Trophic habits of the invasive crab *Libinia dubia* H. Milne Edwards, 1834 from the Gulf of Gabès (Tunisia)

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Handling Editor: Agnese MARCHINI

Abstract

Feeding habits of the invasive spider crab *Libinia dubia* from the Mediterranean Sea were studied in the Gulf of Gabès (Tunisia) using the frequency of occurrence and points methods. The population was sampled at least monthly between November 2015 and October 2016. Stomach contents of 384 specimens were analyzed. Results indicate that *L. dubia* is an herbivorous species exhibiting clear preferences for algae (ALG) and Magnoliophyta (MAG) (62.03%, 7.13 points and 59.36%, 5.3 points respectively) although Echinodermata (ECH), Porifera (POR), Cnidaria (CNI), Mollusca (MOL), Polychaeta (POL), Crustacea (CRU), and fish (FIS) were accidentally consumed along with Bryozoa (BRY), sediment (SED), and unidentifiable materials (UNM). The diversity of ALG ingested was studied in detail: Chlorophyceae were found in 87.93% of stomachs containing ALG and contributed most of points to the stomach contents (4.18 points) followed respectively by Phaeophyceae (81.03%, 2.27 points) and Rhodophyceae (40.95%, 0.68 points). Very low Vacuity Index was recorded (VI = 2.6%). Ingested items varied significantly with the season (Chi-square test, $\chi^2_{calculated} = 87.86 > \chi^2_{theoretical} = 7.81$, $df = 3$, $p < 0.05$) and crab size ($\chi^2_{calculated} = 14.25 > \chi^2_{theoretical} = 5.99$, $df = 2$, $p = 0.026$). Insignificant differences were registered by studying Carapace Width-Stomach Weight (CW-SW) relationships (T-test, $t_{calculated} < t_{theoretical}$, $p > 0.05$). Kruskal-Wallis test was applied so that the composition of crab diet among groups could be compared (H = 1.1, $df = 3$, $p = 0.77$).

Keywords: biological invasions; establishment; introduction patterns; non-indigenous species; potential impact; stomach contents.

Introduction

Biological invasions are recognized worldwide as an important element of global change and constitute a major threat to the marine environment. The number of Non-Indigenous Species (NIS) has increased during the last decades due to globalization and increases in various activities, such as shipping, fisheries, aquaculture, aquarium trade, and climate change (Cohen & Carlton, 1998; Zenetos *et al.*, 2012; Klaoudatos & Kapiris, 2014; Ojaveer *et al.*, 2018). Some introduced species have been documented to displace or harm native species, alter community structure, and food webs in addition to disrupting hosted ecosystem (Galil, 2011; Galil *et al.*, 2014; Katsanevakis *et al.*, 2014; Ojaveer *et al.*, 2015). Consequently, alteration or loss of ecosystem goods and services by these species is overlooked or underappreciated

(Leppäkoski *et al.*, 2002; Charles Lis & Dukes, 2007; Ojaveer *et al.*, 2018).

The Mediterranean Sea is a hotspot of biological invasion records susceptible to Alien Invasive Species (AIS) where the main invasion vectors are ballast water and transportation through the Suez Canal (Galil, 2000; Galil & Zenetos, 2002; Rilov & Galil, 2009). Among AIS, metazoans are substantially greater for the Mediterranean Sea than anywhere else (Galil *et al.*, 2014). By March 2015, 136 non-indigenous fauna species have been reported from Tunisian marine waters, of which 24% were crustaceans (Ounifi-Ben Amor *et al.*, 2016). The best-known marine alien crustaceans are decapods, and their pervasiveness, importance, and impacts on recipient ecosystems have been well studied (Galil, 2011). The Western Atlantic native crab *Libinia dubia* H. Milne Edwards, 1834, was observed for the first time in the Mediterra-

nean Sea on southern Tunisian coasts nearly two decades ago (Enzenross *et al.*, 1997). This crab was collected in the Gulf of Gabès, near the oil terminal of Skhira, probably introduced via ballast water (Enzenross & Enzenross, 2000). Since then, large populations have spread widely and established in the area, causing negative impacts on local coastal artisanal fisheries such as net clogging, in addition to the loss and alteration of catches (Ben Souissi, 2015; Ounifi-Ben Amor *et al.*, 2016; Rjiba-Bahri *et al.*, 2019). The eradication of this species is no longer considered possible (Ben Souissi, 2015); therefore, biological and ecological studies as well as potential impact studies are urgently required for generating an effective risk assessment, and obtaining data is a crucial tool to help decision makers for developing a successful management strategy.

Epialtid crabs are mainly herbivores (Vasconcelos *et al.*, 2009). According to Corrington (1927), *L. dubia* is a scavenger which feeds on easily obtained plant matter, animal tissue, and detritus. In seagrass beds, this species consumes algae belonging to several genera (Stachowicz & Hay, 1999) and some authors noted its feeding on jellyfish mesoglea (Jachowski, 1963; Phillips *et al.*, 1969; Tunberg & Reed, 2004). Despite that this invasive alien brachyuran crab has been reported since the late 1990s and represents a real threat to the sustainability of artisanal fishing, its biological and ecological impacts are poorly documented (Ounifi-Ben Amor *et al.*, 2016; Rjiba-Bahri *et al.*, 2019). This work focuses on the seasonal trophic habits of *L. dubia* in its new area. The expected results of this study target the key threats of this invasive crab, especially its ecosystem engineering capability through predation and habitat modification. This research
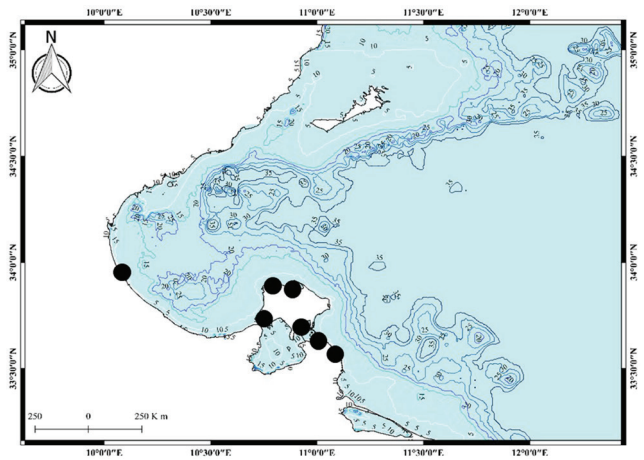

Fig. 1: Location of the study area and sampling sites in the Gulf of Gabès.

will also provide a solid knowledge of crab diet and can help to predict future distribution, tolerance, and food web interactions.

