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Abstract

The sea urchins Paracentrotus lividus and Psammechinus miliaris are submitted to the same environmental conditions in the Bay of Brest. The relationship between seasonal changes in food source quality and their gonad production was investigated in reproducing experimentally these conditions. In a first stage two macroalgae (Palmaria palmata and Laminaria digitata) were tested. P. miliaris showed a stronger preference for P. palmata and over a year-long experiment both urchins progressively preferred P. palmata. Seasonal variations in the chemical composition of P. palmaria

were observed in the Bay of Brest: total carbohydrates were important and the relative maximum (about 50%) was reached between February and August; the lipid level was low and had a relative maximum of about 1% in June and August. Total protein in P. palmaria was high compared to other seaweeds: the maximum value (25%) was observed in June, that was probably due to the maintenance of nitrogen nutrient in the bay.

In the second stage of the study, seasonal changes in biochemical components of ingestion and absorption of the two sea urchins were followed in the laboratory using a monospecific diet of P. palmaria. The patterns of total carbohydrates and lipid absorption were very similar for both sea urchin species. Carbohydrates were absorbed strongly and uniformly, year round. Lipid absorption mimicked the lipid nutrient pattern in the food source. Only changes in protein absorption varied slightly between the two urchin species. Protein absorption was maximal for both species in February and June, but the quantity of absorbed protein was significantly higher in P. miliaris than in P. lividus during February. This increase was concomitant with protein storage in the sea urchin gonads, which peaked in February for P. miliaris and in June for P. lividus. P. lividus had a higher gonad production efficiency, based on gonad yield. The comparison between in situ data and the experimental results suggests that an algal diet more nitrogenous than the in situ algal food source would benefit the herbivorous P. lividus, rather than the more omnivorous species P. miliaris. Although P. milaris has been described as a species with large gonad production potential, P. lividus appears to be a more suitable species for echiniculture conditions.

Key words: sea urchin diet, Palmaria palmata. proximate composition, absorption efficiency, gonadal cycle.

1. Introduction

The sea urchins Paracentrotus lividus (Lamarck) and Psammechinus miliaris (Gmelin) are the two most common sea urchin species on the western coast of Brittany (France). Both species live in sheltered areas of intertidal and sublittoral zones. In an intertidal zone, P. lividus inhabits intertidal rock pools and P. miliaris lives under boulders; in subtidal zones, P. lividus occurs mainly on solid rocks or in seagrass meadows and has been observed on bottom sediments as diverse as gravels, heterogeneous sands or on maerl beds where it can cohabit with P. miliaris (Guillou et al., 2002). Both species have a commercial value. P. lividus populations have dramatically decreased on the northern coasts of Brittany because of destructive harvesting (Allain 1975, Southward and Southward, 1975). Although P. miliaris is smaller in size than P. lividus, it has a greater gonad production potential (Le Gall et al., 1989). Management of their populations could be improved by echiniculture.

Sea urchin biology, in general, has been well-studied all over the world, however studies of urchin populations in western Brittany are rare or incomplete for P. lividus (Allain, 1975, Dominique, 1973) and essentially for P. miliaris (Le Gall et al., 1989, 1990). Although both species have different areas of geographical distribution, they live in the Bay of Brest under similar environmental conditions. Their different temperature optima can lead to different patterns of reproductive cycle in the present environment (Guillou, pers.obs.). Moreover, although they are inherently herbivorous, they can have different diet preferences (Boudouresque and Verlaque, 2001; Kelly and Cook, 2001). The purpose of this study is to use these specific differences to analyze the correlation between food quality and pattern of reproductive cycle in sea urchins.

In the first stage of our study, their dietary preferences among the macrophytes available in situ were tested by an experimental procedure. Sea urchins from the Bay of Brest were maintained in live under conditions as similar as possible to those of their natural habitat. A monospecific diet was desirable for the second stage of the study in which food ingestion rates and absorption rates were evaluated in terms of three major biochemical components: proteins, lipids and carbohydrates. These results were compared to the status of the sea urchins gonad production throughout a year-long experiment. Our approach combined simultaneous analyses of the seawater nutrients, the natural food source biochemistry and the absorption of different components by each species to explain changes in the gonad yield and composition during an annual cycle. The physiological responses of each species (food ingestion and absorption, reproductive growth) were also measured and compared with the goal of improving the culture of these two sea urchin populations.

2. Materials and methods

2.1 Sampling and maintenance

The reproductive cycle of adult Paracentrotus lividus and Psammechinus miliaris in the Bay of Brest was investigated from February 1997 to December 1998. The individuals were collected monthly by dredging or SCUBA divers from a site situated in the southern part of the Bay of Brest (Guillou et al., 2002) on substratum covered by maerl (a substrate composed of the living thalli of the calcareous red alga, Lithotamnion corallioides (P. and H. Crouan)). This substratum promotes the development of epiphytic macrophytes assemblages dominated by Rhodophyceae.

