. nobilis* ocupan un nivel trófico superior al de su hospedador, siendo la capacidad para seleccionar el alimento la razón de este incremento trófico. La invasión de *L. lallemandii* alteró el porcentaje de contribución de las fuentes de alimentación a la dieta de los consumidores. Mientras que el porcentaje de contribución de las fuentes de alimentación no se vio alterado para las firmas isotópicas de δ^{13}C, *L. lallemandii* produjo variaciones en el porcentaje de contribución para las firmas isotópicas de δ^{15}N. Esta macroalga representó una de las principales fuentes de alimento para *P. nobilis*, con una contribución real del 19.4% en praderas invadidas. Además, *L. lallemandii* disminuyó ligeramente el nivel trófico de los consumidores. Este estudio proporciona los primeros datos isotópicos e información trófica del cangrejo guisante *N. pinnotheres*.

Palabras clave: *Pinna nobilis*, *Pontonia pinnophylax*, *Nepinnotheres pinnotheres*, contribución de las fuentes de alimentación, isótopos estables, mezcla de modelos, *Lophocladia lallemandii*, *Posidonia oceanica*.

INTRODUCTION

Interactions between native and introduced species represent a threat to biodiversity and ecosystem functioning (Galil, 2007). In fact, competitive exclusion by invasive species has been reported as a cause for the progressive regression of seagrasses (Williams, 2007). These important habitats are very sensitive to environmental degradation and physical disturbance (Hemminga and Duarte, 2000). The most widespread seagrass species in the Mediterranean Sea is *Posidonia oceanica* (L.) Delile. *P. oceanica* communities include primary producers such as red algae, epibionts and vagile biota (Templado *et al.,* 2004). Faunal communities associated with seagrass encompass many taxonomic groups, including bivalves such as *Pinna nobilis* Linnaeus (1758) (García-March *et al.,* 2002). The bivalve *P. nobilis* is a long-lived species (Butler *et al.,* 1993; Galinou-Mitsoudi *et al.,* 2006) that is endemic in the Mediterranean Sea, being one of the largest bivalves in the world (García-March, 2003). However, it is an endangered species (EEC 1992; Centoducati *et al.,* 2007) due to the increasing human impact on the seabed (Richardson *et al.*, 1999). The fan mussel *P. nobilis* is a benthic suspension feeder that can colonise different substrates such as bare sand bottoms (Katsanevakis, 2007) and soft-bottom areas overgrown by seagrass meadows at depths ranging from 0.5 to 60 m (Zavodnik *et al.*, 1991). In these areas, the fan mussel lives partially buried in the sand, usually anchored among *P. oceanica* shoots and hidden by the leaves of the canopy (García-March *et al.*, 2002).

P. nobilis is the host of two species of crustacean decapods: the shrimp *Pontonia pinnophylax* (Otto, 1821) and the pea crab *Nepinnotheres pinnotheres* (Linnaeus, 1758) (Rabaoui *et al.,* 2008). The decapods belonging to these *genera* also inhabit other organisms, such as mussels (Sun *et al.*, 2006), oysters (Mercado-Silva, 2005; Baeza, 2008), hydroids (Marin *et al.,* 2007), holothurians (Peter and Manning, 2003) and ascidians (Vicente, 1984). Previous studies have highlighted the association between the fan mussel and *P. pinnophylax* (Calafiore *et al.,* 1991; Richardson *et al.,* 1997; Kennedy *et al.,* 2001a; Lagana *et al.,* 2007), whereas only one study conducted by Rabaoui *et al.* (2008) has addressed the association between the fan mussel and the pea crab *N. pinnotheres*.

Invasive species represent a risk to natural ecosystems by damaging biodiversity and altering the structure and functioning of ecosystems (Boudouresque and Verlaque, 2002; MacDougall and Turkington, 2005). The red macroalga *Lophocladia lallemandii* (Montagne) F. Schmitz is an alien species that was introduced into the Mediterranean through the Suez Canal and is widespread throughout the tropics and subtropics (Boudouresque and Verlaque, 2002). *L. lallemandii* grows on a wide range of substrates (Patzner, 1998; Ballesteros, 2006; Ballesteros *et al.*, 2007), including *P. oceanica* meadows (Ballesteros *et al.*, 2007), where it induces shoot mortality and affects the invertebrate community (Patzner, 1998; Ballesteros, 2006). Therefore, invasion by *L. lallemandii* affects the characteristics of microhabitats and faunal communities (Piazzi *et al.,* 2002).