Materials and Methods

Study area and sampling effort

The study was carried out in the Gulf of Gabès, located in south-eastern Tunisia. It extends for about 750 km from Ras Kapoudia at the parallel 35°N to Tunisian-Libyan border (Fig. 1). Nocturnal and diurnal surveys were conducted at seven sites between November 2015 and October 2016 at depths from 3 to 47 m (Table 1). Foregut

Table 1. Details of sampling surveys along the Gulf of Gabès. N: night collection, D: daytime collection.

Nº	Sampling survey date	Collection time	Latitude	Longitude	Depth (m)
1	11/11/2015	N	33.659126° N	10.931991° W	16
2	28/12/2015	N	33.65934° N	11.070179° W	20
3	06/01/2016	D	33.708763° N	10.744194° W	7
4	12/01/2016	D	33.649624° N	10.940231° W	15
5	11/02/2016	D	33.722756° N	10.730804° W	3
6	26/02/2016	D	33.643622° N	11.015762° W	10
7	18/03/2016	D	33.972963° N	10.131019° W	22
8	29/03/2016	D	33.642764° N	10.927185° W	16
9	16/04/2016	D	33.645337° N	10.977653° W	12
10	25/04/2016	D	33.659126° N	10.931991° W	16
11	25/05/2016	N	34.152004° N	11.206305° W	47
12	26/05/2016	N	33.917283° N	10.740074° W	20
13	24/06/2016	D	33.649624° N	10.940231° W	15
14	19/07/2016	D	33.65934° N	11.070179° W	20
15	28/08/2016	N	33.468954° N	11.235832° W	18
16	07/09/2016	N	33.659126° N	10.931991° W	16
17	03/10/2016	N	33.5903° N	11.320975° W	32

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contents of 384 crabs (201 females and 183 males) captured by gillnets and trammel nets were used to study the diet of *L. dubia*.

Experimental Protocol

In the laboratory, Carapace Width (CW) and Total Weight (TW) of each specimen were measured to the nearest 0.01 mm and 0.01 g, respectively. Stomachs removed from live crabs were weighed (SW), examined immediately or preserved in absolute ethanol 1:1. Stomach contents were extracted, weighed (SCW), and placed into a Petri dish. Fragments of the digestive tract were removed and stomach contents were then analyzed under a binocular microscope. Items were identified to the lowest taxonomic level possible. The trophic guild of *L. dubia* was studied using the frequency of occurrence method, commonly applied for crab stomach contents analysis (Williams, 1981). The points method, Importance in Weight Index (IWI) and Vacuity Index (VI) were also used to study the diet of *L. dubia* (Hyslop, 1980; Woods, 1993).

- Frequency of occurrence (FO) = SFI/NES x 100; FO = Number of stomachs which contained a food item (SFI) divided by the total number of filled or non-empty stomachs observed (NES) multiplied by 100.
- Points method: Each stomach was allocated 0-20 points according to its fullness: full stomach, 20 points; 3/4 full, 15 points; 1/2 full, 10 points; 1/4 full, 5 points; empty, 0 points. We also allocated intermediate scores. Then, points were assigned to the different items of stomach contents. For example, if a stomach received 20 points because of its fullness, and 3/4 of its contents were made up of algae, then the algae category was allocated 15 points.
- Importance in Weight Index (IWI) = (Wi/Wt) x 100; IWI = weight of each type of food item i (Wi) divided by the total weight of all food items (Wt) multiplied by 100.
- Vacuity Index (VI) = ES/TS x 100; VI = number of empty stomachs (ES) divided by the total number of examined stomachs (TS) multiplied by 100.

The relationship between crab size (CW) and the weight of its stomach (SW) was assessed by the regression analysis and could be expressed using the power function $Y = a X^b$. Therefore, values were adjusted to the potential type model where the two parameters a and b were calculated by the least-squares method.

Diet composition was evaluated with respect to the size; crabs were grouped into three classes: carapace width CW < 50 mm (class1, small specimens), 50 mm < CW < 60 mm (class 2, medium sized specimens), CW > 60 mm (class 3, large specimens). Some foregut contents were photographed using an electronic microscope connected to a pc (Leica DC software).

Statistical analyses

The nonparametric Kruskal-Wallis test (H-test) was used to compare the feeding habits among groups, which, in the present study, consisted of ovigerous females, non-ovigerous females, males, and overall (all categories, juveniles and adults combined) (Katz, 2006). Diet compositions in relation to season and crab size were tested using the Chi-square test (χ^2 test) (Zar, 1999). Differences were recorded as significant at $\chi^2_{calculated} > \chi^2_{theoretical} = 7.81$, $df = 3$, $p < 0.05$ for seasonal variations while we considered variations of food items with regard to the size as significant at $\chi^2_{calculated} > \chi^2_{theoretical} = 5.99$, $df = 2$, $p < 0.05$. All χ^2 values used in our work are in accordance with χ^2 distribution critical values table. These values correspond to the cross between the degree of freedom (*df*) and the tail probability *p*. Student's test (T-test) was used to evaluate CW- SW relationship and to determine whether sex or reproductive state of the crab influenced diet, $t_{calculated} > t_{theoretical}$, $p < 0.05$.

Results

Stomach contents analysis

The stomach contents of 384 spider crabs, ranging from 30.18 to 74.23 mm carapace width (CW), were examined (Table 2). 12 items were recorded, classified, and identified according to their degree of digestion. Digested items were impossible to identify (unidentifiable materi-

Table 2. Metric and mass measurements (min-max, mean ± SD) of *L. dubia* from the Gulf of Gabès [CW: Carapace Width (mm), TW: Total Weight (g), SW: Stomach Weight (g), SD: Standard Deviation].

	CW (mm)	TW (g)	SW (g)
Non-ovigerous females	34.05 - 68.4	16.11 - 128.14	0.33 - 3.41
Mean ± SD	50.68 ± 5.81	73.64 ± 21.26	1.54 ± 0.48
Ovigerous females	39.03 - 66.04	45.85 - 149.15	0.04 - 0.81
Mean ± SD	55.21 ± 5.3	92.41 ± 25.75	0.34 ± 0.19
Males	30.18 - 74.23	13.38 - 232.24	0.27 - 5.98
Mean ± SD	50.2 ± 9.16	72.94 ± 38.38	1.55 ± 0.87
Overall	30.18 - 74.23	13.38 - 232.24	0.04 - 5.98
Mean ± SD	50.74 ± 7.65	74.48 ± 31.13	1.47 ± 0.74

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als, UNM) and were recorded in 172 stomachs (45.99%). Algae (ALG) were found in 232 stomachs (62.03%), the major percentage of occurrence in this study, followed by Magnoliophyta (MAG) (222 stomachs, 59.36%). In contrast, Cnidarians (CNI) were found only in four stomachs (1.07%) (Fig. 2A). Despite the wide variety of items recorded in the stomach contents of *L. dubia*, ALG, MAG, and UNM predominated as indicated by the points method (Fig. 2B). Results of frequency of occurrence and those of points method relative to animal preys show that this type of food seemed not to be chosen but ingested occasionally or accidentally (Fig. 2A, 2B).

