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RESEARCH ARTICLE

Influence of seasonal food abundance and quality on the feeding habits of an opportunistic feeder, the intertidal crab *Pachygrapsus marmoratus*

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Abstract The abundance of trophic sources on the intertidal zone is discontinuous and their supply can vary both in a predictable or unpredictable way. The Mediterranean semi-terrestrial crab *Pachygrapsus marmoratus*, is known, as adult, to entirely rely on the intertidal trophic sources, and, consequently, it faces the fluctuations of nutritional sources and quality. To clarify the relationships between the feeding habits of an Italian population of *P. marmoratus* and the temporal variation of its food sources, we carried out a 2-year sampling protocol. Data on seasonal variation in composition of intertidal food item assemblages, on the average content in N and C of the commonest algae, on seasonal changes in crabs feeding habits were collected and compared using a suite of multivariate and univariate techniques. Results showed that *P. marmoratus* takes advantage of the recruitment phase of the most common invertebrates, affecting and controlling the abundance even of those species whose adults are out of its reach. It can act both as a herbivore, and as a carnivore that shifts between bivalves, more abundant in spring/summer, and the periwinkle *Melaraphe neritoides*, during winter. In conclusion, we provide evidences on the food choice of this common rocky shore species, which is very plastic and capable of relying on many trophic sources, possibly influencing the abundance and/or the population structure of a number of intertidal populations.

Introduction

The intertidal habitat is known to be dominated by very diverse invertebrate assemblages, mainly belonging to marine taxa (Stephenson and Stephenson 1949; Lewis 1964). The reason why this habitat is productive and diverse is usually explained by the high availability of trophic supply. There are at least four trophic sources. The first is the biofilm that covers the intertidal rock surface, formed by cyanobacteria, diatoms, settling stage of invertebrates and protozoa (Costerton et al. 1978; Wahl 1989; Christensen and Characklins 1990; Anderson 1995). This source is mainly exploited by gastropods (Steneck and Watling 1982; Underwood 1984; Hawkins et al. 1989), echinoderms (Ogden et al. 1989; Johnson and Mann 1993) and juvenile and larval stages of fish and crustaceans (Hudon 1983; Mallin et al. 1992; Edgar and Shaw 1995). The second trophic source is represented by macroalgae, which represent a food source for gastropods (Lubchenco 1978, 1980, 1983; Watson and Norton 1985, 1987), amphipods (Cruz-Rivera and Hay 2000a, b) and decapods (Kennish et al. 1997; Cannicci et al. 2002). The third food source is made by stranded algal detritus propagules, and larvae

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from adjacent marine environments. This food source is of primary importance for bivalves and cirriped crustaceans (Seed 1969), but it can be exploited by mobile opportunist feeders, such as crabs (Cannicci et al. 2002). The invertebrates themselves, which are mainly exploited by gastropods (Vadas et al. 1994) and crabs (Hughes and Seed 1995; Cannicci et al. 2002) represents the fourth trophic source in intertidal habitats.

As generalist feeders, crabs can take direct advantage of all above-mentioned food sources. In fact, as megalopae and juvenile instars they can rely on diatoms (Jensen and Asplen 1998), while most of the adult crabs are non-specialised herbivores and/or omnivores, known to rely on both algae (Kennish et al. 1996; Kennish and Williams 1997), recruiting larvae and stranded matter (Cannicci et al. 2002) and animal preys, such as mussels, gastropods and other crabs (Seed and Hughes 1995; Cannicci et al. 2002). On subtropical and temperate shores, food supply to the intertidal zone exhibits seasonal variations that crabs must necessarily cope with. In Hong Kong, for instance (Kennish 1996, 1997), seasonal fluctuations in food supply had significant consequences for diet, development and life cycle of the intertidal crab *Grapsus albolineatus*. No such information is known for the intertidal crabs of the Mediterranean basin, that exerts significant seasonal variations of energetic supplies. In fact, some easily exploitable foods, such as many macroalgae, goes through an early spring blossom, while their abundance in the autumn–winter months is strongly reduced (Benedetti-Cecchi 2000). Moreover, recruitment at the intertidal zone by pelagic larvae is also known to be seasonal, as for sessile crustaceans (*Chthamalus* spp., Pannacciulli and Relini 1999; Benedetti-Cecchi et al. 2000).

This study aims at assessing the food source utilisation of one of the most common omnivorous invertebrate of the Mediterranean rocky shores, namely the crab *Pachygrapsus marmoratus* (Fabricius, 1787), which is known to exploit most of the trophic sources of the intertidal zone during its adult life (Vernet-Cornubert 1958; Cannicci et al 2002; Flores and Paula 2002a). We hypothesise that this non-specialised crab is able to vary its feeding habits to cope with fluctuations of nutritional sources and, thus, can affect the abundance of a wide number of intertidal organisms.

Materials and methods

Study site

The study was conducted on the *P. marmoratus* population of Calafuria (43°28′N, 10°20′E), Ligurian Sea, Italy. This is a moderately exposed shore about 5-km length and mainly composed by large sandstone platforms broken into large blocks by deep crevices. A large number of crabs colonises the whole shore. The average tidal amplitude is about 40 cm, but the range of intertidal organisms, including *P. marmoratus*, extends up to 70 cm above the mean low water level, due to the effect of waves and barometric pressure on the sea level (Benedetti-Cecchi and Cinelli 1993; Menconi et al. 1999).

Experimental design and sampling procedures

Two sites, i.e. two similar intertidal areas, about 100 m long and 70 cm wide, were selected about 500 m apart from each other. *P. marmoratus* sampling was carried out for the duration of 2 years (2000–2001) using the same protocols for crab collection, assemblage estimation and stomach content analysis. During the first year of study, sampling was on three dates for each of the four seasons. Preliminary data analysis revealed the strongest differences in diet composition between spring and autumn. Therefore, in the second year, we choose to sample three times during the spring and autumn seasons (i.e. three sampling dates at the beginning of June and three in late October to the beginning of November, respectively). Crabs and sessile assemblages were sampled in the same sites used in the previous year. This increased the sampling effort, allowing collection of a higher number of crabs in order to test for difference in seasonal diet across crab sex.

