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Relationships between algal food and gut and gonad conditions in the Mediterranean sea urchin *Paracentrotus lividus* (Lam.)

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Abstract

A study was conducted on the population of *Paracentrotus lividus* (Lamarck, 1816) in a Mediterranean infralittoral bottom of southern of Spain to characterize the relationships between the macroalgal food ingested and the gut and gonad conditions of individuals. Over a full annual cycle (November 2008 to October 2009) composition of the gut content was analyzed monthly and seasonally, the gastrointestinal (GII), repletion (RI) and gonadosomatic (gonad) (GI) indices were calculated, and the stage of gonad maturity of the population was assessed. The GII and RI were found to be strongly correlated, so only the RI was considered in the subsequent analysis. The prevalence of Phaeophyceae and local species of this macroalgal group in the gut content of the sea urchins throughout the year suggests that in conditions of abundant resources, with high levels of algal diversity and no effective limits on supply, brown algae are the main component of the natural diet of *P. lividus*. Comparison of the physiological indices and the algal fractions of gut content identified relationships throughout the year between RI, the stage of gonad maturity and GI. Specifically, these indices were found to be associated with the abundance of Rhodophyta ingested, although in different ways: GI was related to the consumption of fleshy (non-calcified) red algae and the RI, though less statistically significantly, to the consumption of calcified (articulate and encrusting).

Keywords: Feeding index, Gonadosomatic index, Macroalgal diet, *Paracentrotus lividus,* Mediterranean Sea, South Spain.

Introduction

Paracentrotus lividus (Lamarck, 1816) is usually considered an herbivore species and there have been many observations of this feeding behavior in the natural environment (i.e. Kitching & Ebling, 1961; Kempf, 1962; Neill & Larkum, 1966; Niell & Pastor, 1973; Allain, 1975; Régis, 1978b; Traer, 1980; Verlaque & Nédélec, 1983; Kitching & Thain, 1983; Privitera *et al.,* 2008). As a consequence, it is assumed that this species can have a substantial impact on algal communities through intervening in the formation of barren grounds (Lawrence, 1975; Verlaque & Nédélec, 1983; Kitching & Thain, 1983; Verlaque, 1984; Frantzis *et al.,* 1988; Benedetti-Cecchi & Cinelli, 1995; Benedetti-Cecchi *et al.,* 1998; Bulleri *et al.,* 1999, 2002; Privitera *et al.,* 2008) and exerting a control over algal populations (Palacín *et al.,* 1998; Sala *et al.,* 1998). However, this species has also been observed to exhibit opportunistic feeding behavior, with a tendency to omnivory, even becoming essentially carnivorous at least in experimental conditions (Régis, 1978a; Fernandez & Boudouresque, 2000). Moreover, in barren grounds, they may ingest considerable quantities of encrusting coralline algae. Delmas & Régis (1986) pointed out that in the lack of erect macrophytes, they feed by grazing on *Lithophyllum incrustans,* also ingesting its associated epiphytic organisms. Further, this sea urchin species is already known to be able to obtain partially dissolved or fine particulate food through the tegument, this even having been associated with a significant lengthening in the spines, increasing its surface area (Delmas & Régis, 1985; Pancucci & Panayotidis, 1994).

As regards feeding preferences (see the review of Boudouresque & Verlaque, 2007) *P. lividus* can be found eating species such as *Rissoella verruculosa*, *Cymodocea nodosa*, *Cystoseira amentacea, Padina pavonica* and *Undaria pinnatifida*. This species of sea urchin also consumes all parts of the seagrass *Posidonia oceanica*, namely, the leaves either fresh or dead, and the rhizomes and roots (Traer, 1980; Verlaque & Nédélec, 1983), as well as being a consumer of remains and detritus. Regarding the ingestion of *Lithophyllum incrustans* (Delmas & Régis, 1986), this latter variant in the diet is particularly important for newly established individuals in the benthos af-

ter metamorphosis (1 mm in diameter), which ingest this encrusting alga and other endolithic species. Later, individuals change their feeding habits, consuming filamentous Rhodophyta when their diameter is 3 to 7 mm, and shrub-like species, such as *Halopteris scoparia, Padina pavonica, Corallina elongata* and *Cystoseira* spp, when they are between 7 and 10 mm. The adult diet develops after reaching 10 mm of diameter, at which point brown algae or leaves of *Posidonia* predominate in their diet (Verlaque & Nédélec, 1983; Verlaque, 1984).

Since the biomass of algal populations fluctuates throughout the year, the availability of food resources for sea urchins is not completely constant. As a consequence, physiological indices such as those describing the condition of the gut and gonads have certain time-dependent relationships with food intake. This is the case of the repletion index (RI) (Lawrence *et al.,* 1965) that varies in different ways depending on the season (Fernandez & Boudouresque, 1997). However, RI does not significantly differ between individuals as a function of whether or not they feed on preferential species, or between animals inhabiting barren grounds with respect to those living in habitats where there are erect macrophytes (Régis, 1978b; Fernandez & Boudouresque, 1997).

Similarly, there has been found to be considerable variability in gonad condition, while the persistence of mature gametes through the year has been observed in populations with interannual variations in gonad development. Interpopulation variations have been described by various different authors. Byrne (1990) pointed out that infralittoral populations have larger gonads and a longer maturity period than intertidal ones. Furthermore, this author concluded the gonad growth occurs during the coldest part of the year, coinciding with months with shorter days - both temperature and daylight being parameters that determine the gonad growth during winter -, and that the photoperiod does not affect the release of gametes. Bayed *et al.* (2005) detected that on the northern Atlantic coast of Morocco the increase in gonad index occurs between January and March, coinciding with the beginning of a period of algal production, concurrent with increases in water temperature and rates of food consumption. This increase in gonad index is followed by a single spawning period between March and June, possibly triggered by the phytoplankton bloom in the area. In the Mediterranean Sea, Lozano *et al.* (1995) indicated that the maturation of the gonads occurs during the winter and the main spawning during the spring or early summer, suggesting that the abundance of phytoplankton has an impact on the onset of lay. Sellem & Guillou (2007) have established the maturation period to be between April and June for populations in Tunisia, although the gonad indices also seem to vary with year and location. The hydrodynamic conditions play an important role, in such a way that in more exposed areas energy is diverted into maintenance, at least in part, at the expense of reproduction. Low energy conditions encourage breeding and in such circumstances it is possible to find larger individuals.