Since ALG were the most common dietary component in foregut contents of *L. dubia*, the diversity of this alimentary item was studied. Chlorophyceae were found in 87.93% of stomachs containing ALG and contributed most of the points to the stomach contents (4.18 points) followed by Phaeophyceae (81.03%, 2.27 points) and Rhodophyceae (40.95%, 0.68 points) (Fig. 2C, 2D). All recorded items were identified to the lowest taxonomic level possible (Table S1) and some were photographed using an electronic microscope connected to a pc (Leica DC software) (Fig. 3).

Seasonal analysis of foregut contents

Seasonal analysis of stomachs contents revealed that all items were recorded throughout the year, albeit with variable frequencies, except CNI that were observed only in summer, and Polychaeta (POL) that were absent in winter (Table 3). Frequency of occurrence of invertebrates and fish did not exceed 20% for each season considering each group alone (Table 3). The major diversity of items in crab stomachs was observed during summer

(Table 3). Frequency of occurrence of different items recorded in foregut contents of *L. dubia* varied significantly with respect to season ($\chi^2_{calculated}$ = 87.86 > $\chi^2_{theoretical}$ = 7.81, df = 3, p < 0.05) (Table 3).

During the summer, Chlorophyceae dominated the diet, while Phaeophyceae were the most common during autumn. Rhodophyceae were the least consumed ALG in all seasons except in spring (Table 3). The Chi-square test indicated that the frequency of occurrence of different types of ALG consumed differed significantly among seasons ($\chi^2_{calculated}$ = 66.74 > $\chi^2_{theoretical}$ = 7.81, df = 3, p < 0.05) (Table 3).

CW-SW relationship

The CW-SW relationship, with R^2 = 0.27, indicated that not all sampled specimens had completely filled stomachs (Fig. 4A). Ovigerous and non-ovigerous females had different feeding habits: the CW-SW relationship in non-ovigerous females was highly correlated (R^2 = 0.77) compared to ovigerous females (R^2 = 0.013) (Fig. 4B, 4C). The highest correlation in CW-SW relationship was recorded for males (R^2 = 0.79) (Fig. 4D). Student test values showed that all differences in CW-SW relationships were not significant ($t_{calculated}$ < $t_{theoretical}$, p > 0.05).

Seasonal variations in the quantity of food ingested and Importance in Weight Index

Analysis of stomach contents weight (SCW), totaling 363.13 g, showed that the main food groups ingested were ALG and MAG (270.17 g and 69.68 g, respectively) whereas other food items and UNM did not exceed 10 g
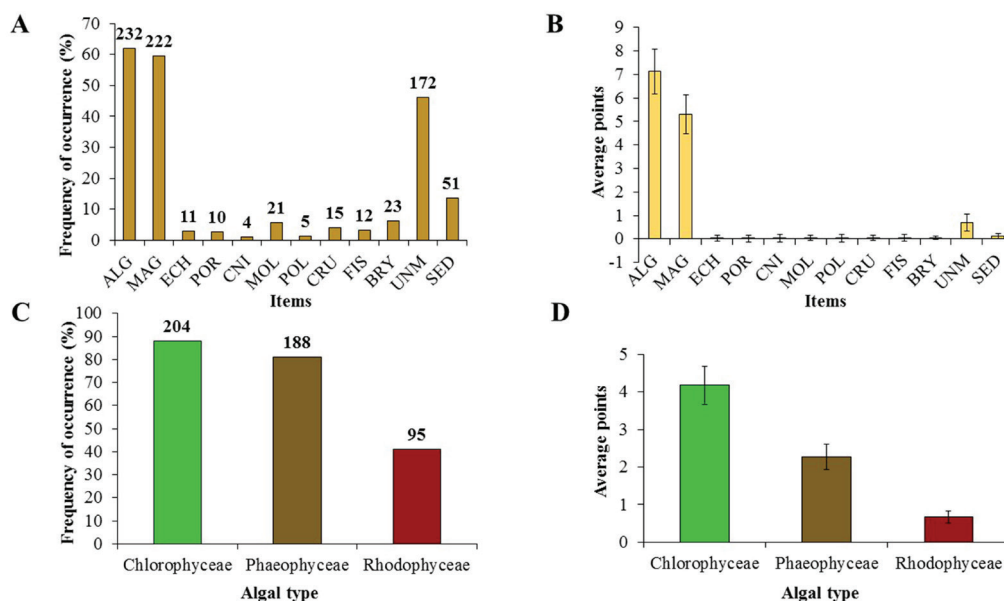

Fig. 2: Frequency of occurrence and average points contributed to the diet of *L. dubia* from the Gulf of Gabès. ALG, algae; MAG, Magnoliophyta; ECH, Echinodermata; POR, Porifera; CNI, Cnidaria; MOL, Mollusca; POL, Polychaeta; CRU, Crustacea; FIS, Fish; BRY, Bryozoa; UNM, Unidentifiable materials; SED, Sediment. The numbers above the bars in subfigures A and C represent the absolute frequency of stomachs filled with the corresponding item. Lines in subfigures B and D indicate two standard errors showing 95% confidence interval.

Table 3. Seasonal frequency of occurrence of recorded items in stomach contents of *L. dubia* from the Gulf of Gabès, N: Total number of filled (non-empty) stomachs.