Analysis of isotopic composition has become an effective method for studying trophic food webs (Pinnegar and Polunin, 2000; Fisher *et al.*, 2001), since organisms assimilate stable carbon and nitrogen isotopes from their food sources (Pinnegar and Polunin, 1999). The values of stable $\delta^{13}C$ isotope have mostly been used to indicate primary food sources, whereas values of stable $\delta^{15}N$ isotope have allowed to determine the trophic level (Post, 2002). Isosource software (Phillips and Gregg, 2003) has made it possible to quantify the source contribution to a mixture by the application of mixing models (Phillips *et al.*, 2005). Previous studies using stable isotopes ($\delta^{18}O$-$\delta^{16}O$; Mg:Ca-Sr:Ca ratios; skeletal $\delta^{18}O$-skeletal $\delta^{13}C$) in the shell of the fan mussel *P. nobilis* shell have mainly been performed to estimate growth and age (Richardson *et al.*, 1999) and to reconstruct sea surface temperatures and ontogenetic records of metabolic CO_2 (Kennedy *et al.,* 2001b). However, only in one study that analysed $\delta^{13}C$ and $\delta^{15}N$ signatures was it demonstrated that the fan mussel and its guest shrimp *P. pinnophylax* had assimilated similar food sources and, consequently, were at a similar trophic level (Kennedy *et al.*, 2001b). In contrast, studies on the diet and trophic relationships of *P. nobilis* and its guest pea crab *N. pinnotheres* have not been undertaken.

Therefore, this study aimed mainly to determine the effect of invasive *L. lallemandii* on the contribution of food sources to the diet of the fan mussel *P. nobilis* and its two guests *P. pinnophylax* and *N. pinnotheres* and their trophic associations.

MATERIALS AND METHODS

Sampling area

This study was carried out in two different environments: *P. oceanica* meadows non-invaded and invaded by the red macroalga *L. lallemandii*. The non-invaded meadows were three sites located in Espardell (7-10 m depth; SE of Ibiza; 38°48'10''N, 1°28'42''E), Esponja (20-25 m depth; SE of Ibiza; 38°52'34''N, 1°25'37''E) and Talamanca (7-10 m depth; SE of Ibiza; 38°54'50''N, 1°28'13''E) (Fig. 1A). The invaded meadows were three sites in Sa Dragonera Natural Park (7-10 m depth; SW of Mallorca Island, Balearic Islands; 39°34'48''N, 2°20'54''E) where *L. lallemandii* had invaded the *P. oceanica* seagrass meadows and epiphytised the *P. nobilis* individuals (Fig. 1B).

Sample collection

At each site, ten linear transects of 30x3 m were sampled by scuba diving. The sites were separated by hundreds of metres. All transects were laid over seagrass beds at 7-10 m depth (except for the Esponja sampling station, which was at a mean depth of 22.5 m); sand patches were also present. A total of 2700 m^2 were sampled for each treatment (*P. oceanica* invaded and non-invaded). According to the European Council Directive 92/43/EEC on the conservation of natural habitats and wild fauna and flora, the fan mussel *P. nobilis* is listed as an endangered species and is under strict protection (Annex IV), and all forms of deliberate capture or killing of this bivalve are prohibited (EEC 1992; Centoducati *et al.*, 2007). Therefore, in order to minimise the impact on local populations, only 24 individuals of *P. nobilis* were randomly collected (11 individuals in a *P. oceanica* meadow invaded by *L. lallemandii* and 13 individuals in a non-invaded meadow) in summer 2007 by experienced scuba divers under license from the appropriate institutions (government of the Balearic Islands). The size range of the samples was kept as constant as possible.