In summary, many variables have been related with the intra- and interannual variations of feeding and gonad indices. It seems that habitat characteristics (Byrne, 1990), hydrodynamic conditions (Sellem & Guillou, 2007), seawater temperature (Byrne, 1990; Bayed *et al.,* 2005; González-Irusta *et al.,* 2010), algal production (Bayed *et al.,* 2005), food availability (Guidetti *et al.,* 2003; Sánchez-España *et al.,* 2004), phytoplankton bloom (Lozano *et al.,* 1995; Bayed *et al.,* 2005; González-Irusta *et al.,* 2010) and photoperiod (González-Irusta *et al.,* 2010) are of particular importance.

This study set out to further our understanding of temporal relationships (monthly and seasonal) between the physiological indices mentioned above, and also between these indices and the composition of food ingested (based on analysis of the gut content), to identify whether these relationships can explain the temporal variations in the indices and, in particular, to establish the impact of feeding on gonad development and whether it is a determinant of the breeding period in this species of sea urchin.

Material and Methods

Study site and sampling

The sampling station was located in Salobreña, Granada province, southern Spain (36º 44' 39''N; 3º 36' 10''W). The specimens were collected at depths between 3 and 10 m, on a human-disturbed rocky bottom made up of blocks and pebbles, which was gently sloping and mainly colonized by the so-called "biocenosis of infralittoral photophilic algae" (Pérès & Picard, 1964). The rocky substrate is also affected by the grazing activity of the sea urchins *Paracentrotus lividus* and *Arbacia lixula*, so that there are wide and irregularly distributed barren grounds, belonging to the "overgrazed facies with encrusting algae and sea urchins". These shallow benthic communities, impoverished and altered in various different ways, occupy wide areas along the Mediterranean shores of southern Spain.

The specimens of *P. lividus* were collected monthly, between November 2008 and October 2009, by scuba diving. Each monthly sample consisted of 30 individuals and these were stored at -20ºC. Prior to freezing, measurements were taken of the total wet weight and of the test diameter at the ambitus without spines, using a Vernier caliper, to select only individuals larger than 3 cm. Through dissection, the digestive tract, excluding Aristotle's lantern, was separated from the gonads. Both components were wet weighed, fixed and conserved in formaldehyde 4%. In each monthly sample, gut content was observed in 10 randomly selected individuals, with the wet weight of the gut being recorded before evacuation. All weights were measured using an electronic bal-

ance after leaving samples to drain for two minutes to remove excess water.

Gut content analysis

The gut content removed was spread evenly in a Petri dish for observation through a stereoscopic microscope at 40x. The content from each individual was analyzed selecting five replicas of a frame of 7 x 9 mm, amounting to a total of 600 observations. In each of these, identifiable remains of macrophytes as well as unidentified species were recorded. The components of the gut content were compared with algal field specimens collected from the sampling station. Some of the remains were fecal pellets, the morphology and composition of which was checked against fecal pellets from laboratory-reared sea urchins fed with specific species of macroalgae collected at the sampling site.

The relative abundance (%) of each food item was determined as a function of the area covered by the item in each frame observation. Similar methods have been used previously by others authors (Cobb & Lawrence, 2005; Privitera *et al.,* 2008). The method has the advantage of providing semi-quantitative data on the abundance of each food item and not only on the occurrence.

Physiological indices

Two feeding indices have been calculated. The gastrointestinal (gut) index relates the digestive system weight to the total wet body weight of the individual, indicating what proportion of this total weight is due to the gut (GII = wet weight of gut x 100/ wet body weight) and the repletion index (RI = wet weight of gut content x 100/ wet body weight) as has been used by Guillou & Michel (1994) and Privitera *et al.* (2008). To assess gonadal condition we calculated the gonadosomatic (gonad) index (GI = wet weight of gonads x 100/ wet body weight), the usual approach adopted in other studies reported in the literature (Guettaf & San Martín, 1995; Sánchez-España *et al.,* 2004; González-Irusta *et al.,* 2010).

Gonad maturation

Gonad maturation was assessed according to the method described by Byrne (1990) distinguishing six stages in the development of the gametes and gonads, in both males and females: I, recovery; II growing; III, premature; IV, mature; V, partly spawned and VI, spent. These stages were identified by observation with an optical microscope equipped with Nomarski interferential contrast, cross-checked with the corresponding histological reference section.

Statistical analysis

Abundance of food items was used to characterize the gut content of the sea urchins. For univariate analysis, one-way ANOVA and Kruskal-Wallis tests with month and season as factors were used to explore the variation in diet over time of both the separate food items and the algal groups. The differences existing between the sexes with respect to the condition indices and the diet composition was tested using Mann-Whitney U and Student's t tests. Also, a comparison analysis between paired samples was carried out using the Wilcoxon signed rank test, and the Spearman rank correlation test between pairs of variables.

Then, for multivariate analysis (Sneath & Sokal, 1973; Legendre & Legendre, 1979) classification analysis was carried out using the Euclidean distance matrix and the *Unweighted Pair Group with Arithmetic Mean* (UPGMA) clustering method. Correspondence analysis (CA) and principal component analysis (PCA) were performed on arcsine transformed data [$x' = \arcsin\sqrt{(x\%/100+0.5)}$]. All univariate statistical tests were conducted using STATISTICA for Windows version 6.0 (StatSoft 2001) and multivariate analysis with NTSYSpc for Windows version 2.0 (Rohlf, 1998).

Results

Diet composition

In the total of 120 specimens, the following classes of food items were observed: Ha, *Halopteris scoparia*; Di, *Dictyota* spp.; Br, unidentified brown pieces, possibly *Cladostephus verticillatus, Dictyopteris polypodioides, Padina pavonica* or *Colpomenia sinuosa*; Ce, *Ceramium* spp.; Ge, *Gelidium* spp.; Pl, *Plocamium cartlagineum*; As, *Asparagopsis armata* and its "*Falkenbergia rufolanosa"* phase; Ca, crust and articulated calcified Rhodophyta, such as *Corallina elongata, Jania rubens, Amphiroa* sp., *Lithophyllum incrustans, Mesophyllum* sp. or *Peyssonnelia* spp.; Ch, *Chaetomorpha* sp.; Cl, *Cladophora* spp.; and Ul, *Ulva* spp. and other non-identified remains of Chlorophyta, possibly *Anadyomene stellata* or *Codium* spp. Remaining unidentified items were classed as Ui. The percent relative abundance (mean ± se) of different food items identified each month in the gut content is listed in Table 1. The grouping into categories was carried out in order that the data statistically tested were more robust, given the volume of samples analyzed and degree of certainty in the identification of the food components. Over the whole period studied (Fig. 1) brown algae represented 44% of the gut content, *Halopteris scoparia* being the most abundant species (16.16%), with a value close to unidentified brown remains (16.29%). The red algae constituted the second most common group of species corresponding to 28.52%, calcified remains (13.83%) being the most abundant component of this category. By contrast, green algae only represented 8.76%, so constitute the group with the lowest contribu-