Crab collection

Crabs were collected using a standardised catch-per-effort technique (Krebs 1989). For 2 h, two researchers in 2000, and four in 2001 (allowing collection of a sufficient number of crabs to test for difference in seasonal diet across crab sex), caught by hand all crabs actively feeding within each site. Crabs were immediately put in a buffered formaldehyde solution (4%) to stop the digestion and to preserve the stomach contents. All collections were carried out with calm sea conditions and during evening spring low tides, when the crab's maximum peak of activity is recorded (Cannicci et al. 1999).

Collected crabs were then brought to the laboratory, sex and carapace length (CL) were assessed, using a Vernier calliper, and stomachs were then removed and preserved for the analysis of the natural diet.

Intertidal assemblages composition

Since *P. marmoratus* is known to feed within short area of maximum 50–75 cm of radius surrounding its refuge

(Cannicci et al. 1999), we assessed the assemblage composition at the same sampling sites to compare food abundance and stomach contents. Five replicate quadrats (20 cm × 20 cm) were randomly chosen in each experimental area and placed within 50–70 cm from the crevices where the crabs sheltered. We recorded the percent cover for sessile organisms and small low-mobility animals (algae, invertebrates and periwinkles) and number of individuals per quadrat for larger mobile animals (limpets). Percent cover estimates were visually obtained by dividing each quadrat into 25 sub-quadrats of 4 cm × 4 cm and assigning to each of them a score from 0 (absence of a particular taxon) to 4 (the sub-quadrat was totally covered by a particular taxon) and adding up the 25 estimates (Dethier et al. 1993; Meese and Tomich 1992). Unidentified taxa were brought to the laboratory and classified to the lowest possible taxonomic level.

During the first sampling campaign of the second year, six samples (about 2–3 g each) of the thallus of the most abundant macroalgae and cyanobacteria were collected to assess their content in organic N and C. Samples were analysed with a Carlo Erba Elemental Analyser NA1500 Series 2.

Diet analysis

The diet composition of *P. marmoratus* was assessed by a single researcher following Cannicci et al. (2002). Each stomach was assigned a score from 0 to 4, according to the degree of fullness. Then, each stomach was opened, the content placed on a Petri dish and the respective proportions of algal and animal matter estimated using a binocular microscope (magnification from 10× to 60×). After this preliminary estimate, we separately analysed the animal and plant fractions according to the following procedures.

Animal matter

Animal matter was identified under 60× magnification to the lowest taxonomic level and then quantified by the Percentage Points method (Wear and Haddon 1987).

Cyanobacteria and algal matter

Three sub-samples from the homogenised stomach content were mounted on slides. For each of them, the three most particle covered fields were identified and observed under 100× magnification. The occurrence of each food item was recorded using an ocular grid marked with 100 cells. The relative abundance of each algal taxon was then determined by dividing the number of intersections scored per item by the total number of intersection points for all species.

Statistical analyses

Multivariate methods were used to analyse seasonal changes in both assemblages and diet composition. In the case of intertidal assemblages, similarity matrixes were computed using Bray–Curtis distance on $\log_{10}(x + 1)$ transformed data. For stomach content analyses, we used Gower distance on untransformed data. This measure of resemblance was used since it takes into account of its computation the joint absence of food item in the stomachs of crabs collected in the same patches. Distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was employed to test (at a significance level of $\alpha = 0.05$), the null hypotheses of no differences among intertidal assemblages across seasons and of no differences in dietary preferences of crabs of different sex among and within seasons. All analyses were based on 4,999 unrestricted permutations of the raw data (Anderson and ter Braak 2003). Non-metric multidimensional scaling (nMDS; Field et al. 1982; Clarke 1993), was used as an unconstrained method of ordination to visualise multivariate patterns. In addition, the canonical analysis of principal coordinates (CAP; Anderson and Willis 2003; Anderson and Robinson 2003) was used as a constrained ordination procedure to visualise patterns by reference to seasonal differences in diet composition. Multivariate analyses were performed using PRIMER v.5.1 (Clarke and Gorley 2001) and the FORTRAN programs PERMANOVA (Anderson 2005) and CAP (Anderson 2004).

A three-way analysis of variance (ANOVA) was employed to test for differences in the percentage intake of particular food items between sexes (a fixed factor in the design) collected at different dates (a random factor in the design, nested within the factor season, fixed and crossed to sexes). Prior to analyses, the homogeneity of variances was assessed using Cochran's test and data were transformed to $x' = \arcsin(x)$ to remove heteroscedascity (Underwood 1997). When appropriate, Student–Newman–Keuls (SNK) tests were used for multiple comparisons of the means.

ANOVAs were performed using GMAV 5 program (University of Sydney, Australia).

For the diet selectivity of *P. marmoratus*, the Wilcoxon–Mann–Whitney non-parametric test was used to assess differences between the relative abundance of the various items within the intertidal community and in the natural diet of crabs along the annual sampling protocol. To simplify the statistical comparison of the cyanobateria and the algal community, algal species were pooled into functional groups, based on the definition of Steneck and Dethier (1994). Thus, the following abbreviations will be used: F.G. 1 for cyanobacteria; F.G. 2 for filamentous algae; F.G. 3 for foliose algae; F.G. 3.5 for corticated foliose algae; F.G. 4 for corticated macrophytes; F.G. 5 for leathery macrophytes; F.G. 6 for articulated calcareous algae; F.G. 7 for crustose algae; F.G. 7.5 for crustose coralline algae. The groups used for the animal prey abundance were chosen at different taxonomic levels, depending on the relative abundance of the different taxa within the diet.