The fan mussels were quickly transferred to the laboratory where they were carefully dissected ac-
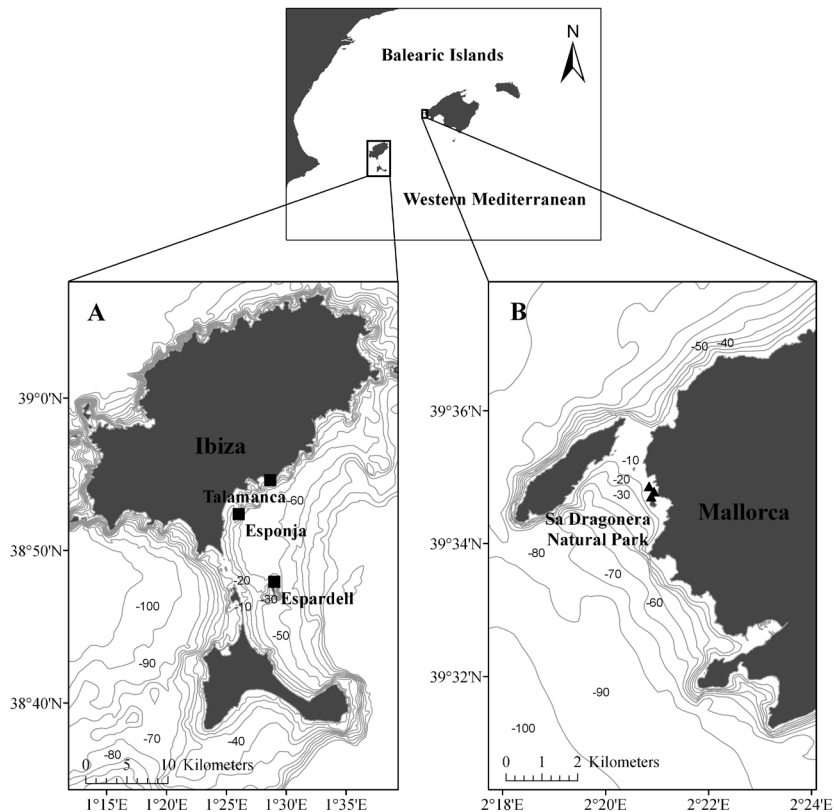

FIG. 1. – Geographic location of the study areas in the western Mediterranean. Continuous lines show isobaths every 10 metres up to 100 metres. A) Sampling areas that showed non-invaded seagrass meadow. ■ indicates the location of the study sites Espardell, Esponja and Talamanca; B) Sampling area that showed *P. oceanica* meadows invaded by *L. lallemandii*. ▲ indicates the three sites selected for the study in Sa Dragonera Natural Park.

cording to the procedure described by Yonge (1953). For each *P. nobilis*, the maximum shell width (W_m), maximum shell length (L_m) and maximum length of the posterior adductor muscle scars (L_{ad}) were measured (Rabaoui *et al.*, 2007). Age was determined by counting the number of abductor muscle scar rings (R) on the shell. However, because the muscle scar ring of the first year is absent or inconspicuous (Richardson *et al.*, 1999), the age was estimated as the number of rings plus one. In addition, the presence or absence of decapod guests was recorded for each fan mussel. The muscle of each *P. nobilis* and the muscle of the associated guests (*P. pinnophylax* and *N. pinnotheres*) were extracted. After extraction, tissues were immediately frozen and stored until further processing for stable isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$).

Potential common food sources were also collected: particulate organic matter (POM), sediment organic matter (SOM), *P. oceanica* leaves as a representation of the potential contribution of its remains, epiphytes of *P. oceanica* leaves (EPoL) and the invasive alga *L. lallemandii*. Water samples (10 l) for POM determination were collected at the maximum depth (~10 m) and filtered through pre-combusted fibreglass filters (Whatman GF/C) at 450°C for 4 h. SOM was collected by scuba divers from a quadrat surface of 400 cm^2 using steel quadrats. EPoL samples were manually isolated from *P. oceanica* leaves at the laboratory using stainless-steel tools, and were pooled and treated as a single sample for each meadow (Cardona *et al.*, 2007). SOM and EPoL were acidified with HCl (2 N) drop-by-drop (the cessation of bubbling was used as the criterion to determine the amount of acid to add) and left for 3 h (Carabel *et al.*, 2006).

Isotopic analysis and processing

Muscle and food source samples were dried at 60°C to constant weight and then ground to a fine powder using a mortar and pestle. Homogeneous dried powder (2 ± 0.1 mg) of each sample was placed in cadmium tin cups and then combusted to determine ^{13}C and ^{15}N stable isotope composition by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a THERMO DELTA X-PLUS mass spectrometer. Samples of an internal reference material were analysed after every eight samples to calibrate the system and to compensate for drift over time. The global standard for $\delta^{13}C$ is CO_2 and for

$\delta^{15}N$ is atmospheric nitrogen. The reference material used for the stable isotopes analyses was the Bovine Liver Standard (BLS: 1577b; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD 20899). The analytical precision was based on the standard deviation of BLS replicates: 0.02‰ and 0.10‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Stable isotope abundances were measured by comparing the ratio of the most abundant isotopes ($^{13}C{:}^{12}C$ and $^{15}N{:}^{14}N$) in the samples with the international isotopic standards. Carbon and nitrogen stable isotope ratios were expressed in δ notation in terms of parts per thousand (‰) deviations from the standards, according to the following equation:

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

where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratio.