Season	Autumn N = 100	Winter N = 96	Spring N = 85	Summer N = 93	Chi- square test (χ^2 test), *df* = 3	
Items	%	%	%	%	χ^2	*p*
ALG	86.0	51.04	54.12	54.84	7.26	0.064
MAG	78.0	42.7	58.82	56.99	5.56	0.13
ECH	5.0	1.04	3.53	2.15	0.58	0.9
POR	1.0	1.04	5.88	3.22	5.77	0.12
CNI	0.0	0.0	0.0	4.3	18.73	< 0.05
MOL	11.0	5.2	4.7	1.07	4.29	0.23
POL	2.0	0.0	1.18	2.15	2.3	0.51
CRU	4.0	2.08	4.7	5.38	2.62	0.45
FIS	8.0	1.04	2.35	1.07	4.19	0.24
BRY	14.0	5.2	3.53	1.07	7.61	0.054
SED	20.0	8.33	16.47	9.68	0.2	0.97
UNM	74.0	21.87	76.47	12.9	28.74	< 0.05
Algal type	%	%	%	%	χ^2	*p*
Chlorophyceae	87.21	79.59	89.13	96.08	7.64	0.52
Phaeophyceae	97.67	91.84	47.83	72.55	17.04	0.91
Rhodophyceae	27.9	59.18	73.91	15.69	42.06	0.07

and 14 g respectively (Fig. 5A). These results corresponded to an Importance in Weight Index (IWI) of 74.4% for ALG, 19.19% for MAG while UNM and other food items did not exceed 4% and 3% respectively (Fig. 5B).

The quantity of ALG ingested was more abundant in autumn/summer in comparison to winter/spring. Of a total of 270.17 g of ALG consumed, Chlorophyceae, Phaeophyceae, and Rhodophyceae contributed to a total weight of 113.75 g, 87.49 g, and 68.93 g, respectively. Similarly, the highest IWI was observed for green ALG, followed respectively by brown and red ALG (Fig. 5C, 5D).

Diet composition in relation to the size, Vacuity Index, and comparison of food habits

Frequency of occurrence of different food items varied significantly with respect to size ($\chi^2_{\text{calculated}}$ = 14.25 > $\chi^2_{\text{theoretical}}$ = 5.99, *df* = 2, *p* = 0.026). The different food items occurred in all carapace width classes but with variable frequencies which increased with crab size. ALG and MAG were the most important groups present in the diet of all size classes. UNM and animal preys were the most common for the larger sized classes (Fig. 6A). Frequencies of occurrence of Chlorophyceae, Phaeophyceae, and Rhodophyceae were high for medium sized and large crabs with very small differences between the two classes (Fig. 6B). These results show that larger specimens have the ability to eat more than smaller ones. Differences were not significant for different kinds of ALG

consumed with respect to size ($\chi^2_{\text{calculated}} < \chi^2_{\text{theoretical}}$ = 5.99, *df* = 2, *p* = 0.14).

Among the 384 stomachs examined, only ten were empty, which corresponds to a very low total Vacuity Index (VI = 2.6%). Proportions of empty stomachs varied only in two seasons with a maximum record in spring (VI = 9.57%) and a minimum in autumn (VI = 0.99%). For females and males, this parameter varied respectively during spring (VI = 15.25%) and autumn (VI = 2%) while no empty stomachs were recorded during winter and summer (Fig. 6C). The VI of night samples was 0%, whereas for daytime samples, stomachs were half full or empty with a total VI of 7.94%. For females and males, this parameter was 13.04% and 1.75%, respectively (Fig. 6D).

A comparison of food habits of males, females (ovigerous and non-ovigerous), and combined sexes is presented in Table 4. Results show that crabs fed on the same food items. Frequencies of occurrence of the different food items were higher in non-ovigerous females except for UNM, which dominated in ovigerous females, unlike animal preys that were completely absent in the stomach contents of this latter group (Table 4).

The VI was 37.5%, 0%, and 0.54% for ovigerous females, non-ovigerous females, and males, respectively. Kruskal-Wallis test indicated that percentage of empty stomachs varied significantly with respect to males, females (ovigerous and non-ovigerous), and combined sexes (H = 17.5, *df* = 3, *p* < 0.05). There were also significant differences in the frequency of occurrence of ALG, MAG, UNM, and other animal foods consumed among
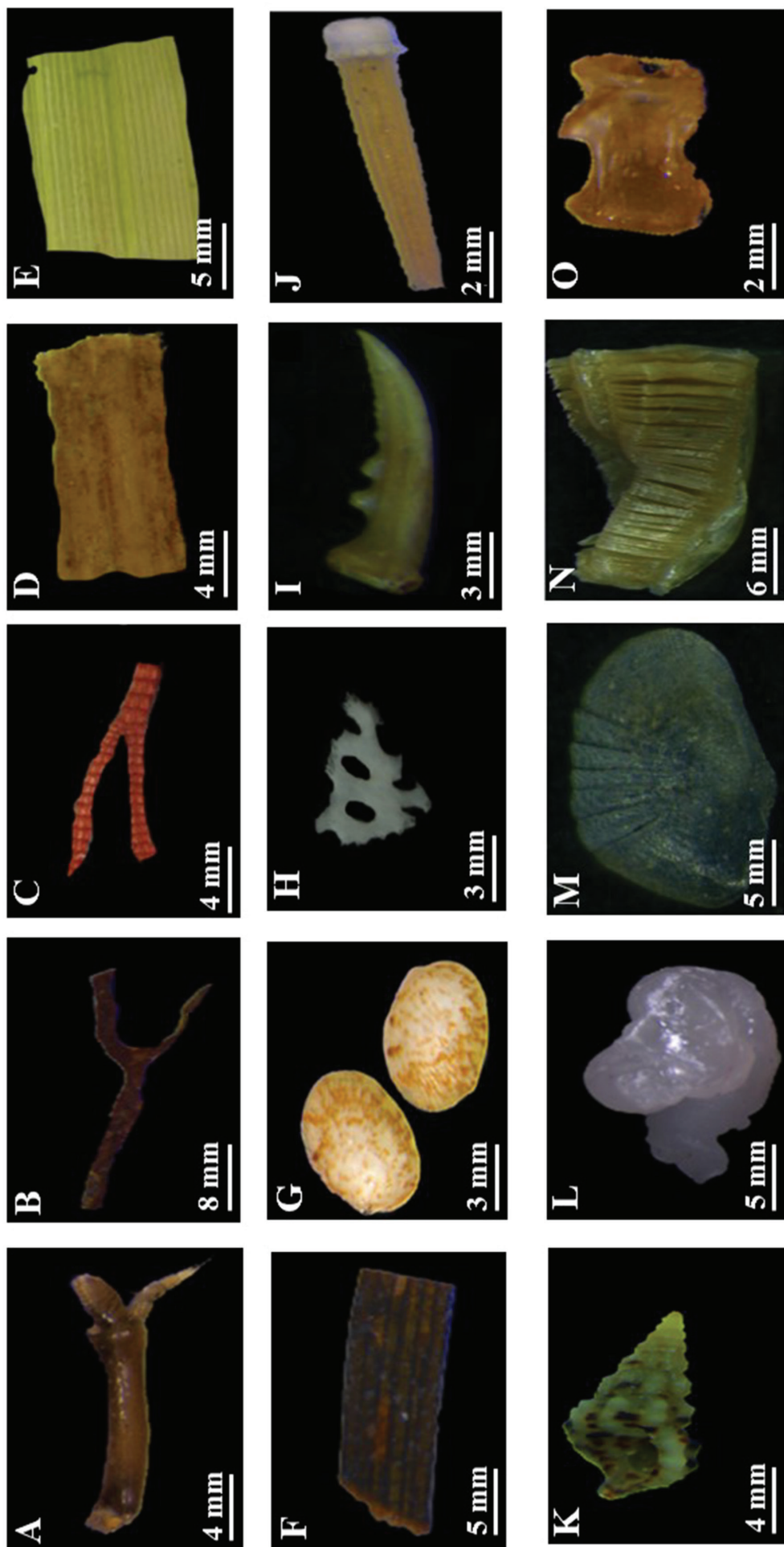
Fig. 3: Main and accidental food items recorded in the stomachs of *L. dubia* from the Gulf of Gabès. A, Non-identifiable Phaeophyceae; B, *Cystoseira* sp.; C, *Polysiphonia elongata*; D, *Cymodocea nodosa*; E, *Zostera marina*; F, *Posidonia oceanica*; G, *Glycymeris* sp.; H, *Sertella* sp.; I, Claw of brachyuran; J, Sea urchin spine; K, *Cerithium* sp.; L, *Rhizostoma pulmo*; M, Fish scale; N, Serpulidae; O, Fish vertebra.