Results

Monthly sampling (2000)

Population structure

Out of the 136 total specimens, the number of *P. marmoratus* actively feeding was higher in the summer months (July–Sept.: mean ± SE = 16.3 ± 5.03 collected crabs), spring (Apr.–June: 12.7 ± 10.0) and autumn (Oct.–Dec.: 15.50 ± 7.8), compared to winter (Jan.–Mar. 6.0 ± 3.5). The overall male/female sex ratio was 0.454, with no differences of seasonal activity between sexes (Kolmogorov–Smirnov Test, ks = 0.28, $P = 0.83$) and monthly means of 5.91 ± 2.33 and 4.91 ± 1.97 for females and males, respectively. The population structure of collected samples differed among seasons, with small crabs being more abundant than large ones in winter and autumn and a more even distribution of crabs among the size classes in spring and summer (*G* test, $\chi^2 = 52.2$; $P < 0.001$, Fig. 1a–d).

Intertidal assemblages

The most abundant autotroph species along *P. marmoratus* feeding areas was *Rivularia atra*, F.G. 1, which dominated the splash zone. In the lower intertidal zone, the most abundant species were the brown algae *Padina pavonica*, F.G. 3.5, and *Cystoseira* sp., F.G. 5, together with the articulated calcareous red algae, *Jania rubens* and *Corallina elongata*, F.G. 6. Among the red algae, the genera *Laurencia* and *Gelidium* F.G. 4, and species belonging to F.G. 2, such as the genus *Ceramium* were also common. The latter F.G. was well represented within the intertidal pools, mainly by filamentous green algae belonging to the genera *Cladophora* and *Enteromorpha*. Crustose brown algae, F.G. 7, and coralline algae, F.G. 7.5, were also abundant.

The barnacle *Chthamalus stellatus* was by far the most abundant invertebrate, covering about 90% of *P. marmoratus* feeding grounds. Among molluscs, the most abundant species present throughout the intertidal zone were the periwinkles *Melaraphe neritoides*, *Osilinus turbinatus* and *Gibbula* spp. and the limpets *Patella* spp. at lower levels. The bivalve *Mytilus galloprovincialis* represented only about 3% of the total cover.

PERMANOVA revealed that the assemblage significantly differed, consistently with sites, among seasons (Table 1). Pairwise comparisons of seasons revealed that seasonal differences were mainly driven by significant differences between spring and autumn ($t = 1.74$; $P = 0.013$) and, to a lesser extent, by differences between summer and autumn ($t = 1.60$; $P = 0.036$). The relative abundance of cyanobacteria was higher during the winter months (Fig. 2a, lower bars), while blossoms of filamentous algae, mainly belonging to the genera *Enteromorpha, Cladophora*, *Ceramium* and *Sphacelaria*, were recorded both in the spring-summer period and in Nov.–Dec. (Fig. 2b, lower bars). On the other hand, the relative abundance of the commonest invertebrate did not show any significant fluctuation, although a weak seasonal variability could be assessed for *M. neritoides* and *M. galloprovincialis* (Fig. 3, lower bars).

Natural diet

Among algae species, the most ingested were the cyanobacteria, especially *R. atra*, and algae belonging to F.G. 2 (Fig. 2a, b; upper bars). Only these latter filamentous algae were significantly more abundant in the stomach than on the intertidal zone (Wilcoxon–Mann–Whitney test: $z = -2.636$; $P = 0.009$; Fig. 2b). *P. marmoratus* tended to avoid the leathery macrophytes, such as the *Cystoseira* sp. and other common macrophytes such as *Laurencia* sp. (F.G. 4), although they were abundant on the shore (Wilcoxon–Mann–Whitney test: $z = 2.23$; $P = 0.03$), and, with the only exception of *Ralfsia verrucosa*, to avoid all crustose algae.

The mussel *M. galloprovincialis*, although fairly uncommon on the intertidal zone, was one of the most ingested prey species (Wilcoxon–Mann–Whitney Test; $z = 2.21$; $P = 0.02$; Fig. 3c). Other very common *P. marmoratus* prey were the periwinkle, *M. neritoides*, and other gastropods such as limpets, *Patella* spp., although their abundance in the stomachs was not higher than their frequency on the shore (Wilcoxon–Mann–Whitney test: $z = -1.39$; $P = 0.16$ and $z = -0.79$; $P = 0.42$, for *M. neritoides* alone and the other gastropods, respectively, Fig. 3b, d). *C. stellatus* was far more abundant on the shore than in the stomach content samples (Wilcoxon–Mann–Whitney test: $z = -3.94$; $P = 0.001$, Fig. 3a).