In the experimental study, P. lividus and P. miliaris individuals were collected by dredging in March 2000 in the same site. In the laboratory, the sea urchins were divided into three replicate groups consisting of 10 individuals of each species, to measure feeding rates. Additionnal tanks maintained in the same experimental conditions were used for measurements of sea urchin gonad indices and biochemical analyses on the gonad tissues. A homogeneous size-class, representative of the dominant size-class of each population (Guillou et al., 2002), was selected: P. lividus: 32-36 mm (34.3 ± 1.8) and P. miliaris 22-25 mm (24.1 ± 1.5). The sea urchin groups were placed in tanks (60 × 40 × 30cm) supplied with fresh running seawater from the Bay of Brest passed through on a sand-filter at temperatures which ranged from 9 °C in winter up to 17 °C in summer. A plastic grid of 2mm meshes on the evacuation exit of each tank prevented the loss of algae or faeces. The photoperiod was adjusted weekly with a timer by means of a set of neon tubes placed directly over the tanks (one 30-watt tube per two tanks). Three replicate groups were used to measure feeding rates.

A preliminary test for food preferences for the two species was completed using: two green algae Cladophora rupestris (Linnaeus) Kützing and Enteromorpha ramulosa (Linnaeus), two red algae Palmaria palmata (Linnaeus) O. Kuntze, Solieria chordalis (C. Agardh) J. and Plocamium cartilagineum (Linnaeus) P. Dixon, and two brown algae Laminaria digitata (Hudson) Lamouroux and Bifurcaria bifurcata (Ross). Three preferred algae for the two sea urchins species were : P. palmata, S. chordalis and L. digitata (Vachet and Guillou, pers. comm.). Because they were easier to collect on a regular basis, P. palmata and L. digitata were used during the long-term study. These algae were collected weekly from a site near the laboratory facilities.

2.2 Feeding rates

2.2.1 First stage 2000-2001

In order to select which alga (Palmaria palmata or Laminaria digitata) was preferred by the two urchins, algal ingestion rates of Paracentrotus lividus and Psammechinus miliaris were recorded weekly in the laboratory from March 2000 to July 2000 then from September 2000 to June 2001. Each group of ten sea urchins was fed 10 g (WW, dried off in blotting paper) of bits of P. palmata and 10 g of bits of L. digitata which were added simultaneously in the tanks. Any food remaining after three days was weighed and biomass was measured to the nearest 0.01 g (WW, dried off in blotting paper). The ingested biomass (in g WW per urchin per day) was calculated by subtraction. The loss of algal biomass during the time period between feeding and collection has been estimated prior to the experiment by weighing algae in three different tanks at different temperatures. The algal loss was low, 0.4 ± 0.7 % and 1.4 ± 1.3 % at 12 and 17°C respectively.. The 10 g algal ration added was always in excess of the amount consumed both during and between the experiments. Tanks were cleaned after each feeding session.

2.2.2. Second stage 2001-2002

In the second part of the study, the ingestion rates and defaecation rates of Paracentrotus lividus and Psammechinus miliaris, fed on the preferred alga only, were recorded twice a month from October 2001 to August 2002. Each group of ten sea urchins were fed with 15 g WW of the preferred alga. All food offered, food remaining after 3 days and faeces collected through a sieve were weighed. The faeces loss during the experiment was estimated according to the procedure used for algae. This loss was 2 ± 3 % and 8.8 ± 1.2 % at 14 and 17°C respectively. For better precision, the biomasses were expressed in dry weight to the nearest 1 mg. Because the offered biomass was fresh and the water concentration varies seasonally in the alga, it was converted to dry weight using the relationship between DW and WW calculated at each feeding session. To do this, three samples of the alga were first blotted dry in the paper, weighed, and then dried to constant weight

(48h at 60°C). The ratio of the wet weight /dry weight of these samples was calculated for the conversion. Algal biomass ingested and faeces produced and absorption, calculated as the difference between algal biomass ingested and faeces produced, were expressed in mg DW. urchin^{-1}.day^{-1}. Absorption rate was the ratio between absorption and the ingested biomass multiplied by 100.

2.3 Environmental parameters

Seawater samples were collected at a station close to the seawater intake that supplied the tanks in the laboratory and which was at less than 0.5 nautical mile from the seaweed sampling site. Samples were collected weekly using the methods recommended by the French monitoring network in coastal environments (SOMLIT: http://www.obs-vlfr.fr/somlit).
Seawater was collected two meters below the surface at high tide and when the tide coefficient was 70 ± 10. Temperature was measured with a conductivity meter (LF 197). Seawater ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) were measured according to the method described in Strickland and Parsons (1972), and modified for a Technicon autoanalyser with an accuracy of 5%.

2.4 Reproductive cycle

On each in situ sampling (from February 1997 to December 1998), 20 individuals were brought back to the laboratory and dissected. Their gonads and tests were dried to constant weight (48h at 60°C). Gonad indices were calculated as the ratio of the dried gonad to the eviscerated test dry weight, and multiplied by 100.