In order to determine the trophic level of the organisms, the following formula was applied:

$$\text{Trophic Level} = (\delta^{15}N_{consumer} - \delta^{15}N_{\text{First trophic level}})/3.4+1$$

where 3.4‰ is the assumed $\delta^{15}N$ trophic enrichment factor (Le Loc'h *et al.,* 2008).

Food source contribution (%)

In order to determine the percentage contribution of the food sources to the fan mussel *P. nobilis* and its guests *P. pinnophylax* and *N. pinnotheres*, feasible contributions for every source were estimated by isotope mixing models using IsoSource software version 1.3.1 (Phillips and Gregg, 2003). The red macroalga *L. lallemandii* was considered as a feasible food source in order to analyse its relative contribution and its effects on the diet of the consumers *P. nobilis* and its guests *P. pinnophylax* and *N. pinnotheres*, regardless of the situation (invaded or non-invaded). By calculating the difference between the hypothetical contribution of *L. lallemandii* in a non-invaded meadow and the *L. lallemandii* contribution in an invaded meadow, the real contribution of *L. lallemandii* was obtained.

The model was used to estimate the potential contributions of the primary producer groups to the fan mussels and their guests. At increments of 1% and a tolerance of 0.1, the mean of the isotopic values, 1st to 99th percentiles, and the range of probable

TABLE 1. – Morphometric features of *Pinna nobilis* specimens and the presence of its associated guests, the shrimp *Pontonia pinnophylax* and the pea crab *Nepinnotheres pinnotheres*. Maximum shell width (W_m), maximum shell length (L_m), maximum length of the posterior adductor muscle scars (L_{ad}), and number of muscle scar ring (R) are represented. ● indicates the presence of guests of only one sex. ▲ indicates the presence of the couple guests (male and female simultaneously).

		Pinna nobilis cm			age-years	Guests *P. pinnophylax*	*N. pinnotheres*
		W_m	L_m	L_{ad}	R		
Individuals from invaded *Posidonia oceanica* meadow							
	1	16.0	41.4	19.8	7	▲	
	2	15.9	37.2	18.0	6	▲	
	3	15.6	29.0	14.0	3	●	
	4	18.0	37.0	15.8	5		
	5	13.0	26.9	13.3	3		
	6	16.1	37.4	18.3	7		
	7	13.2	26.8	12.0	3	●	
	8	15.0	27.0	13.2	3	▲	
	9	17.4	36.7	16.4	5	▲	
	10	14.6	38.0	19.0	7	●	
	11	15.3	39.0	16.4	6	▲	
Individuals from non-invaded *Posidonia oceanica* meadow							
	12	12.0	31.0	14.4	3	▲	
	13	13.9	25.8	11.2	2		
	14	15.8	44.0	20.02	7	▲	
	15	14.6	29.6	14.4	4	●	
	16	14.9	27.9	12.8	3	●	
	17	11.3	24.5	13.3	4		
	18	24.0	69.2	41.0	11		
	19	18.2	41.0	24.0	3	▲	●
	20	21.0	61.1	29.5	10		●
	21	22.1	63.6	31.0	10		
	22	15.4	38.0	17.8	6		
	23	17.6	42.0	18.8	6		
	24	16.0	31.0	14.2	4		

contributions to the organisms were determined for every primary producer (Decottignies *et al.,* 2007; Ince *et al.,* 2007; Pitt *et al.,* 2008). In the absence of consumer-specific isotope discrimination factors for these two consumers, an assumed discrimination of 0.5‰ was applied for carbon (Pitt *et al.*, 2008). Nitrogen could not be incorporated into the model since the discrimination levels were unknown, and applying an assumed 3–4‰ value did not yield results because the values of the consumers lay outside the polygon created by the potential sources (Ince *et al.*, 2007; Pitt *et al.*, 2008).

Statistical analysis

Statistical analysis was carried out using SPSS☐ (v. 16.0 for Windows☐). One-way ANOVA was performed in order to analyse the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures in the *P. nobilis* individuals inhabiting *P. oceanica* meadows invaded by the red alga *L. lallemandii* and the fan mussels that occurred in non-invaded seagrasses. One-way ANOVA was also used to determine the $\delta^{13}C$ and $\delta^{15}N$ differences be-

tween the *P. pinnophylax* individuals that colonised *P. nobilis* inhabiting invaded meadows and those in non-invaded meadows.