Table 1. Abundances (% mean ± se) of the food items in the gut content of the sea urchin over the study period. Ha, *Halopteris scoparia*; Di, *Dictyota* spp.; Br, unidentified brown pieces; Ce, *Ceramium* spp.; Ge, *Gelidium* spp.; Pl, *Plocamium cartilagineum*; As, *Asparagopsis armata* and *Falkenbergia rufolanosa*; Ca, crust and articulated calcified Rhodophyta; Ch, *Chaetomorpha* sp.; Cl, *Cladophora* spp.; Ul, *Ulva* spp. and other non-identified remains of Chlorophyta; and Ui, remaining unidentified items.

	Ha	Di	Br	Ce	Ge	Pl	As	Ca	Ch	Cl	Ul	Ui
Total	16.16±0.7	11.59±0.7	12.29±0.6	0.64±0.2	9.00±0.5	0.70±0.2	4.35±0.5	13.84±0.7	0.42±0.1	1.34±0.4	7.02±0.6	18.64±0.5
N08	13.89±2.7	12.89±2.7	26.75±1.3	0.26±0.3	9.53±1.7	-----	3.96±1.4	10.18±3.1	0.97±0.4	1.02±1.0	----	20.51±1.5
D08	16.72±0.8	5.15±1.0	16.77±1.1	0.41±0.3	9.65±1.6	5.06±1.3	2.02±0.6	17.39±2.7	0.72±0.5	0.2±0.2	3.26±1.1	22.59±1.9
J09	16.01±2.2	9.05±1.8	14.67±2.1	0.54±0.4	5.14±1.5	0.6±0.6	6.2±2.8	12.41±3.2	0.3±0.3	2.89±1.2	13.03±2.7	19.1±2.7
F09	16.62±2.7	7.55±2.3	10.52±1.7	0.91±0.6	11.56±1.9	0.57±0.4	5.92±1.5	14.56±2.1	0.64±0.5	3.76±1.7	11.24±2.2	16.09±1.4
M09	15.54±2.7	13.69±1.8	14.16±2.2	1.39±0.8	13.94±1.5	----	2.11±1.0	10.14±2.3	----	5.86±3.0	6.27±1.7	16.84±1.9
A09	23.44±2.1	14.23±2.2	15.44±2.0	0.87±0.5	5.55±1.8	1.25±0.6	2.28±0.7	1543±1.5	-----	0.44±0.3	5.3±0.9	15.7±1.5
M09	13.72±2.2	9.36±2.4	16.02±1.5	0.37±0.3	8.03±2.1	-----	2.92±1.0	24.62±2.3	0.96±0.5	0.35±0.4	7.07±2.0	16.52±1.8
J09	16.09±2.0	17.69±2.4	11.82±1.2	-----	11.55±1.3	0.21±0.2	1.36±0.4	13.79±1.5	1.38±0.8	0.16±1.2	6.82±0.9	19.09±1.3
J09	13.51±1.2	11.89±1.3	16.03±1.1	1.69±1.2	7.56±1.5	0.2±0.2	8.65±3.0	13.16±1.8	-----	-----	8.27±1.7	18.99±1.0
A09	16.36±2.4	20.85±2.8	16.66±2.6	0.17±0.2	7.21±1.9	0.52±0.4	4.58±2.0	8.23±1.7	-----	0.33±0.3	5.22±1.5	19.83±1.6
S09	17.81±2.0	7.92±1.4	18.32±1.2	-----	7.84±2.2	-----	8.29±2.3	12.07±1.5	-----	0.76±0.8	5.27±1.7	21.66±2.3
O09	14.18±3.1	8.73±1.6	18.3±1.6	1.07±0.4	10.4±2.1	-----	3.84±1.0	14.04±2.4	-----	0.25±0.3	12.45±1.6	16.69±1.2

tion and can be considered the least important in the diet of the sea urchin population studied. The unidentified remains group represented 18.63% of the total. There were significant differences between months in Phaeophyceae, calcified Rhodophyta and Chlorophyta (Kruskal-Wallis test, $p<0.01$), but this is not the case of non-calcified Rhodophyta ($p>0.05$). Of these algal groups, only Phaeophyceae showed significant seasonal differences ($p<0.01$); indeed, this is the only algal group in which seasonal abundance seems to be determinant in the sea urchin feeding behavior. The monthly analysis identified significant differences in the different items: Di, Br and Ca (one–way ANOVA, $p<0.01$), Ge and Ch (Kruskal-Wallis test, $p<0.05$) and Pl, Cl and Ul ($p<0.01$), whereas in Ha, Ce and As no significant differences were identified ($p>0.05$) meaning that abundance levels of these items in the gut content remain similar through the year. These levels are high in St, as mentioned above, but low in Ce and As. Analyzing these results seasonally the differences in Di, Br, Pl, Ca and Cl retained similar levels of significance (Fig. 2). In summer, the ingestion of Di was clearly higher than the other food items; in autumn the dominant category was Br, while in winter the ingestion of Pl and Cl were higher; and in spring, the highest intakes also corresponded to Ca. No significant differences were detected between sexes with respect to the ingestion of food items (Student's t test, $p>0.05$; Mann-Whitney's U test, $p>0.05$).

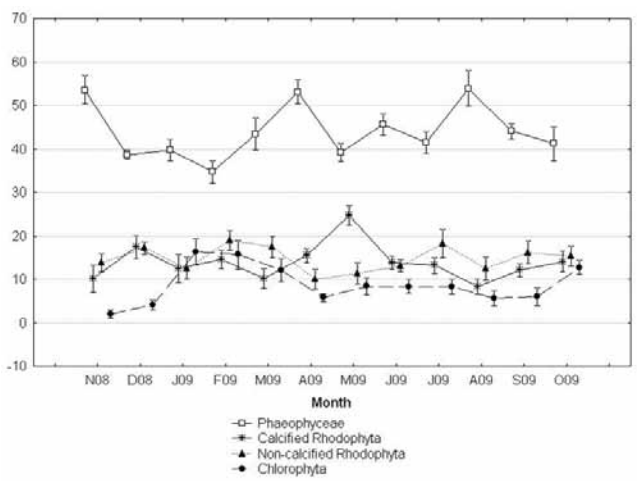

Fig. 1: Temporal trends in abundances (% mean ± se) of the different algal groups present in the gut content of the sea urchins studied.