Five times during the second stage of the experimental feeding experiment (24^{th} October 2001, 21^{th} December 2001, 5^{th} February 2002, 7^{th} June 2002, 19^{th} August 2002), five urchins of each species were isolated from the additional tanks to determine the gonad index according to the previous experimental protocol and to analyze the biochemical composition of the gonad.

2.5 Biochemical composition

The biochemical composition of the preferred alga, faeces and gonads were determined at the same time as gonad indices. The contents in carbohydrates, proteins and lipids of each compartment (alga, faeces and gonads) were determined. Three samples of algae and three samples of faeces from each urchin species were analyzed. Alga samples were rinsed and epiphytes removed before the analysis. Each sample of algae and faeces was divided into two parts. One part was weighed (wet weight) and then dried at 60°C to constant weight for estimation of the water content (difference between wet and dry weight). Ash content was determined on the dried tissue after combustion in a muffle furnace at 500°C for 4h. The second part of each sample was homogenized in distilled water using an

Ultra turax and this homogenate was used for the biochemical analyses. Carbohydrates were analysed using the Dubois procedure (Dubois *et al*., 1956). Nitrogen was determined by the total Kjeldahl method (TKN) (protein content = 6.25 × TKN) (Indergaard and Minsaas, 1991). Total lipid content was determined gravimetrically using the Bligh and Dyer method (1959).

For the gonad analyses four sea urchins were dissected and their gonads collected and homogenized with the Ultra turax. This homogenate was divided in four parts: the first split was used for water content determinations (drying at 60°C to constant weight). The dried material was then combusted at 500°C for 4h to determine the ash content of gonads. The remaining 3 splits were used for measuring the levels of carbohydrates, proteins and lipids using the techniques of Dubois et al. (1956), Lowry et al.(1951) and Bligh and Dyer (1959), respectively.

The proximate organic composition of each compartment was determined using the ash-free dry weight (AFDW). From these data, ingestion rates in terms of organic components, (carbohydrates, proteins and lipids) were expressed in mg DW.urchin^{-1}.day^{-1} for each nutrient.

The quantity of the ingested component was equal to the percentage of this component present in the alga sample at any given period multiplied by the quantity of alga ingested by the sea urchin over the same time. The quantity of excreted component was a function of the percentage of this material in the faeces and the quantity of faeces produced by the sea urchin. The quantity of component absorbed by the organism was the difference between the quantity ingested and the quantities excreted.

The chemical composition of the gonads was corrected by the gonad index at the time of sampling in order to take into account the changes in gonad weight over the length of the experiment. The index was calculated from the percentage of the organic component in the gonad at a given time multiplied by the gonad index at the same sampling.

2.6 Statistics

Changes in ingestion and defaecation rates, gonad index, quantities of ingested components (carbohydrates, proteins, lipids), of absorbed components and chemical composition of the gonad, were tested for each sea urchin species with a one-way analysis of variance (ANOVA) ($P < 0.05$) with the least significant difference test once the homogeneity of variance had been tested. The gonad index of experimental and control animals were arcsine-transformed.

All analyses were done with the statistical software STATGRAPHICS 4.

3 Results

3.1 Environmental variations

Ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) levels increased beginning in October 2001 (Fig.1a). The main peak of ammonium was observed at the end of October (2.4 µM) followed by a nitrite peak at the end of November (0.75 µM) and a nitrate concentration peak in mid-

February 2002 (23.6 µM). Then nitrates decreased in March when chlorophyll *a* showed a small peak (Fig. 1a and b). Nitrites and nitrates dropped to very low levels in March and May respectively, and stayed low until September during the temperature maximum (Fig. 1a).

Ammonium reached its lowest levels from March to the end of June followed by a new peak at the beginning of August. Successive peaks of chlorophyll *a* occurred from mid-May to the end of August (Fig. 1b).

3.2 Reproductive cycle

Field data obtained in 1997 and 1998 on the Paracentrotus lividus reproductive cycle in the Bay of Brest indicated that the time when spawning started, marked by a drop in the GI, differed between years (Fig. 2a). In 1997, the GI reached a maximum in May (GI=7) and then decreased sharply, indicating a short spawning period. In contrast during 1998, the GI decrease was small during winter and spring. Each year, the minimum GI values were observed in June and followed by a rapid increase. Spawning of Psammechinus miliaris occurred from early March to mid-June in 1997 and from mid-April to mid-June in 1998 (Fig. 2b). The GI reached maximum values of 12 and 8 respectively, and a minimum value of 2. This low level reflecting the resting stage remained steady during about 3 months.

For both species P. lividus and P. miliaris, the changes in gonad indices (GI) under experimental conditions confirmed the seasonal variations (Fig. 2a and b). For P. lividus, the GI increased from October 2001 (GI=2) to June 2002 (GI=8). In August, the GI value was still high. Comparison with the field data suggested the spawning event in the experimental study would be around the maximum GI value (8) observed in June. After the onset of spawning, which cannot be precisely defined here, the GI might drop to a low level located in mid-June in both sets of field data (1997 and 1998). Spawning marks were observed visually in the laboratory tanks during this time. Thus, the GI estimated in August would be during the recovery stage of the gonad, as the post-spawning stage, or resting stage, was very short in the field confirming the previous studies on P. lividus (Byrne, 1990; Spirlet et al., 1998). For P. miliaris, GI values increased significantly from December 2001 (GI=5) and reached the highest value (15) in February 2002, after which the GI value decreased and reached a minimum level in August 2002 (GI=2.6). The high level observed in February compared to the field data situated the onset of spawning event close to February. As for P. lividus the two sets of 1997 and 1998 field data indicated that the end of spawning took place in June. The very low value measured in August 2002, and similar to the field observations, suggests the gonads were in resting stage. The field and experimental observations indicate that spawning occurred earlier in P. miliaris than in P. lividus .