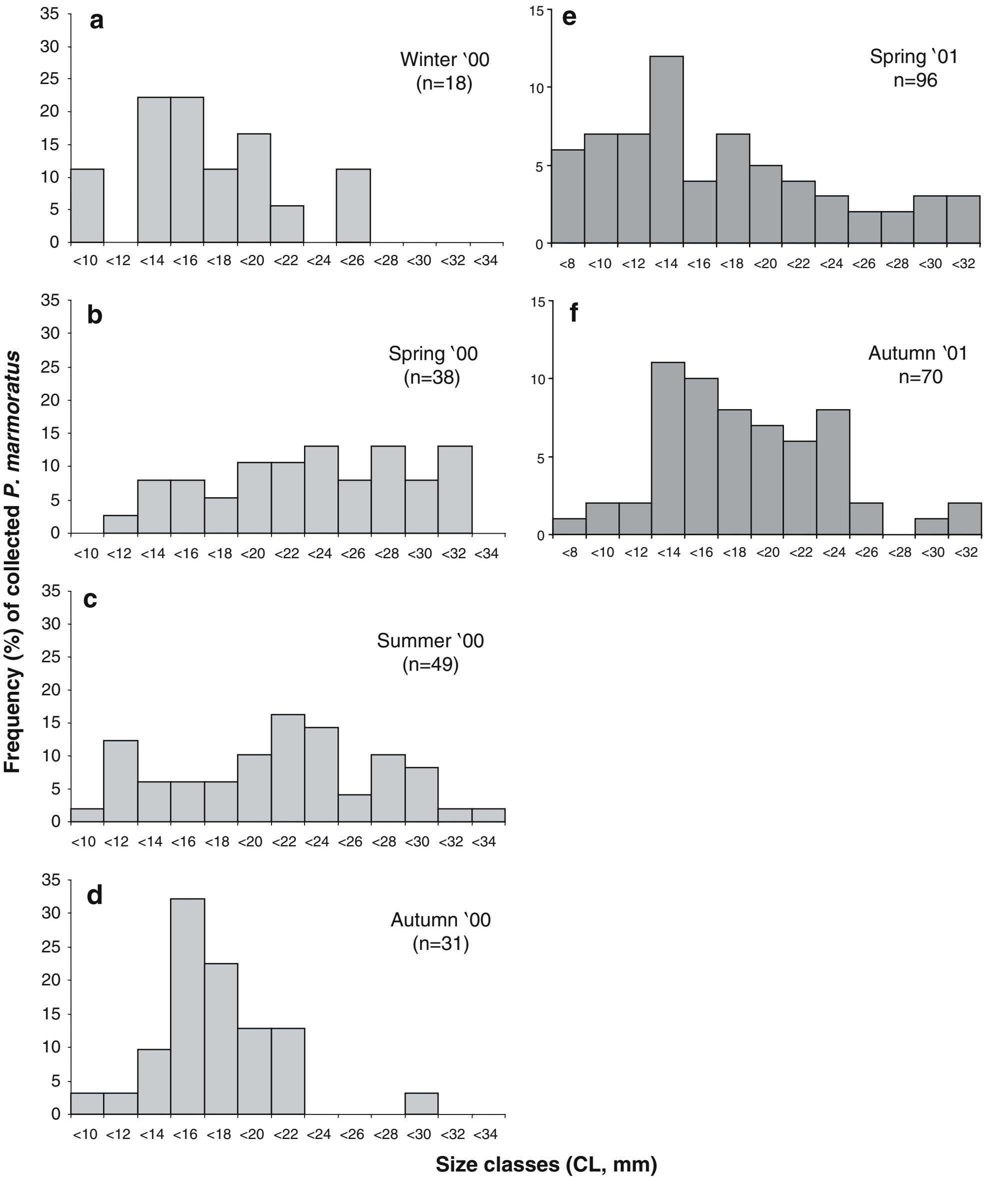

Fig. 1 *Pachygrapsus marmoratus*. Size class distribution (*CL* carapace length), expressed as percentage frequency, of specimens collected in winter (**a**), spring (**b**), summer (**c**) and autumn (**d**) of the first year of sampling and in spring (**e**) and autumn (**f**) of the second year, at Calafuria. n = total number of collected crabs in the season

PERMANOVA revealed significant seasonal differences in the crab stomach contents (Table 2). Pairwise tests revealed that the composition and relative abundances of the ingested items differed between spring and autumn ($t = 1.73$; $P = 0.007$), and between spring and winter ($t = 1.63$; $P = 0.012$). CAP analysis showed a significant effect of season, with a squared canonical correlation of $\delta^2 = 0.2539$ ($P = 0.0194$). Stomachs of crabs collected in spring had positive values on the first Canonical axis, while the stomachs collected in winter and autumn were more scattered (Fig. 4). Since this first axis was positively correlated with the presence of mussels, *M. galloprovincialis*, and many filamentous algae (Table 3) and negatively with the consumption of periwinkles, *M. neritoides*, and two species of cyanobacteria, the analysis showed a seasonal shift between the bivalves and the periwinkles and between the algae belonging to F.G. 2 and cyanobacteria.

Spring and autumn intensive sampling (2001)

Population structure

A total of 166 crabs were collected, 96 in the spring and 70 during the autumn samplings. Size class frequency distribution was different between the two samples

Table 1 Temporal changes of intertidal assemblages within the *P. marmoratus* feeding area

Source	*df*	SS	MS	*F*	*P*
Site = Si	1	2481.22	1.63	0.13	0.15
Season = Se	3	9742.71	2.14	0.01	0.01
Month(Se) = Mo(Se)	8	17637.33	1.45	0.05	0.06
Si × Se	3	5645.49	1.24	0.22	0.25
Si × Mo(Se)	8	17978.52	1.48	0.05	0.05
Residual	48	72965.66	1.63		
Total	71	126450.90	2.14		

Permutational MANOVA on the basis of the Bray–Curtis dissimilarities on log(x + 1)-transformed abundance data from 34 taxa. The analysis refers to the first year of study, where sampling for each of the two sites was undertaken in three sampling dates for each of the four seasons