Classification analysis of the food items identified in the gut content, using the Euclidean distance matrix and the UPGMA clustering method, produced the dendrogram shown in Figure 3, illustrating the relationships between these items. The food components belonging to the brown and green algae categories (Ha, Br, Di, and Ul) with a higher relative abundance in the gut content are grouped and separated from another cluster containing the lower abundance red and green algae (Ce, Cl, As, Pl, Ch, Ge, and Ca). There is some association between brown algae which, together with the unidentified remains, constitutes an important fraction of the sea urchin diet throughout the year. The red and green algae, with the exception of the *Ulva* spp., remain in the second group, their presence in the gut content being much more limited. However, the remains of *Gelidium* spp. and calcified Rhodophyta form a subgroup corresponding to a food fraction of relatively high importance.

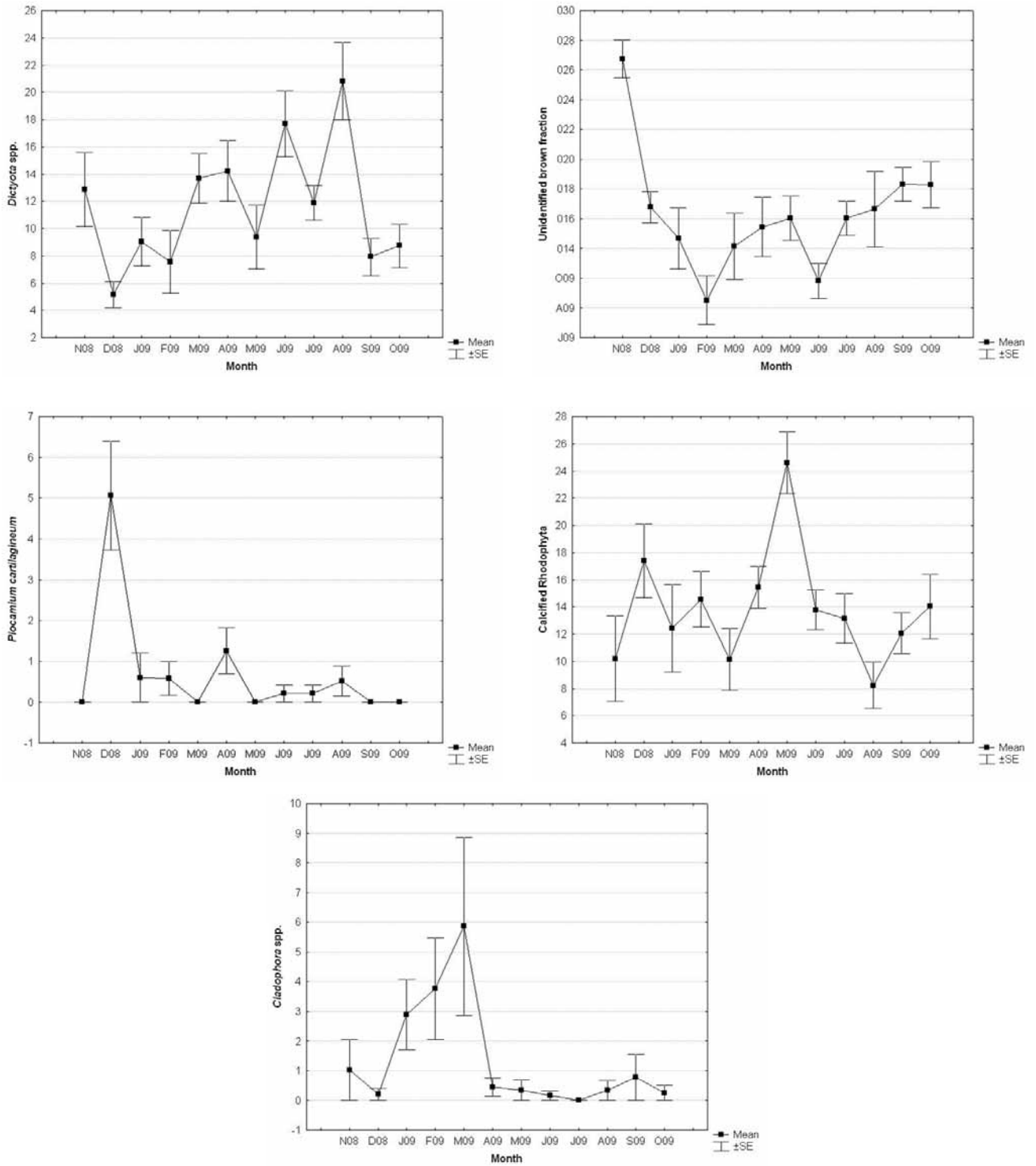

Fig. 2: Temporal trends in abundances (% mean ± se) for selected food items in the gut content of the sea urchins studied.

Gut condition

Over the period studied, there were significant differences between months in GII (% annual mean ± se= 7.7928±0.24) considering the sexes separately and over the total population (Kruskal-Wallis test, $p<0.01$), although there was no clear trend. The lowest values occurred in certain winter months (January, March) and late summer (August, September, October) and the maximum in December, February and June (Fig. 4). It is worth noting that the values in December and February were similar to those obtained in spring and early summer. On the other hand, there were significant differences between the sexes (Mann-Whitney's U test, $p<0.05$). A similar result (Fig. 4) was obtained in the RI (% annual mean ± se= 6.1509±0.23) as regards the monthly variation, although in this case there are no differences between sexes

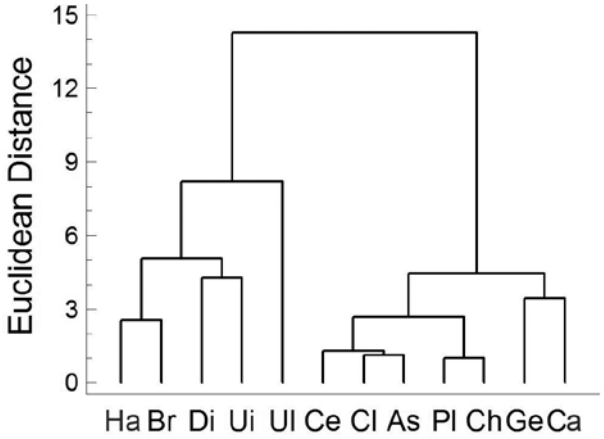

Fig. 3: Dendrogram for all food items. Codes for food items are as in Table 1. Cluster analysis was based on the UPGMA method and Euclidean distances.