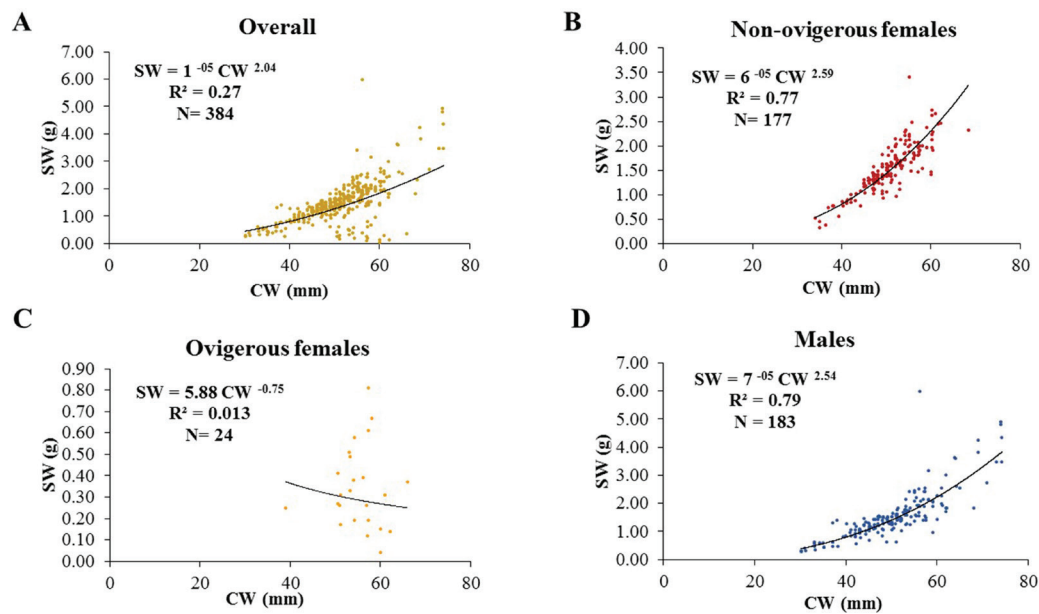*Medit. Mar. Sci.*, 21/2, 2020, 420-432

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Fig. 4: Relationships Carapace Width, CW (mm) *versus* Stomach Weight, SW (g).
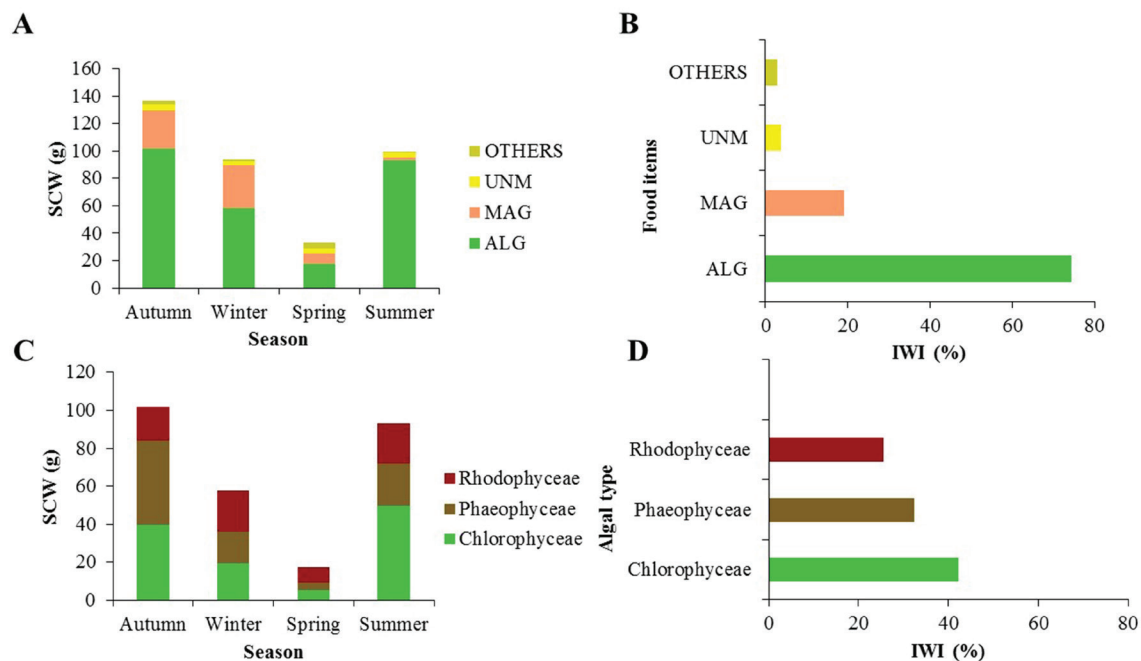

Fig. 5: Seasonal variations in the weight of stomach contents (SCW) and Importance in Weight Index (IWI) of *L. dubia* from the Gulf of Gabès.

the different groups while no significant differences were recorded over all ingested foods (H = 1.1, *p* = 0.77) (Table 4).