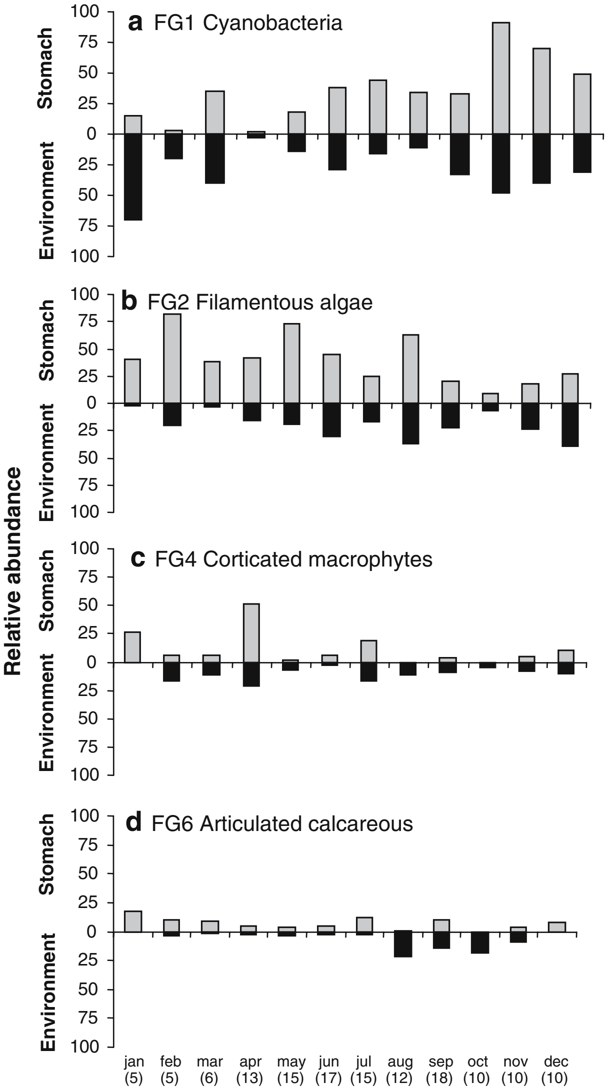

Fig. 2 Relative abundance of algae of functional groups 1 (**a**), 2 (**b**), 4 (**c**) and 6 (**d**) in the stomachs of *P. marmoratus* (*upper bars*) and on the shore (*lower bars*) recorded in the different sampling months of the year 2000. The number of non-empty stomachs analysed for each month is also shown (in *parentheses*)

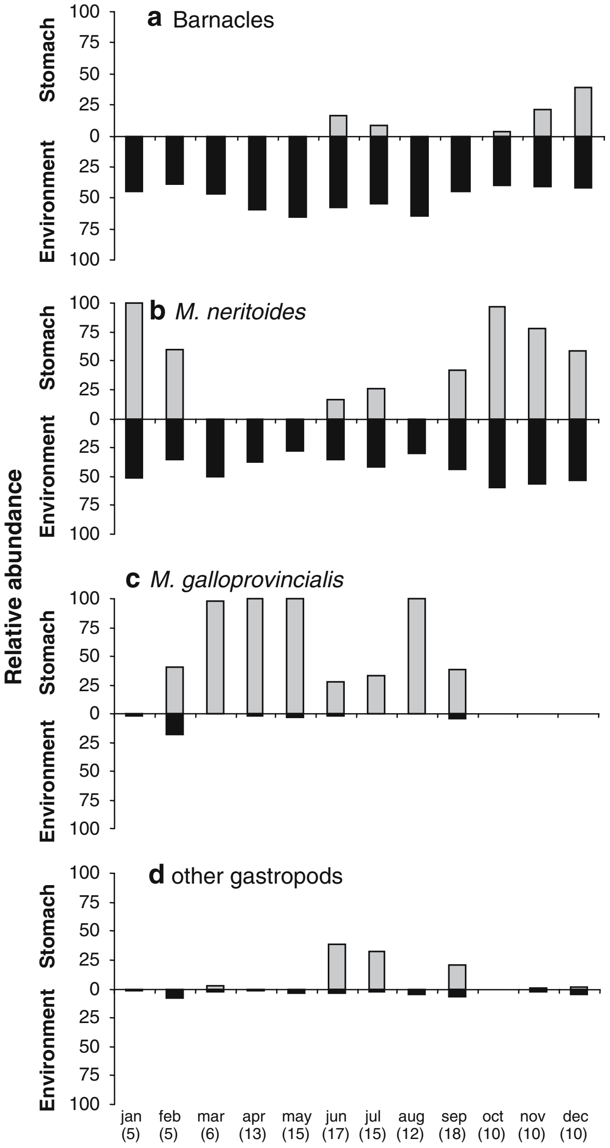

Fig. 3 Relative abundance of barnacles (**a**), *Melaraphe neritoides* (**b**), *Mytilus galloprovincialis* (**c**) and other gastropods (**d**) in the stomachs of *P. marmoratus* (*upper bars*) and on the shore (*lower bars*) recorded in the different sampling months of the year 2000. The number of non-empty stomachs analysed for each month is also shown (in *parentheses*)

Table 2 Results of the two-factor PERMANOVA testing the seasonal differences in *P. marmoratus* diet

Source	*df*	SS	MS	*F*	*P*
Season = Se	3	19.55	6.52	1.85	0.04
Month(Se) = Mo(Se)	8	34.77	4.35	1.23	0.08
Residual	48	168.91	3.52		
Total	59	223.22			

Analysis based on Gower dissimilarity of untransformed data from 20 variables (taxa). The analysis refers to the first year of study, where sampling was undertaken in three sampling dates for each of the four seasons. The data coming from the two experimental areas were pooled

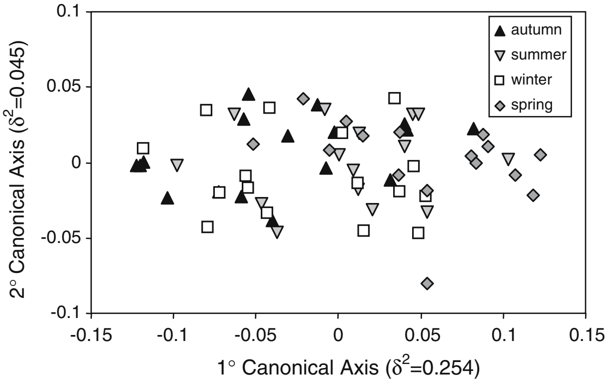

Fig. 4 Two-dimensional scatter plot of the first and second Canonical axes for season of *P. marmoratus* stomach contents in each sampled season of the year 2000

Table 3 Seasonal differences in *P. marmoratus* diet

Positive correlation (spring)		Negative correlation (autumn–winter)	
Mytilus galloprovincialis	0.64	*Rivularia atra*	−0.62
Polysiphonia spp.	0.40	*Melaraphe neritoides*	−0.59
Cladophora spp.	0.38	*Entophisalis* sp.	−0.59
Sphacelaria spp.	0.30		
F.G. 4	0.29		
F.G. 2	0.28		
Jania rubens	0.27		
Enteromorpha sp.	0.23		
Gelidium sp.	0.21		

Correlation coefficients for individual taxa ($|r| > 0.20$) with the first canonical axis for effects of season. The analysis was done using the first $m = 3$ principal coordinate axes (explaining 41% of the variation in the original dissimilarity matrix) on data transformed to fourth roots. A positive correlation indicates association with spring diet composition, whereas a negative correlation indicates an association with both autumn and winter diet (see Fig. 4)

(*G* test: $\chi^2 = 16.34$; $P < 0.05$, Fig. 1e, f), with both smaller and larger crabs more abundant in spring.