RESULTS

Biometry of *Pinna nobilis* and presence/absence of guests

The mean values of maximum shell width (W_m), maximum shell length (L_m), maximum length of the posterior adductor muscle scars (L_{ad}) and number of muscle scar rings (R) of the 24 *P. nobilis* (11 in invaded seagrass and 13 in non-invaded seagrass) were 16.12 ± 0.61, 37.71 ± 2.44, 18.28 ± 1.42 and 5.45 ± 0.53 (mean ± SE), respectively (Table 1).

The fan mussels *P. nobilis* hosted the shrimp *P. pinnophylax* in 54.2% and the pea crab *N. pinnotheres* in 8.3% of the samples. *P. pinnophylax* couples (female and male) were present in 33.33% and a solitary shrimp in 20.8% of the samples. Both guests (shrimp and pea crab) were present in 4.2% of the
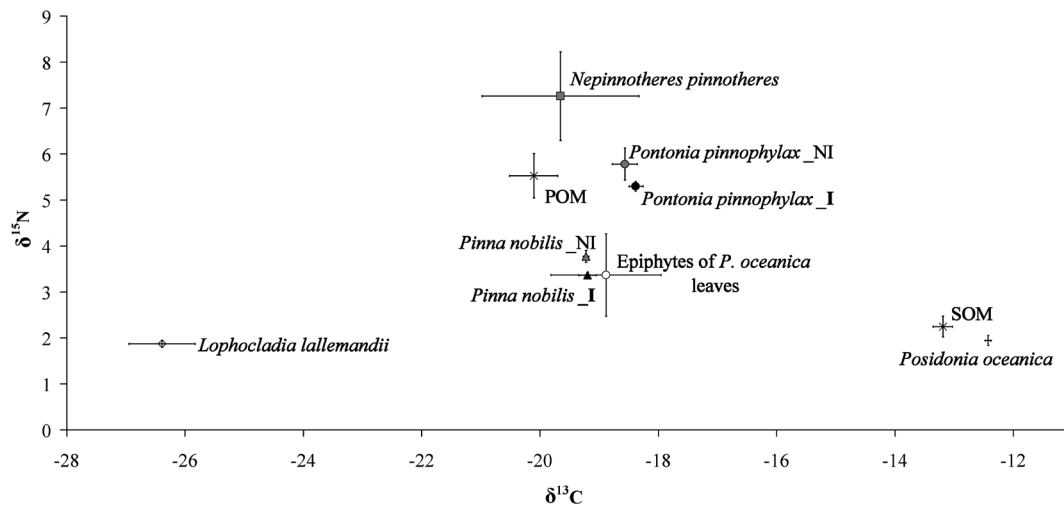
FIG. 2. – Distribution of the carbon and nitrogen stable isotopes ratios (mean ± SE) for the fan mussel *Pinna nobilis*, its decapod guests (*Pontonia pinnophylax* and *Nepinnotheres pinnotheres*) and the potential dietary sources. I: invaded; NI: non-invaded; POM: particulate organic matter; SOM: sediment organic matter.

P. nobilis samples (Table 1). The pea crab *N. pinnotheres* only occurred in two *P. nobilis*, both from non-invaded seagrass. Ten *P. nobilis* did not have any guests, corresponding to 41.7% of the specimens sampled.

Stable isotopes

The $\delta^{13}C$ and $\delta^{15}N$ mean values (±SE) are shown in Figure 2. The consumers *P. nobilis* and its guests *P. pinnophylax* and *N. pinnotheres* showed little variation in the $\delta^{13}C$ values. For δ^5N values, these consumers showed more variation, being ranked as follows: *N. pinnotheres* > *P. pinnophylax* > *P. nobilis*. The isotopic $\delta^{13}C$ and $\delta^{15}N$ signatures for invaded (fan mussels from seagrass invaded by *L. lallemandii*) and non-invaded (fan mussels from non-invaded *P. oceanica* meadow) *P. nobilis* were similar. The

$\delta^{13}C$ and $\delta^{15}N$ values for invaded and non-invaded *P. pinnophylax* were also similar. Nevertheless, the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of invaded *P. nobilis* and *P. pinnophylax* showed values below the isotopic signatures of non-invaded *P. nobilis* and *P. pinnophylax* (Fig. 2 and Table 2). Statistical analysis demonstrated differences between invaded and non-invaded *P. nobilis* $\delta^{15}N$ signatures (ANOVA, $P<0.05$). In contrast, the $\delta^{13}C$ isotopic signatures of invaded and non-invaded *P. nobilis* were not significantly different (ANOVA, $P>0.05$). No differences between $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures were found in the shrimp *P. pinnophylax* (invaded and non-invaded) (ANOVA, $P>0.05$).