3.3 Feeding preference

The feeding rates on Palmaria palmata and Laminaria digitata for the two urchin species Paracentrotus lividus and Psammechinus miliaris from March 2000 to June 2001 are presented in the figure 3 using units of g WW. urchin^{-1}.day^{-1}. With respect to P. lividus (Fig. 3a) three feeding rate trends were observed: from March 2000 to July 2000 (except for April 2000), sea urchins ingested quantities significantly larger of L. digitata than P. palmata, then from September 2000 to June 2001, the ingestion of L. digitata and P. palmata did not differ significantly, and finally, from May 2001 to June 2001, the ingested biomass of P. palmata were higher than those of L. digitata ones (*P* < 0.05).

For P. miliaris, (Fig.3b), two stages could be distinguished: from March 2000 to September 2000, the feeding rates on L. digitata and P. palmata were not significantly different, and in the second stage, from October to June 2001, more P. palmata was ingested than L. digitata (*P* < 0.05). The ingestion rate of P. palmata increased significantly during this last period. This increase coincided with a decreasing consumption of L. digitata over the same period.

Finally the both species presented a similar pattern with a higher attraction for P. palmata with time .

3.4 Ingested and defaecated biomasses during 2001-2002

Based on the previous results, Palmaria palmata was used for the second part of the study. Samples were collected in Dellec Cove, near the seawater sampling station. In both species, changes in ingestion and defaecation rates had a similar pattern, with more pronounced variations in Psammechinus miliaris than in Paracentrotus lividus (Fig. 4a and b). A decrease in ingestion rate was observed from February (60 mg DW urchin^{-1} d^{-1}) to April (30 mg DW urchin^{-1} d^{-1}) in P. lividus, and from January (79 mg DW urchin^{-}

^{1} d^{-1}) to April (20.5 mg DW urchin^{-1} d^{-1}) in P. miliaris. After April, ingestion rates increased through June (50 and 68 mg DW urchin^{-1} d^{-1} for P. lividus and P. miliaris respectively) and remained high through summer.

The pattern of defaecation followed that of ingestion with mimima observed in April. Changes were significantly more pronounced for P. miliaris than for P. lividus.

3.5 Biochemical composition of Palmaria palmata

Biochemical analyses done on Palmaria palmata five times during the year showed seasonal changes in organic component levels (Fig. 5). Carbohydrates increased significantly from October (40.4 % AFDW) to December (53.2 %) then remained constant until August (P < 0.05). Proteins increased significantly from December (12.4 %) to February (24.4 %) and then decreased from June to August (13.7 %). The maximum level of proteins in P. palmata was measured in February and June.

Lipids increased significantly from February (0.4 %) to June (1.1 %) and reached their maximum value in August (1.3 %).

3.6 Quantity of ingested nutrients

The estimated ingestion of carbohydrates remained constant for Paracentrotus lividus throughout the annual cycle, about 20 mg DW. urchin^{-1}.day^{-1} (P> 0.05) (Fig. 6a). For Psammechinus miliaris, the quantity of ingested carbohydrates increased significantly from October (19.6 mg DW.

urchin^{-1}.day^{-1}) to December (26.8 mg DW. urchin^{-1}.day^{-1}) and reached its maximum level in February and June (29.5 mg DW. urchin^{-1}.day^{-1}) (Fig. 6b). Then it decreased from June to August (26.1 mg DW. urchin^{-1}.day^{-1}).

The estimated quantity of proteins ingested by P. lividus and P. miliaris, increased significantly from October (6.3 mg and 6 mg DW. urchin^{-1}.day^{-1}, respectively) to February (9.6 mg and 14.3 mg DW. urchin^{-1}.day^{-1}, respectively). However, in P. lividus the maximum level occurred in June (12.1 mg DW. urchin^{-1}.day^{-1}) ($P < 0.05$), while in P. miliaris it was observed in both February (14.3 mg DW. urchin^{-1}.day^{-1}) and June samples (15.7 mg DW. urchin^{-1}.day^{-1}) which were not significantly different. In both species the quantity of proteins ingested decreased significantly between June and August.

With respect to the lipids, the estimated quantity ingested by each species increased significantly between February (0.17 and 0.18 mg DW. urchin^{-1}.day^{-1}, respectively) and June (0.53 and 0.68 mg DW. urchin^{-1}.day^{-1}, respectively). Maximum levels of lipids were ingested in June and August.