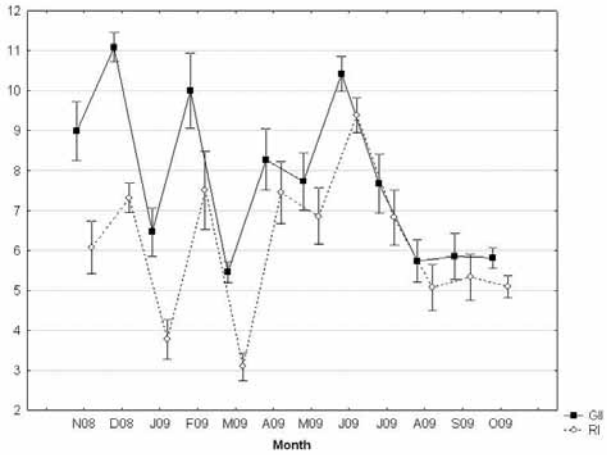

Fig. 4: Monthly changes (mean ± se) in the gastrointestinal (GII) and repletion (RI) indices in the sea urchins studied.

(p>0.05). Although the RI is used more widely in the literature, our results indicate that the two indices can be used more-or-less interchangeably, as can be seen from comparison of the two figures (Spearman rank correlation test, r=+0.90, p<0.001).

Gonad maturation and condition

In females (Fig. 5a), the growth of oocytes (stage II) occurred during October and January. The prematuration (stage III) occurred almost simultaneously in all the population and extended until March at which point a long period of maturation commenced (stage IV), and this extended until September, involving almost all females between March and July. Between April and September individuals were observed to have partially spawned (stage V) and between late summer and early autumn specimens with empty gonads predominated (stage VI). The recovery phase (stage I) seemed to be ephemeral and, accordingly, at any one time few individuals were observed in this stage. Males (Fig. 5b), like the females, went through a period of growth between October and January, an almost simultaneous prematuration phase and a long maturity period (stage IV) which spread from December to September, with individuals who had released some of their sperm appearing from April onwards (stage V). Empty gonads (stage VI) were observed in October and the recovery stage (stage I) was almost undetectable. A high degree of overlap in the timing of the different phases was observed between the sexes with a predominance of stage IV, indicating the occurrence of gonads in maturation much of the year, with the exception of the autumn, and a very clear breeding period between April and September, that appears gradually but comes to an end sharply in October, a month in which a large percentage of the population was found to have empty gonads.

The GI (% annual mean ± se= 3.4547±0.19) was analyzed monthly in a similar way, revealing significant differences (Kruskal-Wallis test, p<0.05) across the whole population during the study period. The monthly variation of GI (Fig. 6) produced peak values in February, coinciding with the first appearance of a great proportion

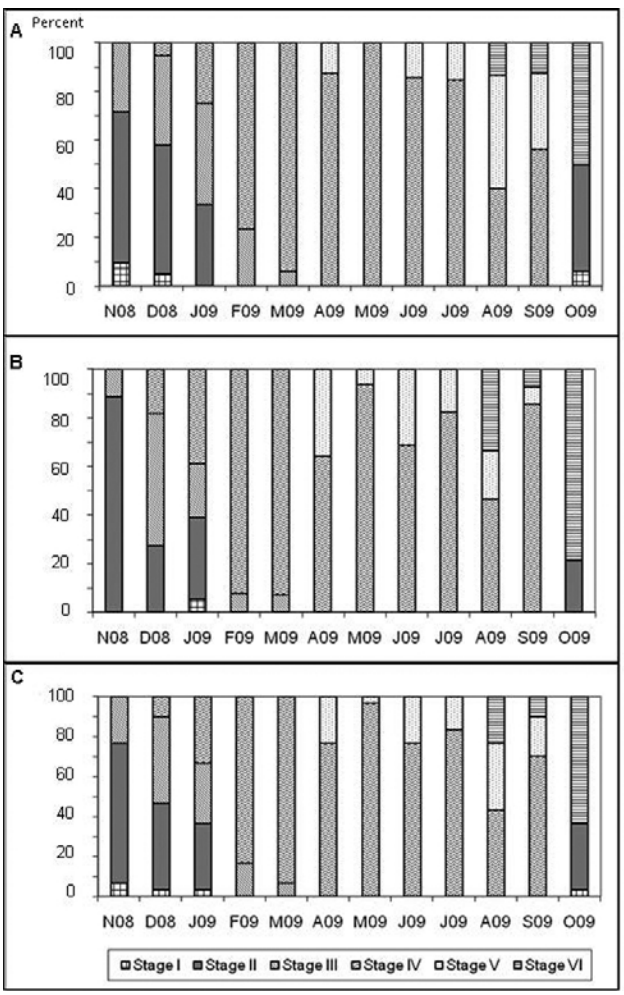

Fig. 5: Temporal trends in the percentage of individuals at each gonadal stage in the sea urchins studied. (A) Females, (B) males, and (C) both sexes.

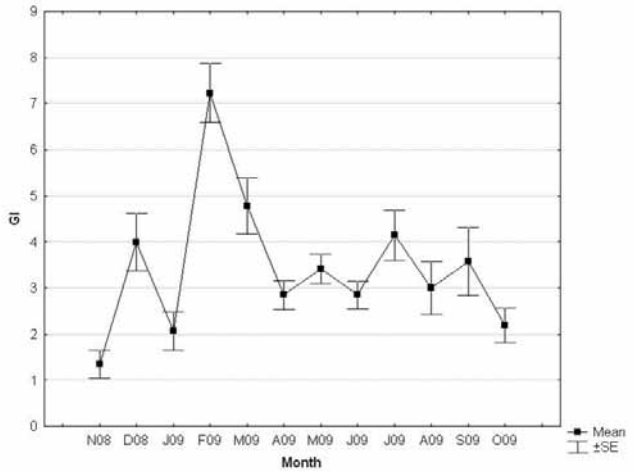

Fig. 6: Time course of the gonadosomatic (gonad) index (GI) in sea urchins, during the study period.