The food sources EPoL and POM ranged in the same group constituted by the consumers *P. nobilis*, *P. pinnophylax* and *N. pinnotheres*. EPoL was positioned near the fan mussel *P. nobilis* and POM

TABLE 2. – Number of samples (n), stable isotopes values ($\delta^{13}C$ and $\delta^{15}N$) and trophic level (TL) of the studied species at invaded and non-invaded locations and the food sources. Results are expressed as mean ± standard error (SE). POM, particulate organic matter; SOM, sediment organic matter; ---, data insufficient.

Species and Food sources		$\delta^{13}C$		$\delta^{15}N$		
	n	Mean	SE	Mean	SE	TL
Pinna nobilis invaded	11	19.20	0.15	3.37	0.06	1.43
Pontonia pinnophylax invaded	13	18.39	0.13	5.30	0.11	2.00
Pinna nobilis non-invaded	13	19.22	0.05	3.77	0.13	1.55
Pontonia pinnophylax non-invaded	8	18.56	0.21	5.78	0.34	2.14
Nepinnotheres pinnotheres	2	19.66	1.33	7.26	0.96	2.57
Lophocladia lallemandii	4	26.39	0.56	1.87	0.08	1.00
Posidonia oceanica	6	12.43	0.05	1.94	0.11	1.01
POM	4	20.11	0.41	5.53	0.48	2.06
SOM	2	13.19	---	2.24	---	1.10
Epiphytes of *P. oceanica* leaves	4	18.89	0.93	3.37	0.89	1.43
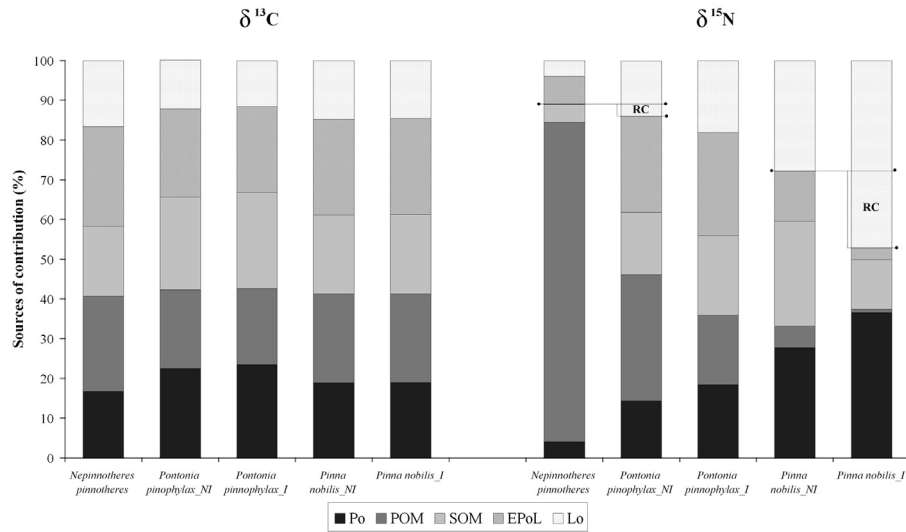
Fɪɢ. 3. – Mean values of potential food sources contribution (%) to the δ^{13}C and δ^{15}N diet of the fan mussel *Pinna nobilis*, the shrimp *Pontonia pinnophylax* and the pea crab *Nepinnotheres pinnotheres* in an invaded and non-invaded situation. I: invaded; NI: non-invaded; Po: *Posidonia oceanica*; POM: Particulate organic matter; SOM: sediment organic matter; EPoL: epiphytes of *Posidonia oceanica* leaves; Lo: *Lophocladia lallemandii*. The real contributions (RC) of *L. lallemandii* are delimited by the dashed lines.

was located near its guests (Fig. 2 and Table 2). The invasive red alga *L. lallemandii* was only sampled in Sa Dragonera where the seagrass was invaded by this red alga, and contained the lowest δ^{13}C isotopic value. In contrast, *P. oceanica* was the most enriched in δ^{13}C. In relation to the δ^{15}N isotopic values, similar results were found in these food sources. SOM was located near *P. oceanica* (Fig. 2 and Table 2).

Two trophic levels were established (Table 2). The first included the primary producers *L. lallemandii* and *P. oceanica*, SOM and primary consumers such as EPoL and invaded and non-invaded *P. nobilis*; the second incorporated the fan mussel guests (invaded and non-invaded *P. pinnophylax* and *N. pinnotheres*) and POM.