Discussion

Alien Invasive Species (AIS) are a major threat for marine biodiversity at many levels and to different extents; they may affect community structure, and/or food webs in addition to having direct effects on native species through competition or predation (Streftaris & Zenetos, 2006; Hulme *et al.*, 2009; Ojaveer *et al.*, 2015). Dietary

habit studies are crucial for establishing nutritional requirements, assessing interactions with other organisms, and for estimating breeding potential (Santos & Borges, 2001). Trophic habits and potential impacts of the invasive crab *L. dubia* on recipient communities are, to date, virtually unexplored. Results included in this paper are a first step to aid the understanding of feeding behavior in *L. dubia* from the Mediterranean Sea.

In its natural range, *L. dubia* seems to be omnivorous, feeding on easily procured plants, animal tissues, and detritus (Corrington, 1927). In seagrass beds, the species consumes macroalgae as a portion of its diet including *Gracilaria tikvahiae* McLachlan, 1979 and algae belong-
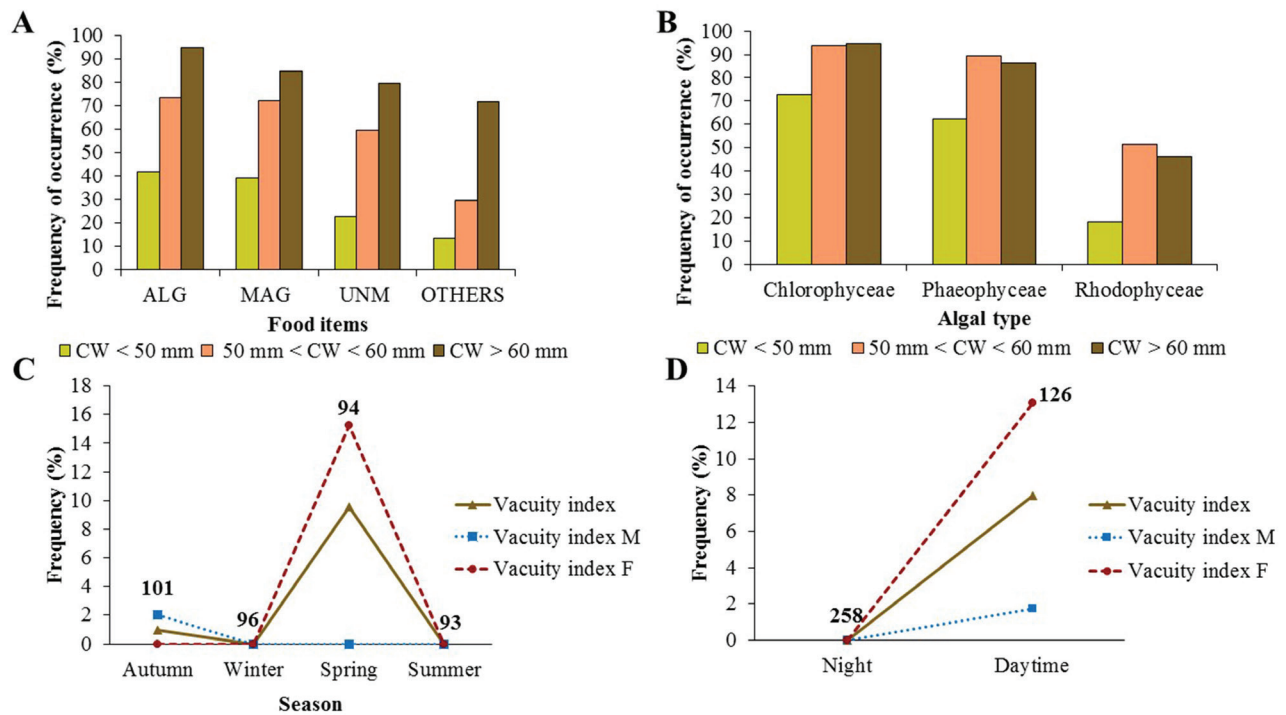
Fig. 6: Frequency of occurrence of food items (A) and algal type (B) with respect to size and Vacuity Index of *L. dubia* from the Gulf of Gabès on seasonal (C) and daily (D) basis (brown: overall; blue: males; red: females). Numbers in subfigures C and D indicate total sample size.

ing to the genera *Ulva*, *Hypnea*, *Chondria*, and *Padina* (Stachowicz & Hay, 1999). Several papers highlight its feeding on mesogleas of the cannonball jellyfish *Stomolophus meleagris* Agassiz, 1862 and the moon jellyfish *Aurelia aurita* Linnaeus, 1758 (Jachowski, 1963; Phillips *et al.*, 1969; Shanks & Graham, 1988; Tunberg & Reed, 2004). Similarly, Carman *et al.* (2017) reported a unique trophic interaction between the highly toxic hydrozoan jellyfish *Gonionemus* sp. and the spider crab *L. dubia*. The crab also feeds on the sea nettle *Chrysoara quinqecirrha* Desor, 1848 (Phillips *et al.*, 1969). Our study identified more than ten items within the trophic guild of *L. dubia* but with clear preferences for ALG and MAG. It is noteworthy that the species does not maintain the same feeding preferences in the Mediterranean Sea as in its original locality, the Western Atlantic.

Brachyurans belonging to the genus *Libinia* also present various trophic habits. The common species *Libinia emarginata* Leach, 1815 shows a universal predation upon *Asterias* in cases of low availability of food items (Aldrich, 1976) whereas its congener *Libinia spinosa* Guérin, 1832 is omnivorous, feeding on sessile, mobile, sedentary, and even agile animals as attested by Barros *et al.* (2008). Crabs of the genus *Libinia* have symbiotic interactions with scyphozoan jellyfish where they are associated with scyphomedusae and may benefit from this association (Carman *et al.*, 2017). Barros *et al.* (2008) recorded a large frequency of plant materials in the stomach contents of *L. spinosa* and suggested the selective use of this item as a food resource. The present study agrees with these findings, and shows that the Mediterranean population of *L. dubia* is mainly herbivorous. In addition, this result is in accordance with previous researches dealing with the feeding preferences of some epialtid crabs. The diet of the spider crab *Acanthonyx scutiformis* (Dana, 1851) is mainly composed of algae (Vasconcelos *et al.*,

Table 4. Comparison of food habits of *L. dubia* from the Gulf of Gabès. Non-of: Non ovigerous females, Of: Ovigerous females. a: calculated test statistic (H statistic) = 1.1, *df* = 3, calculated p-value = 0.77.