Intertidal assemblages

PERMANOVA confirmed the patterns observed in the first year of study, with seasons and dates within spring and autumn that differed significantly and consistently across sites (Table 4). Seasonal changes were clearly portrayed in the nMDS ordination (Fig. 5).

The CAP analysis showed a significant effect of season, with a squared canonical correlation of $\delta^2 = 0.917101$ ($P = 0.0002$). In the spring, we found more filamentous green, such as *Cladophora* sp., red, such as *Ceramium* sp. and *Caulacanthus ustulatus*, and brown algae, such as *Sphacelaria* sp. On the other hand, the dominant algae in the autumn were the crustose coralline algae, F.G. 7.5, the genus *Gelidium* and the genera *Enteromorpha* and *Lophosiphonia* colonising the tidal pools. Regarding invertebrates, both mussels, *M. galloprovincialis*, and limpets, *Patella* spp., showed a higher density during the spring, while in autumn the dominant species were *Chthamalus stellatus* and *Melaraphe neritoides* (Table 5).

Table 4 Temporal changes of intertidal assemblages within the *P. marmoratus* feeding area

Source	*df*	SS	*F*	*P*	$\mathrm{MS}_{\mathrm{denom}}$
Site = Si	1	4644.90	2.57	0.06	D(S)
Season = Se	1	21712.15	3.98	0.02	Res
Date(Se) = D(Se)	4	21829.58	2.40	0.003	T × D(S)
Si × Se	1	2116.11	1.17	0.34	Res
Si × D(Se)	4	7227.25	0.80	0.70	D(S)
Residual = Res	48	109082.56			
Total	59	166612.54			

Permutational MANOVA and post hoc pairwise comparisons on the basis of the Bray–Curtis dissimilarities on $\log(x + 1)$-transformed abundance data from 49 taxa. The analysis refers to the second year of study, where sampling for each of the two sites was undertaken in three sampling dates for each of the spring and autumn seasons

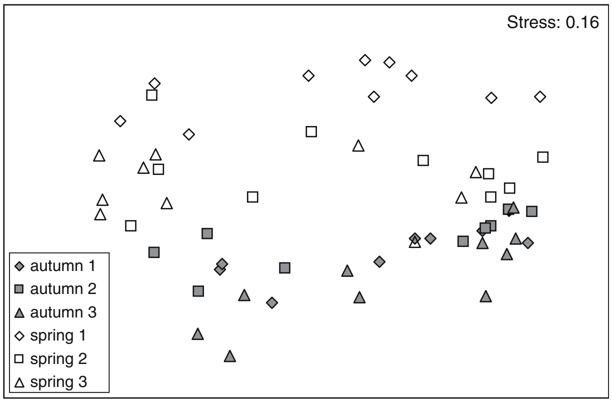

Fig. 5 Second year of study. Non-metric multidimensional scaling (nMDS) plot of assemblages of algae and slow-moving and sessile invertebrates in the two seasons of the year 2001

Natural diet

Only 31 (32.3%) and 10 (14.3%) stomachs in spring and autumn, respectively, were empty. In this second year of study, the filamentous species belonging to F.G. 2 were once more the most ingested algae, constituting about 50 and 40% of the algal component of the stomachs in spring and autumn, respectively, with the genus *Cladophora* alone reaching values of 36 and 32% in the two seasons. Cyanobacteria were consistently ingested throughout the year as well, averaging 45 and 37% of the algal component in spring and autumn, respectively. In the spring, the most abundant animal prey was *C. stellatus* (26.4% of the animal component)

Table 5 Seasonal differences in the intertidal assemblages in the *P. marmoratus* feeding area

Positive correlation (autumn)		Negative correlation (spring)	
F.G. 7.5	0.77	*Ceramium* sp.	−0.83
Gelidium sp.	0.47	*Padina pavonica*	−0.73
Chthamalus stellatus	0.46	*Caulacantus* sp.	−0.65
Enteromorpha sp.	0.38	*Sphacelaria* sp.	−0.57
Calotrix sp.	0.36	*Mytilus galloprovincialis*	−0.54
Melaraphe neritoides	0.34	F.G. 2	−0.51
Jania sp.	0.23	F.G. 3.5	−0.48
Lophosiphonia sp.	0.22	*Patella* spp.	−0.38
		Cladophora sp.	−0.34
		Cystoseira sp.	−0.34
		Annelidae	−0.29
		Rivularia atra	−0.28
		F.G. 4	−0.27
		Acetabularia acetabulum	−0.22

Correlation coefficients for individual taxa ($|r| > 0.20$) with the first canonical axis for effects of season. The analysis was done using the first $m = 14$ principal coordinate axes (explaining 90.33% of the variation in the original dissimilarity matrix) on data transformed to fourth roots. A positive correlation indicates association with intertidal assemblages recorded at autumn, whereas a negative correlation indicates an association with spring assemblages

followed by *P. marmoratus* itself (21.6%), while in the autumn periwinkles, *M. neritoide*s, and conspecific crabs were the most ingested items, respectively 25 and 16% of the animal content.