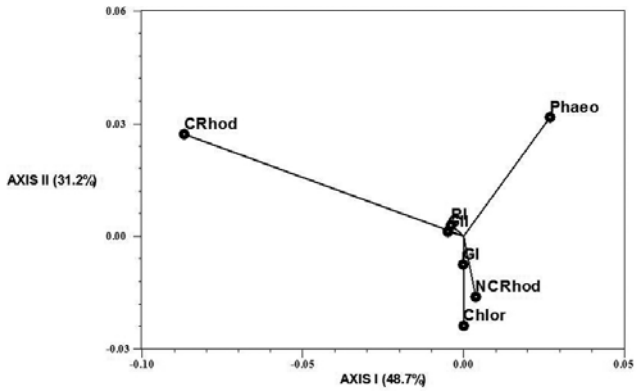

Fig. 7: Results of the correspondence analysis on 2D plot for physiological indices and pooled algal groups of the gut contents in the sea urchins studied. (GI) gonadosomatic (gonad) index; (GII) gastrointestinal index; (RI) repletion index; (Chlor) Chlorophyta; (CRhod) calcified Rhodophyta; (NCRhod) non-calcified Rhodophyta; (Phaeo) Phaeophyceae.

of individuals with gonads at the mature stage, and then fluctuating values during the favorable period for breeding. The lowest values were observed from October to January, also in accordance with the annual evolution of the gonad stages (Fig. 5). As expected, the highest values of GI corresponded to stage IV and the lowest ones to stage I of the gonad cycle (Figs. 5c and 6). No significant differences were detected between the sexes except in stage VI (Mann-Whitney's U test, $p>0.05$).

Gut contents vs gonad maturation and physiological indices

There were no significant differences in most food items as a function of stage of maturity. However, the unidentified brown fraction was significantly higher (one-way ANOVA, $p<0.05$) in stage II specimens, so this food fraction may be largely responsible for gonad growth, although it had lower values in relation to stage IV. Some single food components were significantly higher ($p<0.01$) at certain stages, namely, *Plocamium cartilagineum* in stage III and *Ulva* spp. in stage VI, but in both cases abundances in the diet were clearly lower.

A comparison analysis between paired samples indicated that all the variables showed differences with the same level of significance except for comparing the abundances of *Ulva* spp. with RI and the abundance of *Asparagopsis armata* with GI (Wilcoxon signed rank test, $p>0.05$). However, there were some significant correlations (Spearman rank correlation test, $p<0.05$) between pairs of variables, in particular, negative correlations of *Dictyota* spp. with certain red-algae (Pl and As variables) and with the unidentified brown remains (Br variable), and between them and the GI. Further, this index was positively correlated with abundances of *Cladophora* spp., and the RI with *Plocamium cartilagineum* and *Chaetomorpha* sp.

CA on the transformed data was used to characterize the relationship between the physiological indices (GI, GII and RI) and the feeding variables pooled into four enlarged algal variables (Phaeophyceae, non-calcified Rhodophyta, calcified Rhodophyta and Chlorophyta). The first three axes (Fig. 7) explained 90.64% of the total variance. Axis I mainly separated the abundances in the gut content of Phaeophyceae and calcified Rhodophyta, placing the others variables in the center of the plot. This axis indicated very different patterns of consumption of these two types of algae by *P. lividus*. Axis II separated non-calcified Rhodophyta and Chlorophyta from the other pooled algae and the distance from the score of green algae to the center of the plot was shorter than in the cases of Phaeophyceae and calcified Rhodophyta. Most of the observations were centralized around the physiological indices with values close to that of non-calcified Rhodophyta (Fig. 7), an algal group that seemed to be particularly closely related to these indices. However, in this analysis the physiological indices tended to clump together and, therefore, the relationships of each with the pooled algal variables cannot readily distinguished. To overcome this difficulty PCA was performed with the same transformed variables (Fig. 8). The eigenvalues >1 (the three first components) accounted for 74.35% of the variability. The loadings on the variables indicated that the indices also contribute to the separation of gut algal abundances. The first two components separated the pooled algal variables in a similar way to the CA. The Phaeophyceae were a significant distance from the calcified Rhodophyta ($r=-0.35$, $p<0.001$), non-calcified Rhodophyta ($r=-0.47$, $p<0.001$) and Chlorophyta ($r=-0.52$, $p<0.001$). Regarding the RI and GII indices, as noted above, they were found to be strongly positively correlated with each other ($r=+0.89$, $p<0.001$), but the results indicate that the annual values of RI are not related in a specific way with

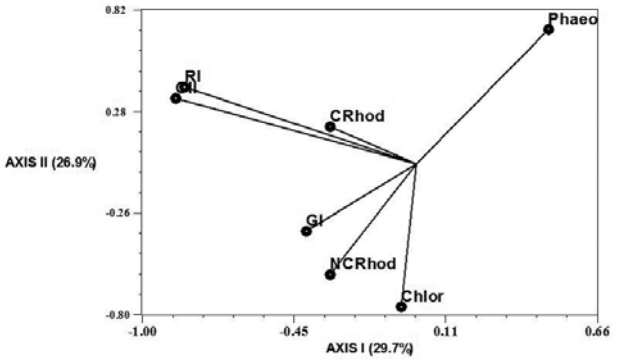

Fig. 8: 2D plot of the principal component analysis for physiological indices and pooled algal groups of the gut contents in the sea urchins studied. Codes as for Fig. 7.