					Kruskal- Wallis test (H test)		
	Non-of	**Of**	**Males**	**Overall**	**Rank sum**[a]	**Average rank sum**	***p***
% Empty stomachs	0.0	37.5	0.55	2.6	17.5	7.0	< 0.05
% Occurrence algae	70.62	40.0	58.24	62.03	58.5	23.4	< 0.05
% Occurrence Magnoliophyta	64.97	60.0	58.24	59.36	60.5	24.2	< 0.05
% Occurrence UNM	59.89	93.33	37.36	45.99	54.0	21.6	< 0.05
% Occurrence others	28.25	0.0	28.02	27.0	19.5	7.8	< 0.05

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2009). In the same context, Varisco *et al.* (2015) described a wide variety of macrophytes in the stomach contents of *Leucippa pentagona* H. Milne Edwards, 1834.

The Mediterranean Sea is one of areas most severely affected by biological invasions worldwide, in terms of number of AIS and rate of introductions (Raitsos *et al.*, 2010; Occhipinti-Ambrogi *et al.*, 2011; Zenetos *et al.*, 2012). Crabs are the most common taxa introduced in the Mediterranean Sea (Galil *et al.*, 2015) and constitute some of the best examples of marine alien species that have had significant impacts on coastal habitats and economies (Klaoudatos & Kapiris, 2014). Tunisian crustacean decapods remain rather poorly studied especially the inshore brachyurans (Zaouali *et al.*, 2013). The Gulf of Gabès, in particular, is most frequently invaded by Indo-Pacific alien crab species and only four non-indigenous crabs have Atlantic origins (Zaouali *et al.*, 2008; Ounifi Ben Amor *et al.*, 2016).

Among alien brachyurans spreading in Tunisian waters, we mention the blue swimming crab *Portunus segnis* (Forskål, 1775) recorded in 2014 (Rifi *et al.*, 2014; Rabaoui *et al.*, 2015). This Indo-Pacific species leads to a biological explosion or a 'bloom' in the coastal area of the Gulf of Gabès (Crocetta *et al.*, 2015). According to Ben Abdallah-Ben Hadj Hamida *et al.* (2019), *P. segnis* appeared to be omnivorous with a preference for animal materials whereas Annabi *et al.* (2018) obtained a trophic position of 3.32 confirming the carnivorous feeding behavior of the species. This trophic guild seems to be highly diverse and demonstrates the ability of *P. segnis* to explore more feeding resources in the Gulf of Gabès compared to *L. dubia*.

Alien brachyuran crabs commonly expand their range through dispersal of their planktonic larvae by advection of ocean currents (Klaoudatos & Kapiris, 2014). However, it is not the case of *L. dubia* where its actual biogeographical distribution is still limited to the Gulf of Gabès despite its occurrence in this area for two decades. Indeed, the duration of total larval life in *L. dubia* is quite short (approximately nine days) (Sandifer & Van Engel, 1971) compared to its congener *L. spinosa* where each zoeal stage lasts ca. 8-10 days (Boschi & Scelzo, 1968). It is also the case for the common species of the genus *L. emarginata* which needs at least 14 days to reach first crab stage (Johns & Lang, 1977). According to Sandifer & Van Engel (1971), the large-scale variation in the duration of larval stages between *L. dubia* and *L. spinosa* may be partially explained by the differences in experimental conditions. By contrast, and despite its recent occurrence in Tunisia, *P. segnis* invaded the entire Gulf of Gabès, became very abundant and rapidly reached the northern Tunisian coasts (Bdioui, 2016). In portunids, the duration of metamorphosis is relatively long (14-19 days) allowing a wider dispersion of larval stages (Arshad *et al.*, 2006). However, the duration of the planktonic phase and the transition to the benthic stage are relatively short in *L. dubia*, which may constitute an obstacle for its large expansion and explain its occurrence only in the Gulf of Gabès.

Habitats have an essential effect on invasion success

and the development of benthic communities (Vaz-Pinto *et al.*, 2014). Up to 10 m deep, substrate composition of the Gulf of Gabès is sandy. Exceeding this depth, the substrate is mainly muddy (Ben Othmen, 1973) causing some difficulties in the movement, since *L. dubia* belongs to spider crabs commonly known as slow-moving (Hartnoll, 1993; Sallam *et al.*, 2007).

In dietary composition studies, researchers use foregut contents to identify the food consumed but, as yet, there is no established methodology for the quantitative analysis of gut contents in crustaceans that feed on macroscopic food items (Ben Abdallah-Ben Hadj Hamida *et al.*, 2019). Several authors adopted the frequency of occurrence as a method of analyzing stomachs contents in crabs (Williams, 1981; Comoglio & Amin, 1999; Barros *et al.*, 2008). In the literature, other techniques such as the points method was used (Hyslop, 1980; Woods, 1993). In order to correctly describe the diet of a predator, Hyslop (1980) suggested experimentation with several methods. According to the recommendation of this author, we used both points and frequency of occurrence methods in this work for the analysis of foregut contents of *L. dubia*. The latter technique is suitable for most types of food ingested and was adopted to describe broadly the different alimentary items (Comoglio & Amin, 1999). Its disadvantage is that small animals, unidentifiable materials, and sediment may occur frequently but in small quantities (Comoglio & Amin, 1996) which totally agrees with the present study. UNM were recorded in 45.99% of stomachs but with a weight of 13.38 g out of a total of 363.13 g of food items consumed, corresponding to a very low Importance in Weight Index (IWI = 3.68%). For this study, we excluded sediment (foraminiferans and sand grains) from the alimentary spectrum of *L. dubia*. Branco & Verani (1997) demonstrated that the occurrence of sand grains in stomach contents suggested a deposit feeding behavior whereas Carqueija & De Gouvêa (1998) did not agree with the idea that sediment is an alimentary item and supported its accidental ingestion attached to other food elements. Qualitative analysis in addition to quantitative analysis confirmed that *L. dubia* is an herbivorous crab and that animal preys were accidentally ingested when associated with marine vegetation.

Biological invasion studies aim to specify the most suitable environments for invasion, and identify traits associated to establishment success and high invasiveness (Marchetti *et al.*, 2004; Fleming & Dibble, 2015). Azzurro *et al.* (2014) discuss the external morphology as a criterium for explaining the success of biological invasions, since it is considered as a proxy for a species ecological position in a community. In the case of *L. dubia*, indeed, some morphological characteristics could explain its successful establishment into a new area for more than 20 years. Compared to native crabs, *L. dubia* exhibits the following differences: a spiny carapace, decorating and grooming behaviors, and cheliped regeneration capacity (Rjiba-Bahri *et al.*, 2019), which improve defense and survival capability of the species. These traits, in addition to feeding habits, may justify its transition from NIS to AIS.