PERMANOVA revealed the significance of the interaction term Sx × D(Se), indicating difference in diet composition between sexes that varied across dates within seasons (Table 6). Pairwise comparisons between sexes within each level of the factor D(Se) detected significant differences for the third spring sampling date and for the second autumn sampling date. ANOVA, using the above-described three-factor mixed design, detected the same significant effect of the interaction among sex and date on the intake of green filamentous algae ($F_{4,84} = 6.32$; $P = 0.001$) and on the abundance of total animal matter in the stomachs ($F_{4,84} = 2.40$; $P = 0.05$). SNK tests showed that males ingested significantly more green filamentous algae than females both in the third spring sampling date (relative abundance 42.21 ± 11.01 and 3.94 ± 2.56, for males and females, respectively) and in the second autumn sampling date (27.80 ± 9.98 and 4.57 ± 3.05, for males and females, respectively). The opposite trend was observed in ingestion of animal matter, significantly preferred by females both in the third spring sampling date (26.04 ± 9.51 and 71.87 ± 8.76, for males and females, respectively) and in the second autumn sampling date (25.00 ± 6.68 and 41.66 ± 6.30, for males and females, respectively).

Table 6 Results of the three-way PERMANOVA and pairwise comparisons of temporal changes of *P. marmoratus* diet across sexes

Source	*df*	SS	*F*	*P*	MS_{denom}
Sex = Sx	1	1.55	0.38	0.96	Sx × D(S)
Season = Se	1	5.19	1.78	0.10	D(S)
Date(Se) = D(Se)	4	11.63	1.20	0.20	Res
Sx × Se	1	2.43	0.60	0.83	Sx × D(S)
Sx × D(Se)	4	16.27	1.67	0.01	Res
Residual = Res	84	204.28			
Total	95	241.35			

Pairwise tests	*t*	*P*
Females vs. males within autumn 1	0.67	0.83
Females vs. males within autumn 2	1.99	0.004
Females vs. males within autumn 3	1.08	0.32
Females vs. males within spring 1	0.78	0.72
Females vs. males within spring 2	0.76	0.68
Females vs. males within spring 3	1.58	0.04

Analysis based on Gower dissimilarity of untransformed data from 21 variables (taxa). The analysis refers to the second year of study, where sampling was undertaken in three sampling dates for each of the spring and autumn seasons

The C/N ratio of the collected algal tissues varied among the different intertidal species (Fig. 6). The majority of algae, especially the filamentous green genera *Enteromorpha*, *Cladophora* and *Chaetomorpha*, were characterised by very low N contents and only 6 taxa had a C/N ratio of about 20 or less: *Hildebrandia* sp. (6.36 ± 0.12); *Gelidium* sp. (8.39 ± 0.25); cyanobacteria (11.82 ± 0.19); *Ulva* sp. (11.89 ± 0.41); *Herposiphonia* sp. (19.18 ± 2.87) and *C. elongata* (22.50 ± 3.53).

Discussion and conclusions

In our study area, *P. marmoratus* exploited the intertidal food sources throughout the whole year, as showed in other studies along the Portuguese coasts (Flores and Paula 2001, 2002a). However, in contrast with those Western Atlantic populations, free raging, actively feeding crabs were more abundant during the summer, less abundant in the spring and the autumn and very scarce in the winter. Thus, at the Ligurian Sea this crab significantly reduces its activity in winter. A similar pattern has been observed for North Adriatic populations (Cannicci et al. 2002). Moreover, the population site structure differed among seasons, with the spring/summer samples more evenly distributed among size classes. Strong differences in frequency distributions among and within seasons of different years are not surprising in a species characterised by a wide range of variation in spawning and recruitment timing

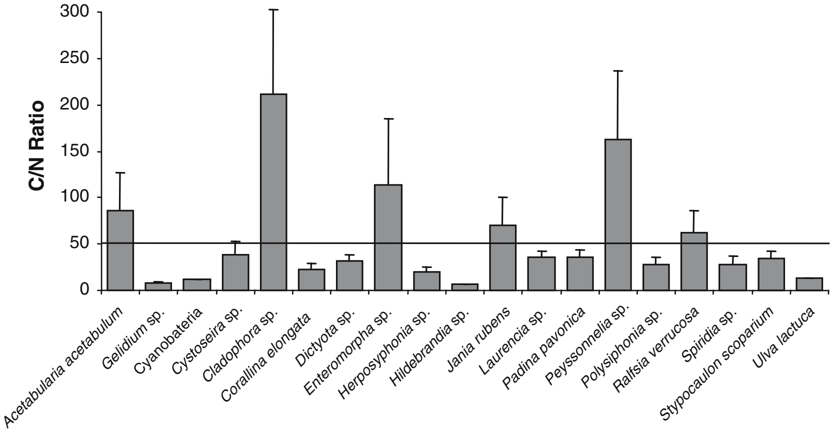

Fig. 6 Values of C/N ratio (±95% c.l.) in the tissues of most abundant macroalgae sampled at the study shore. The *horizontal line* around 50 represents the overall mean C/N ratio of the total sample

and intensity (Vernet-Cornubert 1958; Flores and Paula 2002a, b). In Southern France, the only other Mediterranean area where *P. marmoratus* has been studied in details, the spawning period shifted from early to late spring at different shores, while the peaks of recruitment shifted in terms of months in different years (Vernet-Cornubert 1958). The same information comes from central Portugal, where Flores and Paula (2002a, b) found considerable differences in recruitment timing and intensity at two rocky shores a few km apart from each other.

In this study, the feeding habits of *P. marmoratus* were assessed by means of stomach content analysis. Although, it is well known that the resistance of the different items to digestion can affect their abundance in the crab foregut, many authors stated that no assimilation takes place in the cardiac region of the crab stomach (Dall and Moriarty 1983), and that residence time, although different between plant material and animal tissue, is short for all kinds of items, concluding that stomach content analysis is still of great importance in assessing feeding habits. Moreover, this methodology is known to provide only partial information about intake and real assimilation of the different food items and the approach of stable isotope analysis is far more appropriate for those kinds of studies (Post 2002). However, by assessing which species are removed and ingested by *P. marmoratus* it is possible to obtain a strong indication about its natural diet and, most of all, it is important to determine the impact that this consumer has to the natural populations it relies on. In fact, even if some of the ingested species could not be assimilated, they are still removed from the shore.