3.7 Total absorption rate and quantity of absorbed components

The total absorption rate was high for both species (Fig. 4). In Psammechinus miliaris a period of low absorption occurred in May (60.1 ± 6.36 %) between two periods of high, but significantly different, absorption rates, the first from October to the end of April (82.1 ± 5.5%) and the second from the mid-June to the end of August (77.6 ± 4.9 %). In Paracentrotus lividus the absorption rate was homogeneous over the year

(87.6 ± 3 %) and was significantly higher than even the high absorption rate periods of P. miliaris (P<0.05). With respect to the different components, the absorption of carbohydrates was significantly higher in P. miliaris than in P. lividus (97 ± 1% versus 86 ± 7% in). The protein absorption did not vary significantly between the two species (78 ± 9% and 80.5 ± 7% for P. miliaris and P. lividus respectively).

The amount of an absorbed biochemical component was considered relative to the ingested and defaecated biomass of the same component (Fig. 7). The quantity of absorbed carbohydrates was not significantly different during the annual cycle for each species but was significantly different (*P* <0.05) between both species with 20.7± 1 and 26.4 ± 4 mg DW.urchin^{-1}.day^{-1} for P. lividus and P. miliaris respectively. Both species exhibited similar changes in the absorption of proteins. The quantity of absorbed proteins increased significantly from October (4.9 and 4.8 mg DW.urchin^{-1}.day^{-}1 for P. lividus and P. miliaris respectively) to February (7.12 and 9.9 mg DW.urchin^{-1}.day^{-}1) and then from February to June (11.2 and 13.8 mg DW.urchin^{-1}.day^{-}1). This increase was followed by a decrease from June to August (4.3 and 5.7 mg) (*P* < 0.05).

For both P. lividus and P. miliaris, the absorption of lipids was only quantifiable in June and August (0.37 and 0.46 mg DW.urchin^{-1}.day^{-}1 in P. lividus and P. miliaris, respectively). because of the scarcity of this component in the alga.

3.8 Biochemical composition of the gonad

The quantity of carbohydrates in the gonad increased significantly for both species, from December to February and then decreased from February to June ($P < 0.05$) (Fig. 8).

The protein content in Paracentrotus lividus gonads increased steadily and significantly from October and reached its maximum level in June ($P < 0.05$); it decreased between June and August, but remained superior to October and December values. For Psammechinus miliaris, the quantity of proteins in the gonads increased significantly from December to February then decreased steadily and significantly to August. The level in August was lower than that in October ($P < 0.05$).

The quantity of lipids in gonad samples increased significantly for P. lividus from December to February, decreasing thereafter into August. For P. miliaris, an important significant increase was observed between December and February, followed by successive significant decreases in both June and August ($P < 0.05$).

4. Discussion

4.1 Palmaria palmata as a nutritional source

One of our first objectives was to determine the preferred alga by the two sea urchin species in order to use a monospecific, natural diet for subsequent experiments. A previous study (Vachet, unpublished) suggested the sea urchins had a preference for two algae already used commonly in echiniculture: Palmaria palmata and Laminaria sp. (Basuyaux and Blin, 1998; Kelly, 2001, Spirlet et al., 2000). In the present study, sea urchins were fed P. palmata and L. digitata for more than one year. Analysis of the results showed that, in the short term (6 months), there was a variable consumption rate of the two algae, by the sea urchin species. Over longer time periods, there was a progressively greater consumption of P. palmata by both urchin species. In this first experiment, this change in feeding preference was not directly correlated to changes in alga composition or in sea-urchin maturity as the feeding response during the period of intense modifications in algae and in sea-urchin gonads (April-June) was significantly different between 2000 and 2001. Lemire and Himmelman (1996) have classified different algae according to their ability to support somatic and gonadic growth (using hierarchal cluster analysis), and reported that both these algae contributed strongly to the fitness of another urchin species, Strongylocentrotus droebachiensis. Vadas et al. (2000) in a similar study, also concluded that P. palmata among four species of preferred macroalgae “induced the quickest and highest” enhancement in gonad index values. The improvement in gonad yield has been credited to the high protein levels measured in this alga (Fleurence, 1999 and Martinez and Rico, 2002), an explanation discussed by other investigators (see review Morgan et al., 1980 and Hagen Rødde et al. 2004). L. digitata contains a low proportion of protein and a relatively high proportion of complex carbohydrates (Otero-Villanueva et al., 2004) that can explain the poorer sea urchin ingestion, absorption and assimilation efficiencies.