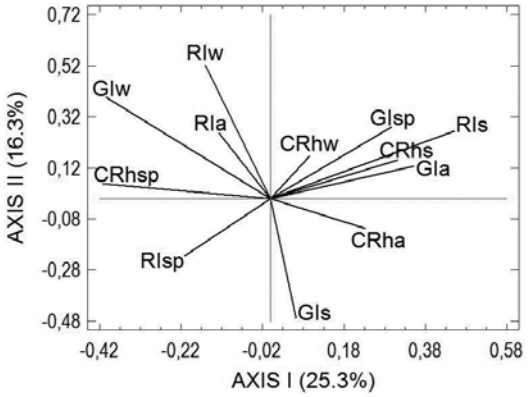

Fig. 9: 2D plot of the principal component analysis for repletion index, gonadosomatic (gonad) index and calcified Rhodophyta of the gut contents pooled per seasons in the sea urchins studied. (CRha) calcified Rhodophyta-autumn; (CRhs) calcified Rhodophyta-summer; (CRhsp) calcified Rhodophyta-spring; (CRhw) calcified Rhodophyta-winter; (GIa) gonad index-autumn; (GIs) gonad index-summer; (GIsp) gonad index-spring; (GIw) gonad index-winter; (RIa) repletion index-autumn; (RIs) repletion index-summer; (RIsp) repletion index-spring; (RIw) repletion index-winter.

any of the algal groups. Specifically, the correlations between the RI and the algal groups, analyzed over a complete annual cycle, were not statistically significant, although the correlation was somewhat stronger in the case of calcified Rhodophyta ($r=+0.17$, $p<0.10$). However, the PCAs carried out separately in each algal group by seasons show that the RI was correlated with the consumption of calcified Rhodophyta (Fig. 9) in spring ($r=+0.37$, $p<0.05$) and in summer ($r=+0.54$, $p<0.01$) as well as with non-calcified Rhodophyta (Fig. 10) in winter ($r=+0.37$, $p<0.05$). In spring an increase in RI can be observed as the consumption of calcified Rhodophyta grows, and, moreover, both decline together in summer, suggesting a relationship between these variables. The occasional increase in RI during a specific period in the winter could, on the other hand, be assumed to a greater consumption of certain non-calcified Rhodophyta in this period. The GI was positively correlated, considering the complete annual cycle, with non-calcified Rhodophyta ($r=+0.21$, $p<0.05$) and GII ($r=+0.19$, $p<0.05$) (Fig. 8), but negatively correlated with Phaeophyceae ($r=-0.25$, $p<0.01$). The seasonal analysis shows that the correlation with the consumption of non-calcified Rhodophyta (Fig. 10) was strongest in winter ($r=+0.46$, $p<0.01$), the season when GI shows the greatest increase (Fig. 6). Gonad condition seems to depend more on the consumption of non-calcified Rhodophyta than on the other algal taxa and, notably, it was only correlated with RI in winter ($r=+0.59$, $p<0.001$). That is, our data indicate that a greater overall consumption of algae by sea urchins may be only partially decisive in determining higher gonad weight at one or various points in the annual cycle.

Discussion

In the field, there is no doubt that *P. lividus* is not an extremely selective species as for the consumption of one or other particular species of algae. Leaving aside some old and less accurate observations of gut content (Fischer, 1864; Mortensen, 1943), algae which have been identified as predominant in the gut of this sea urchin include Ulvales, *Codium*, Ectocarpales, *Dictyota,* Gelidiales, Ceramiaceae and Rhodomelaceae (Kempf, 1962), and algae belonging to the Phaeophyceae (Neill & Larkum, 1966), in particular *Cystoseira* spp., *Sargassum vulgare* and *Dictyopteris polypodioides*. Verlaque & Nédélec (1983) also report this fraction as the most abundant (41%), followed by Rhodophyta (19%), *Posidonia oceanica* (16%) and Diatomophyceae (9%) making moderately important contributions to the diet and Chlorophyta being much rarer (2%). According to these authors relatively few species account for 63-78% of the diet, specifically *Sphacelaria* spp., *Dictyota* spp., Ectocarpaceae, *Corallina* spp., *Padina pavonica, Cystoseira brachycarpa* (as *C. balearica*), *Laurencia microcladia* and *Halopithys incurva*. However, San Martín (1987) attributes a greater contribution to Chlorophyta (46%) with species such as *Monostroma grevillei, Ulva* sp. and *Codiun fragile*, followed by Rhodophyta *Gracilaria bursa-pastoris* (16%). At the same time, this author highlights the lower abundance of Phaeophyceae, represented by Ectocarpaceae (6%) and *Colpomenia peregrina* (3%), a result that can be attributed to the study having been carried out in a coastal lagoon (Thau Lagoon). All of these observations have been made in the Mediterranean Sea and do not appear to differ from the findings of studies carried out in the Atlantic. For example, Niell & Pastor (1973) also assign the highest percent abundance in the gut contents to Chlorophyta, specifically the *Ulva* spp. (>60%) and *Cladophora* spp. (15%), followed by some Phaeophyceae such as *Cystoseira granulata* (40-50%), and calcified Rhodophyta (>25%) in third place. *Ceramium* spp. and

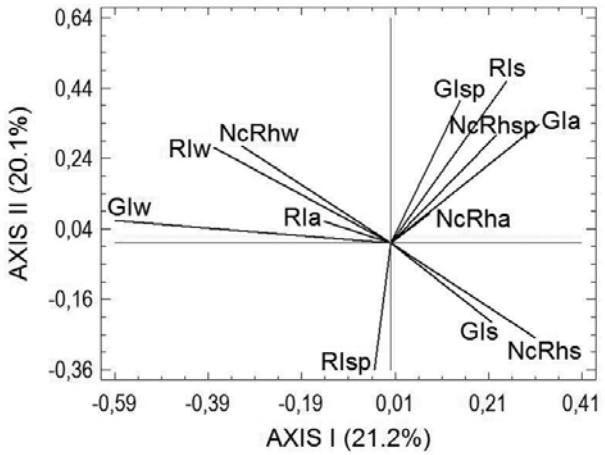

Fig. 10: 2D plot of the principal component analysis for repletion index, gonadosomatic (gonad) index and non-calcified Rhodophyta of the gut contents pooled per seasons in the sea urchins studied. (NcRha) non-calcified Rhodophyta-autumn; (NcRhs) non-calcified Rhodophyta-summer; (NcRhsp) non-calcified Rhodophyta-spring; (NcRhw) non-calcified Rhodophyta-winter; (GIa) gonad index-autumn; (GIs) gonad index-summer; (GIsp) gonad index-spring; (GIw) gonad index-winter; (RIa) repletion index-autumn; (RIs) repletion index-summer; (RIsp) repletion index-spring; (RIw) repletion index-winter.

a few non-calcified Rhodophyta were found to represent less than 20%.