Our population proved to feed on a wide range of algae, definitely avoiding only the leathery macrophytes, the encrusting coralline algae and the foliose macrophytes. Its diet relied on cyanobacteria and filamentous algae, but articulated coralline, of the genera *Jania* and *Corallina*, and corticated macrophytes, especially *Gelidium* spp., were also commonly ingested. Despite the low N/C ratio of their tissues, showed by our analysis, and their low calorific content (Coen 1988), fast-growing filamentous macroalgae are known to be preferred by both herbivorous (Coen 1988; Kennish et al. 1996) and omnivorous crabs (Barry and Ehret 1993; McDermott 1998). Many authors suggested that crabs try to minimise the intake of secondary metabolites instead of maximising nutritive gains, and compensate the lower calorific and protein intake with a high feeding rate on these fairly common macrophytes (Coen 1988; Kennish at al. 1996). On the other hand, low mobility crabs are known to be able to degrade secondary metabolites and to consume a wide range of brown algae (Stachowicz and Hay 1999). The selection of algae with low secondary metabolites content by *P. marmoratus*, seems however confirmed by the active avoidance of the crustose alga *Hildebrandia* sp. This species is the richest in nitrogen among our samples, but it is also known to contain polyphenolics (Kennish et al. 1996) and was always avoided, while another crustose alga, *Ralfsia verrucosa*, was commonly ingested. *P. marmoratus* is likely to be forced to choose filamentous algae for another reason. The claws of many algivorous and omnivorous Grapsidae proved to be quite inefficient in manipulating foliose and leathery macrophytes (Kennish at al. 1996; Cannicci et al. 2002), while they show high efficiency when collecting filamentous and crustose algal species. Although *P. marmoratus* is a true omnivore, feeding also on animal prey, this crab showed a strong preference for algae rich in nitrogen, such as various cyanobacteria.

Our study demonstrated that food resources availability varied across time within *P. marmoratus* feeding areas, as expected from the literature on Mediterranean rocky shore (Airoldi et al. 1995; Menconi et al.

1999; Benedetti-Cecchi 2000). The most preferred algae, filamentous algae, cyanobacteria and *Gelidium* spp., although reduced, did not disappear from the shore during winter or summer, being available throughout the year. Despite this, *P. marmoratus* algal intake shifted from filamentous algae, in spring–summer, to cyanobacteria in autumn–winter. Seasonal changes in intertidal crabs diet were previously known, but they were associated with strong seasonal differences in algal availability, such as the case of *Grapsus albolineatus* at Hong Kong (Kennish et al. 1996; Kennish 1997), where filamentous algae nearly disappear in the summer and crustose algae dominate the shore and, consequently, crabs' diet.

Seasonal lack of selected algae is not an explanation for diet shift in our population, but the high seasonal variability of availability of filamentous algae can be important. In fact, these algae are a good nutrient source only at high intake rates, thus they are not a suitable choice during winter and autumn, when crabs reduce their activity and have shorter feeding excursions. Moreover, although low in energetic value, the filamentous greens eaten by *P. marmoratus* are all ephemeral and are more energetic when they are fast growing, i.e. in spring–summer, becoming even more appealing (Kennish et al. 1996). Filamentous green algae were, however, the food item of choice also throughout the second year of sampling, especially in males, which showed to consume them in higher rates with respect to females, both in spring and autumn. Although less abundant in autumn–winter, the filamentous genera *Cladophora* and *Enteromorpha* are still common in tidal pools of the higher littoral fringe and can be harvested safely by the crabs even in rough sea conditions. Moreover, the preference of males for this food can still be due to its relatively high energetic content, which is necessary to defend territories and actively interact with crabs of both sexes, as described by Cannicci et al. (1999).

On the other hand, cyanobacteria such as *R. atra* form important colonies on the rocky substratum and can be readily scraped throughout the year and are richer in proteins than the average of other algae. It is worth noting that *P. marmoratus* exerted a higher predation on *Melaraphe neritoides* in winter, while it ingested more mussels, during the first spring/summer period, and barnacles, in the second. Winter prey are thus small periwinkles which require high manipulating times and assure low net gains (Hughes 1988), hence they can be the only possible food when crabs are forced to the higher shore levels by rough sea, and some additional protein intake, such as cyanobacteria, can be also necessary.

Females were shown to be more ready to prey upon invertebrates. This higher predation rates can be explained with the need of protein for oogenesis, which, in this species, occurs both in winter and early spring, with females ready to spawn from the early days of March throughout the spring months (Vernet-Cornubert 1958).

Both the temporal peaks in recruitment and the ingested items, i.e. parts of shells and plates belonging to very small specimens, indicate that *P. marmoratus* takes strong advantage of the recruitment phase of invertebrates, which is known to occur between April and October with two seasonal peaks around June–July and September (Panaciulli and Falautano 1999; Benedetti-Cecchi et al. 2000; O'Riordan et al. 2004). *P. marmoratus* seems to readily compensate for the known differences in recruitment timing and intensity of the various populations and concentrated its predation effort on *M. galloprovincialis*, in the first year, and *Chthamalus stellatus* in the second one.

In conclusion, the present study shows that *P. marmoratus*, which is by far the commonest intertidal crabs of the Mediterranean rocky shores, is a plastic feeder capable of relying on many trophic sources and affecting the abundance and population structure of a number of intertidal populations, which were thought to be not influenced by its occurrence. *P. marmoratus* is both herbivorous, forced to high intake rates by the characteristics of its food, and a predator readily taking advantage of the recruitment phase of the commonest invertebrates. This behaviour is likely to influence and/or control the population structure and abundance even of those species whose adults are out of its reach.

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