Our study showed an increase in total protein in the alga, P. palmata between October and December, and maximum values were reached in February and June (24.4 % AFDW). These values were close to the maximal values reported from other studies in Brittany (about 25% from March to May in the southern part (Galland-Irmouli et al., 1999) and 22 to 20.4% between February and April in the northern part (Rouxel et al., 2001)) and were superior to values reported from the northern Spanish alga populations (18 % between March and May (Martinez and Rico, 2002)). The main difference between all these populations was the maintenance of a high protein level during June in P. palmata from the Bay of Brest, while the protein level decreased to 10 % in other populations along the coast of Brittany and declined to 2 % at the Spanish sites. The maintenance of a high protein content in P. palmata was probably related to the seawater nitrate concentration. Nitrate is the most available N source and is the main inorganic nutrient involved in algal nutrition (Chapman and Craige, 1977). A rapid increase in protein contents of P. palmata follows high concentrations of seawater nitrate (Morgan and Simpson, 1981). In our study, the increase was concomitant with the increase in seawater NO_3^- and NO_2^- concentrations and the maximum protein content occurred during the peak of NO_3^-. The overall seawater nitrogen concentrations in the Bay of Brest (maximum $NO_3^- + NO_2^-$ ⁝ 24 µM) was higher compared to those on the Spanish coast (9 µM).

P. palmata in our study remained very rich in proteins even in June. These proteins serve as a reserve source used for growth, maintenance and reproduction by the alga. In Brittany, the reproductive stage of P. palmata occurs during winter and the maximum growth rate, during winter and spring (Le Gall, 2002). Thus in June the protein content should have been low in the alga as it is the case in the Spanish coast, except if a nitrogen source was still present in the seawater., Two indices suggests the higher level of nitrogen in the Bay of Brest; the first is the presence of low but not insignificant concentrations of NH_4^+ which can also be utilized by the algae to contribute to the maintenance of growth (Martinez and Rico, 2002). The second is the occurrence of successive peaks of chlorophyll *a*, corresponding to phytoplankton blooms, from May to the end of August. These summer peaks of low intensity typical of the Bay of Brest ecosystem (http://www.obs-vlfr.fr/somlit) suggest sufficient nutrients were present to support bloom conditions, which could benefit the macroalgae also.

4.2 Changes in ingestion and defaecation rates

In the two sea urchin species, monthly variations were observed for both ingestion and defaecation rates. The possible loss in alga and faeces biomass during the experiment was too low to explain the main changes. The difference in timing for the start of an ingestion rate decrease (in January for P. miliaris and in February for P. lividus) may also be related to the relative stage of maturity in each species. During 2002, the highest GI reported here, and corroborated by the earlier field data (Fig. 2), showed that the maturity stage occurred earlier in P. miliaris than in P. lividus with the bay of Brest environmental conditions. Some previous studies have shown that echinoid feeding rates decrease before spawning (Fuji, 1967, De Ridder and Lawrence, 1982). The reason for this phenomenon may be physiological or due to the gonad size increase into the coelomic space during the gametogenesis. The first hypothesis is plausible for both species, but the second only concerns P. miliaris, since the P. lividus GI was high in April when feeding activity increased again. In both species, the increase in food consumption was concomitant with a water temperature increase in mid-April, suggesting temperature can control the sea urchin feeding rates also (see review Lares and Mc Clintock, 1991).

The defaecation rate changes in both species mimicked, in general, changes in ingestion rates. The total nutrient absorption rates were high (mean annual values of 78% and 62 % for P. lividus and P. miliaris respectively) but not superior to the values observed in P. lividus by Frantzis and Grémare (1992), often above 80%. P. miliaris presented absorption rates significantly lower and seasonal changes in ingestion and defaecation rates more pronounced than P. lividus For P. miliaris, total nutrient absorption was significantly lower after the spawning event, than between October and April during the gametogenesis stage. This process is probably related to progressive increase of reserve storage for gametogenesis.

4.3 Changes in nutrient absorption rate: connection with the proximate composition of food and gonad

Absolute changes in absorption rate differed for each nutrient, but the patterns were very similar for both species. The carbohydrates were absorbed uniformly throughout the year, in contrast to the absorption of proteins and lipids, which changed seasonally. The absorption of proteins significantly increased from October to June, and then decreased from June to August when the absorption of lipids increased. These changes in sea urchin nutrient absorption were linked to several factors: the total concentration of the nutrient in the food, the specific composition of lipids, carbohydrates and proteins, the physiological requirements of the sea urchin for a particular nutrient, and the digestive characteristics of the sea urchin, (especially its enzymatic equipment). Without data on changes in specific composition of the nutrients and their digestibility in the sea urchins, this discussion was only based on the relationship between the proximate organic composition of the alga and its absorption by the sea urchins with a particular attention to the gonad production.

For the two sea urchin species in our study, carbohydrate absorption was not affected by diet as has been previously described in Watts et al., (1998) for Lytechinus variegatus (L). In our study, the carbohydrate absorption did not vary during the year-long experiment, even though this component increased significantly in Palmaria palmata from October to December. The carbohydrate absorption rate strongly suggest that these sea urchins were efficient in digesting the available carbohydrates. However, overall lower carbohydrate absorption recorded for *P. miliari*s suggest that *P.lividus* has better enzymatic conditions for digesting the insoluble carbohydrate fraction (which can represent about 55% of the dry weight of P. palmata) (Lahaye, 1991; Hagen Rødde et al., 2004). Total carbohydrate absorption was probably not affected by physiological demand for reproduction because the maximum need in this component (essentially as glycogen, Monteiro-

Torreiro and Garcia-Martinez, 2003) would have been between February and June for P. lividus, and December and February June for P. miliaris.