Our results are relatively similar to those of Verlaque & Nédélec (1983): Phaeophyceae (44 vs 41%), Rhodophyta (28.5 vs 19%) and Chlorophyta (8.7 vs 2%). However, there are some differences in our data with respect to that of Privitera *et al.* (2008): Phaeophyceae (44 vs 30.8%), calcified Rhodophyta (13 vs 23.7%), non-calcified Rhodophyta (14.6 vs 28.2%) and Chlorophyta (8.7 vs 2%). The differences between our findings and those of these and other authors can be attributed to the availability of algal resources, explained by local or habitat differences, and also the fact that their observations were made with a significantly smaller numbers of individuals and for shorter periods of time. In particular, these factors may explain the smaller contribution of some algal taxa, favoring higher proportions of others. In this sense, our survey provides more consistent results, as it covers a complete annual cycle, minimizing effects that seasonality can have on the percentage contribution of each algal fraction and the way it can affect the availability of one or other species. The Verlaque & Nédélec (1983) study was carried out during the spring-summer-autumn period, so it is the most suitable to compare with ours. These authors attribute great importance to the seasonality of the species and identify *Dictyota* spp. as characteristic of the gut contents of the sea urchin in spring and *Padina pavonica* in summer-autumn. In our data, presence of *Dictyota* spp. is also seasonal, rising in the spring and becoming even more pronounced in summer when consumption is at its highest. The intake of *Cladophora* spp. and *Plocamium cartilagineum* is also markedly seasonal (with peaks in spring and winter respectively), although both species are among the least abundant, so their total contribution to the sea urchins diet is relatively small. The fact that *Halopteris scoparia* abundances are relatively high and constant in the intestinal content can be considered as a local feature, reflecting the fact that sea urchins exploit a resource that is available all year round. Indeed, this finding contrasts with the results of other authors in that this species was absent or reported to have a very low abundance (Niell & Pastor, 1973). The high contribution of Chlorophyta to the gut content reported by Niell & Pastor (1973) and San Martín (1987) could be attributed, as noted above, to the fact that their data was only collected over a relatively short period of time. However, it could also be due to populations belonging to habitats subject to disturbance in which colonization by green algae is favored. *P. lividus* is able to survive even in relatively polluted habitats in which it adopts an opportunistic strategy (Régis, 1978a; Delmas & Régis, 1986) that could in such cases be based primarily on consumption of these green algae. The prevalence of Phaeophyceae in the gut content of this species is consistent with experimental studies on feeding preferences and intake of certain algae, such as *Undaria pinnatifida* (San Martín, 1987), *Cystoseira* spp. (Knoepffler-Peguy *et al.,* 1987), and *Colpomenia sinuosa, Padina pavonica* and *Cystoseira mediterranea* (Frantzis & Grémare, 1992).

The annual reproductive pattern of *P. lividus* has been studied in southern Spain (Sánchez-España *et al.,* 2004). This pattern generally includes a long period of gonad maturation (stage IV) mainly running from February through to August, with local variations. In a year-long study in the locality of La Herradura, geographically close to the site of our study, the proportion of the population at this stage of maturity was reported to fluctuate throughout the year. GI was found to reach a maximum in February, but the main peak of the index occurred in July, the rise in February being barely discernible, unlike in our study in which there was a sharp peak in this month. According to these authors, the variations observed from one locality to another in the time the GI reaches its annual maximum, are related more or less directly with the availability of food as indicated by Lawrence & Lane (1982). Differences in GI between locations are often observed (González-Irusta *et al*., 2010) and are also related to other factors such as farm effluents and organic pollution (Allain, 1975; Delmas & Régis, 1986; Cook & Kelly, 2007), temperature (Byrne, 1990), phytoplankton blooms (Lozano *et al.,* 1995; Bayed *et al*., 2005) and salinity and contamination by heavy metals (Bayed *et al.,* 2005). Considering the relationship with the availability of algal resources, we can compare the annual evolution of GI and RI. Bayed *et al*. (2005), in their study on Atlantic

populations of Morocco, indicate that the RI (dry weight) varies significantly over time, with annual peaks being recorded just before (February) and during the spawning period (April), although decreasing in March, when the GI is at its maximum value. In our population, the RI is highest in spring and early summer, especially for large individuals, corresponding to increased feeding activity and greater dedication of resources to reproduction. As in the Moroccan populations, RI increases in February, declines in March and increases again in April, but at our site the maximum value of the annual study appears in June. This June maximum marks the fundamental difference between the two sets of data. On the other hand, the GI follows the RI in that both have peaks in February. The breeding period coincides with the prevalence of gonads at the mature stage (stage IV), extending in our population from February to July (Fig.6) and affecting a high percentage of individuals, which explains the peaks of GI and RI in February, and is consistent with another maximum, namely that of the RI in June. Episodes of RI increase in the course of the annual cycle appear to be well related to obtaining abundant resources that are primarily intended for reproduction and indeed parallels between feeding and gonad indices have been noted previously on several occasions (Régis, 1979). Interestingly, Lozano *et al.* (1995) indicate divergences between the indices in stable habitats and those rich in algal resources and, on the contrary, unstable habitats show concordant indices. In population we studied the two indices are concordant though to a low level of significance. A pattern of oscillations in months with unfavorable temperatures and photoperiods (November to spring), with RI decreases not directly related to the corresponding GI, as reported by Sellem & Guillou (2007) in Tunisia, also seem to appear in our population. The lowest annual values of RI were found in this period and indicate a decrease of feeding activity that can be explained by the existence of short periods of low temperatures as these authors suggest. These previous studies and our results seem to confirm a relationship, albeit weak, between the indices, with some variations occurring in parallel.

High values of RI indicate high rates of consumption, which suggest that there is no effective limit on available food resources. In line with this, seasonal or local abundance of fleshy macroalgae produces high rates of consumption of this type of algae, and when it is in limited supply feeding efforts are diverted to other resources, such as, among others, the encrusting calcified macroalgae (Cobb & Lawrence, 2005; Privitera *et al*., 2008). In our study, RI (and GII) are inversely related to the abundances of Phaeophyceae and Chlorophyta and positively related to non-calcified Rhodophyta in winter and to calcified Rhodophyta in spring and summer. This suggests that, in our population, high rates of consumption are associated with the ingestion of red algae, which in part can be attributed to the temporal fluctuations in the abundance and widespread distribution of articulate and encrusting calcified algae occupying a large proportion of the surface area at the site of our study.

Finally, the GI is significantly correlated with non-calcified Rhodophyta above all in winter ($r=+0.46$, $p<0.01$), i.e., with fleshy red macroalgae. This apparently more direct relationship may be closely related to nutritional requirements, linked to the supply of certain substances essential for growth and gonad maturation by this algae group. The higher values in both indices can be attributed to the importance of the red algae in the feeding and gonad condition of sea urchins.

Acknowledgement

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