Food source contributions

IsoSource results determined the percentage contribution of the potential food sources to the fan mussel *P. nobilis* and its guests *P. pinnophylax* and *N. pinnotheres* from *P. oceanica* meadows, either invaded or non-invaded by the red macroalga *L. lallemandii* (Fig. 3).

For *P. nobilis*, the percentage contribution of each food source to the δ^{13}C signature was almost identical in the two situations (invaded and non-invaded). The greatest contribution based on δ^{13}C came from the EPoL (24.4%), followed by POM, SOM and *P. oceanica* particulate remains. The red macroalga *L. lallemandii* made a minor contribution to the *P.*

nobilis (invaded and non-invaded) δ^{13}C value. However, *L. lallemandii* made a major contribution to the *P. nobilis* δ^{15}N signature (47.2% invaded and 27.8% non-invaded). Consequently, the real contribution of *L. lallemandii* to the *P. nobilis* δ^{15}N signature was 19.4% (Fig. 3). Invaded and non-invaded *P. nobilis* showed differences in the percentage contribution of the food sources based on the δ^{15}N signature. The highest contributions to the δ^{15}N signature in invaded mussels were made by *L. lallemandii* and *P. oceanica* particulate remains, showing an overall contribution of 83.7%. For the non-invaded *P. nobilis* δ^{15}N signature, *L. lallemandii* and *P. oceanica* particulate remains represented 55.5% of the contribution. SOM also made a significant contribution (26.4%) to non-invaded fan mussels (Fig. 3).

On the other hand, in the shrimp *P. pinnophylax* δ^{13}C signature, the food sources SOM, *P. oceanica*, EPoL and POM made similar contributions within the range of 19.1-24.3%. *L. lallemandii* made a minor contribution to *P. pinnophylax* (invaded and non-invaded) in the δ^{13}C signature. In terms of the δ^{15}N signature, the epiphytes of *P. oceanica* leaves made a major contribution (26.0%) to invaded *P. pinnophylax*. POM and EPoL made the highest contribution to non-invaded *P. pinnophylax* at 31.8% and 24.1%, respectively. *L. lallemandii* showed little real contribution (4.1%) to the *P. pinnophylax* δ^{15}N signature (14.0% non-invaded shrimp, 18.1% invaded shrimp) (Fig. 3).

In the pea crab *N. pinnotheres*, strong differ-

ences in the contribution percentages were observed between δ^{13}C and δ^{15}N signatures. All food sources contributed to the δ^{13}C signature, with a range of 16.6-25.1%, the highest being from the EPoL. In contrast, POM (80.5%) made the highest contribution to the δ^{15}N signature.

DISCUSSION

The invasion of *L. lallemandii* altered the δ^{15}N percentage contribution of the food sources to the consumers' diet and slightly decreased the trophic level. The present study revealed that the guests of *P. nobilis* (*P. pinnophylax* and *N. pinnotheres*) occupied a higher trophic level than their host. The food selection capacity of the guests may allow them to increase their trophic level. Isosource results showed that the epiphytes of *P. oceanica* leaves made the highest contribution to the δ^{13}C signatures of *P. nobilis* and its guest *N. pinnotheres*. For *P. pinnophylax* the food sources SOM, *P. oceanica*, EPoL and POM made similar contributions; *L. lallemandii* made a minor contribution. In contrast, according to the δ^{15}N isotopic values, POM and EPoL represented the main food sources for *N. pinnotheres* and *P. pinnophylax*, respectively. In the case of the *P. nobilis* δ^{15}N signature, *L. lallemandii* and *P. oceanica* were the main food source, with *L. lallemandii* presenting a real contribution of 19.4% in the invaded meadow.

Several studies have reported seasonal, spatial, food web, and organism size variations and fractionation among tissues as a consequence of differences in stable isotope signatures (Decottignies *et al.,* 2007). The experimental design of the present work avoided the interference of factors such as geographical differences or physicochemical water parameters, since the samples were collected from the same extended area and during the same period. Moreover, all *P. nobilis* had comparable dimensions. Therefore, the study design avoided biases in the stable isotopic values as far as possible.