Lipid absorption was only observed in June and August when their levels were maximal in P. palmata. With a total lipid content of more than 1% in summer, P. palmata in the Bay of Brest have a relatively high lipid concentration (Sanchez-Machado et al., 2004). There was no significant difference in the mean quantity of lipids absorbed by the two sea urchins during this period. Their absorption reflects the significant increase of lipids in the food source and could not be linked to reproductive needs: the maximum gonad demand for this nutrient was in February for both sea urchin species.

The protein level in P. palmata increased from October to February. This increase was followed by the increase of ingested proteins from October to February for P. miliaris and from October to June for P. lividus. In both species, the maximum level of absorbed protein was observed in June. From February, the quantity of absorbed protein was significantly higher in P. miliaris than in P. lividus. These observations attest to a physiological relationship between the increase in the protein absorption and reproduction, the gonad growth phase being earlier in P. miliaris than in P. lividus. Protein is the major component of P. lividus and P. miliaris gonads (Monteiro-Torreiro and Garcia-Martinez, 2003) and the need for this nutrient increases strongly before spawning (Fenaux et al., 1977; Fernandez, 1998, Monteiro-Torreiro and Garcia-Martinez, 2003). In our study, this requirement was highest in February for P. miliaris and in June for P. lividus and would have been supported by the high protein content in P. palmata production during the same period. The protein conversion from ingested food to gonad biomass is known to be rapid (Fernandez, 1996) and suggests that gonadal growth cannot be effective when only protein reserves are available. The organism needs the protein-rich food also.

The relationship between gonad yield and protein content in algae (Lowe and Lawrence, 1976; Larson et al., 1980; Vadas et al., 2000) or in prepared feeds (see review Pearce et al., 2003) is well-documented. Comparing the GI obtained experimentally with the monospecific P. palmata diet and the GI observed in the field suggested that this protein-rich alga enhances the gonad yield in P. lividus. The results were less clear in P. miliaris. This species is known to be more omnivorous than P. lividus (*op.cit.*) and under natural conditions, P. miliaris feeds on algae and large numbers of encrusting intertidal organisms such as mussels or barnacles (Kelly and Cook, 2001), increasing its protein input.

Our experimental results showing the stronger preference of P. miliaris for the more protein-rich alga P. palmata (as compared to P. lividus) is consistent with the possibility that P. miliaris has a higher protein requirement. Higher protein ingestion may also explain the higher in situ P. miliaris GI values as compared to those of P. lividus (Le Gall, 1989; Kelly, 2000; this study). The enhanced gonad index in P. lividus when fed a monospecific high protein diet suggests that the optimum protein level (Akiyama et al., 2001) to maximize P. lividus gonad production is not reached under natural conditions, compared to P. miliaris. A protein-rich algal diet, atypical for P. lividus, could favour gonad growth in this species, whereas P. miliaris can utilise food of animal origin under natural conditions. The quantity of ingested and absorbed nutriments per urchin per day related to the sea urchin test biomass was higher in P. miliaris than in P. lividus. However, the maximum gonad biomass recorded in 2002 from P. miliaris (0.45g DWW) remained low compared to the maximum gonad biomass from P. lividus (0.70g DWW). In the same way, the conversion efficiency of food to gonadal production at a mature stage (ratio of ingestion rate to gonad growth rate) is better for *P. lividus* than for P. miliaris (20% and 9% respectively). Under echiniculture conditions, gonad production enhancement by protein input from natural food sources is likely to be more productive for P. lividus than P. miliaris.