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Once established, AIS may modify food webs and trophic hierarchies, which in turn affect the flow of energy, matter, and nutrients through the ecosystem (Hänfling *et al.*, 2011; Klaoudatos & Kapiris, 2014). Alien Invasive Crustaceans (AIC), in particular, have a complex trophic role since they are often omnivorous, opportunistic, and can efficiently exploit the most abundant food source available in the invaded food web (Van der Velde *et al.*, 2009; Hänfling *et al.*, 2011). Analysis of gut contents of *L. dubia* reveals that in the Gulf of Gabès the species is herbivorous, feeding mainly on various kinds of ALG and MAG. Macrophytes and especially Chlorophyceae were the most common type of ALG consumed by *L. dubia* followed respectively by brown and red ALG. Rhodophyceae were minimally consumed as shown by the low percentage of occurrence in this study in terms of algal type consumed. Indeed, crabs fed less frequently on Rhodophyceae in summer (15.69%) than in other seasons (27.9%, 59.18% and 73.91% in autumn, winter, and spring respectively). These findings agree with the results obtained for brachyurans belonging to other genera (Woods, 1993; Samson *et al.*, 2007). Nevertheless, stomach contents of *L. dubia* from the Gulf of Gabès contain animal items with very low frequencies of occurrence. These results partially agree with those of the trophic habits of *L. spinosa* and could be explained by consumed items which include a portion of unidentifiable material (Barros *et al.*, 2008).

In this study, correlations recorded in the CW-SW relationships reflect the great food availability for *L. dubia* in its new area and, concurrently, its great fitness in feeding which may be one of the reasons for the successful establishment of the species. However, ovigerous females show a very low correlation ($R^2 = 0.013$, b = - 0.75) confirming their anorexic feeding behavior. Indeed, Sumpton & Smith (1990) and Josileen (2011) attested that ovigerous females spend more time grooming their eggs than feeding.

Plant materials (ALG and MAG) were present in the diets of all size classes albeit with variable frequencies increasing with the size, revealing that feeding ability increases with specimen size. Furthermore, the size of an ingested item is directly proportional to crab size and an optimum prey size increases with carapace size. Therefore, a predator crab selects preys according to their sizes in order to maximize the energy gained (Stevens *et al.*, 1982; Chande & Mgaya, 2004). Ben Abdallah-Ben Hadj Hamida *et al.* (2019) attested that this also applies to Tunisian populations of *P. segnis*, although crustaceans resulted the most ingested prey item for all size classes of the blue swimming crab.

Intensity of feeding is negatively related to the percentage of empty stomachs (Bowman & Bowman, 1980). In this work, the total VI was 2.6% showing slight annual variations. *Libinia dubia* from the Gulf of Gabès decreased its intensity of feeding in spring (VI = 9.57%) especially for females (VI = 15.25%). This behavior can be explained by the reproduction state since most of empty stomachs observed for females were recorded during the spawning period (March-July). Night sampling showed a VI of 0% whereas stomachs collected during daytime were half full and, in some cases, empty. Indeed, *L. dubia* is a nocturnal benthic spider crab (Williams, 1984).

The present work is the first study of the trophic habits of the invasive crab *L. dubia* in the Mediterranean Sea. The number of stomachs examined was sufficient to describe accurately the diet of this decapod, which appears to be almost uniform throughout the year with alternation of ALG and MAG in all seasons, for all size classes, and for both sexes. Results of Kruskal-Wallis test showed that differences in feeding rates among groups were not significant while the Chi-square test revealed high significant differences among seasons and between crab sizes. The results presented here clearly identify *L. dubia* from the Gulf of Gabès as a primarily herbivorous species with accidental ingestion of animal items. This type of trophic niche has implications for a broader understanding of the species as invasive and may be a reason for the successful establishment of the species in its new area during the last two decades. Data presented in this work are relevant and are of interest for invasion biology, risk analysis, and possible management. In addition to technical means available for any management strategy, the study of biology and the evaluation of the potential impacts of an invasive species are essential before any action plan can be formed (Dutartre *et al.*, 2012). Among important impacts that AIC inflict at the community level is competition with native species for food and shelter (Hänfling *et al.*, 2011). The potential impacts of *L. dubia* as an invasive NIS are poorly documented but preliminary data showed a highly competitive level of this species with the local crab fauna of the Gulf of Gabès (Ben Souissi, 2015). Investigations and surveys in the Gulf of Gabès combined to the Local Ecological Knowledge (LEK) as a complementary approach have underlined a direct link between the proliferation of *L. dubia* and the alteration of some important fisheries resources. Huge quantities of spiny spider crabs in nets cause severe injuries to fish species of commercial interest (Rjiba-Bahri *et al.*, 2019). Similarly, *P. segnis* causes damages to the ecosystem, artisanal fisheries catch, and fishing nets (Khamassi *et al.*, 2019). As a relatively new species invading the Gulf of Gabès, *P. segnis* occupies an ecological niche which overlaps not only with native communities but also with *L. dubia*. The resulting competition is mainly for shelter where *P. segnis* can successfully exclude and/or displace *L. dubia*. In addition, food competition is excluded for these two invasive crabs since they are functionally and morphologically different with distinct trophic guilds. Such competition impacts negatively the abundance of native crabs in the area, especially *Carcinus aestuarii* Nardo, 1847 where local fishermen attested in LEK interviews to the decline of *Carcinus* populations (Khamassi *et al.*, 2019).

Since 2017, the Gulf of Gabès is facing a real disaster after the introduction and the spreading of the invasive blue crab *Callinectes sapidus* Rathbun, 1896 (Ben Souissi *et al.*, 2017), and this area is actually a melting pot of four alien invasive crabs: *L. dubia*, *P. segnis*, *C. sapidus*, and *Eucrate crenata* (De Haan, 1835) (Zaouali, 1992).

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The two blue crabs, in particular, are highly competitive and their presence in the Gulf of Gabès in a relatively short time interval indicates the accelerated rate of bioinvasions (Ben Souissi *et al.*, 2017). Invasion diversity in the Gulf of Gabès is, to date, far greater than previously understood, setting the stage for research on biology and potentially ecological, environmental, economic, and social impacts.

Acknowledgements

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Supplementary data

The following supplementary information is available on line for the article:

Table S1. List of food items found within the foregut contents of *L. dubia* from the Gulf of Gabès.

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