The isotopic δ^{13}C and δ^{15}N isotopic values obtained in the present study were within the range of previous studies (Kennedy *et al.*, 2001a; Cardona *et al.*, 2007; Deudero *et al.*, 2009). The variations in some isotopic values such as those in EPoL occurred because seagrass epiphyte δ^{15}N values can vary by 3‰ depending on the proportion of animals and plants that form this epiphyte community (Kennedy

et al., 2001a). According to the δ^{15}N isotopic results obtained in the current work, which determine the trophic level (Post, 2002), the guests of *P. nobilis* (*P. pinnophylax* and *N. pinnotheres*) occupied a higher trophic level than their host. *P. pinnophylax* and *N. pinnotheres* showed more enriched δ^{15}N isotopic values than their host. The difference in δ^{15}N between *P. nobilis* and *P. pinnophylax* was 1.97‰, and between *P. nobilis* and *N. pinnotheres* it was 3.49‰. However, the fan mussel guests had similar δ^{13}C isotopic values. A slight increase in the δ^{15}N isotopic value of *P. pinnophylax* with respect to its host *P. nobilis* has previously been reported (Kennedy *et al.*, 2001b). Nonetheless, this study provides the first isotopic and trophic data for the pea crab *N. pinnotheres*, which had the highest δ^{15}N enrichment of the two guests. The isotopic enrichment of the fan mussel guests in the present work was consistent with the estimated trophic level of the consumers (Le Loc'h *et al.*, 2008). The capacity for food selection may explain the differences in the contribution of the different food sources to their diets, since the morphology of both studied guests allows them to select food sources (Rabaoui *et al.*, 2008). A previous study also demonstrated that the capacity for food selection generated variations in isotopic composition (Decottignies *et al.*, 2007).

Since *P. nobilis* feeds on suspended materials (Kennedy *et al.*, 2001b), *L. lallemandii* could become a new potential food source that contributes to the fan mussel δ^{15}N isotopic signatures; however, *P. nobilis* guests can select food to avoid feeding competition, as reported in other species with the capacity for food selection (Decottignies *et al.*, 2007). In fact, a previous investigation suggested the need to include the qualitative selection capacities of consumers in future interpretations of trophic relationships in marine coastal ecosystems (Decottignies *et al.*, 2007). Isosource results showed that EPoL made the highest contribution to the δ^{13}C signatures of all studied consumers, while for δ^{15}N isotopic values, POM and EPoL represented the main food sources for *N. pinnotheres* and *P. pinnophylax*, respectively. There was greater variability in the contribution of food sources to *P. nobilis* δ^{15}N isotopic values, with *L. lallemandii* and *P. oceanica* remains being the main food sources in the *P. oceanica* meadow invaded by *L. lallemandii*. In agreement with these results, a previous study reported food source contributions in a non-invaded *P. oceanica* meadow in which EPoL contributed to the *P. pinnophylax* diet

and *P. oceanica* remains were a potential food source for *P. nobilis* (Kennedy *et al.*, 2001b).

The invasion of *L. lallemandii* altered the percentage contribution of the food sources to the consumers' diet. In fact, it has been reported that the incorporation of new sources resulted in changes in the contribution of food sources to an organism's diet (Phillips and Gregg, 2003). Whereas the percentage contribution of the food sources to the $\delta^{13}C$ signatures did not really change, *L. lallemandii* produced variations in the percentage contribution to the $\delta^{15}N$ signatures. Differences in the percentage contribution between $\delta^{13}C$ and $\delta^{15}N$ signatures by food sources such as POM and SOM have been reported previously (Sarà *et al.,* 2004). The contributions to $\delta^{15}N$ signatures of invaded versus non-invaded *P. pinnophylax* showed little variation, almost avoiding the effect of the red macroalga *L. lallemandii* (4.1% of real contribution), perhaps due to its capacity for food selection (Rabaoui *et al.*, 2008). In invaded versus non-invaded *P. nobilis*, the food sources showed variations in their percentage contribution to the $\delta^{15}N$ signatures, with *L. lallemandii* making a real contribution of 19.4% in *P. nobilis* located in the invaded seagrass meadows. Therefore, invasion by *L. lallemandii* led to a slight decrease in the trophic level of the consumers *P. nobilis* and the shrimp *P. pinnophylax*.

Changes in the trophism of *P. nobilis* in a *P. oceanica* meadow invaded by *L. lallemandii* could be related to the physiological effects of the lophocladines, bioactive alkaloids from the genus *Lophocladia* (Gross *et al*., 2006). The invasive alga *L. lallemandii* has an injurious effect on the sea grass *P. oceanica* meadow (Ballesteros *et al.*, 2007) and contains lophocladines (Sureda *et al.*, 2008) that might reverberate in the physiology of *P. nobilis*, as demonstrated for other organisms (Boudouresque and Verlaque, 2002; Ballesteros, 2006; Sureda *et al.*, 2006). Future studies are thus required to determine the physiological effect that the invasive red alga *L. lallemandii* might induce in endemic species.

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