LEGENDS

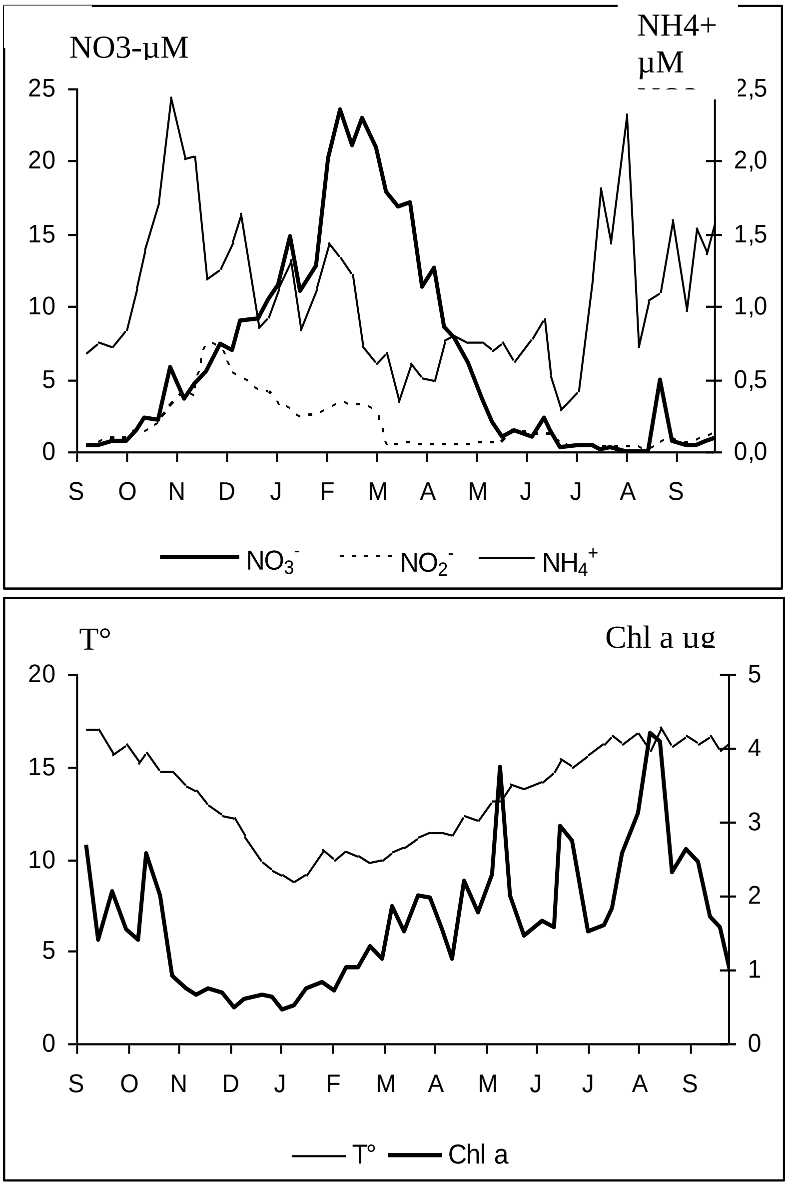

Fig.1.Seasonal changes in the seawater parameters in the Bay of Brest. from September 2001 to October 2002 : a : ammonium, nitrite and nitrate; b : temperature and chlorophyll *a*

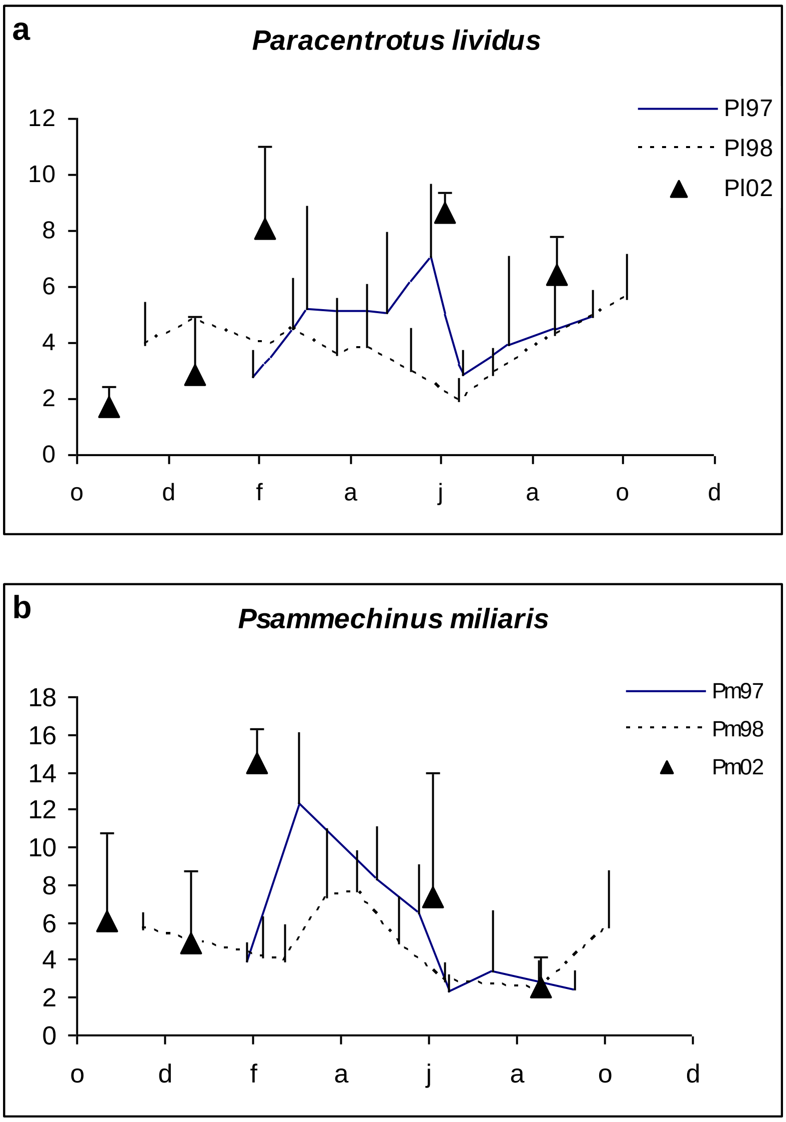

Fig. 2 Gonad indices (in % of dry weight) during the experiment (black triangle +SD) compared to the IG seasonal changes recorded in 1997 and 1998 from *in situ* populations

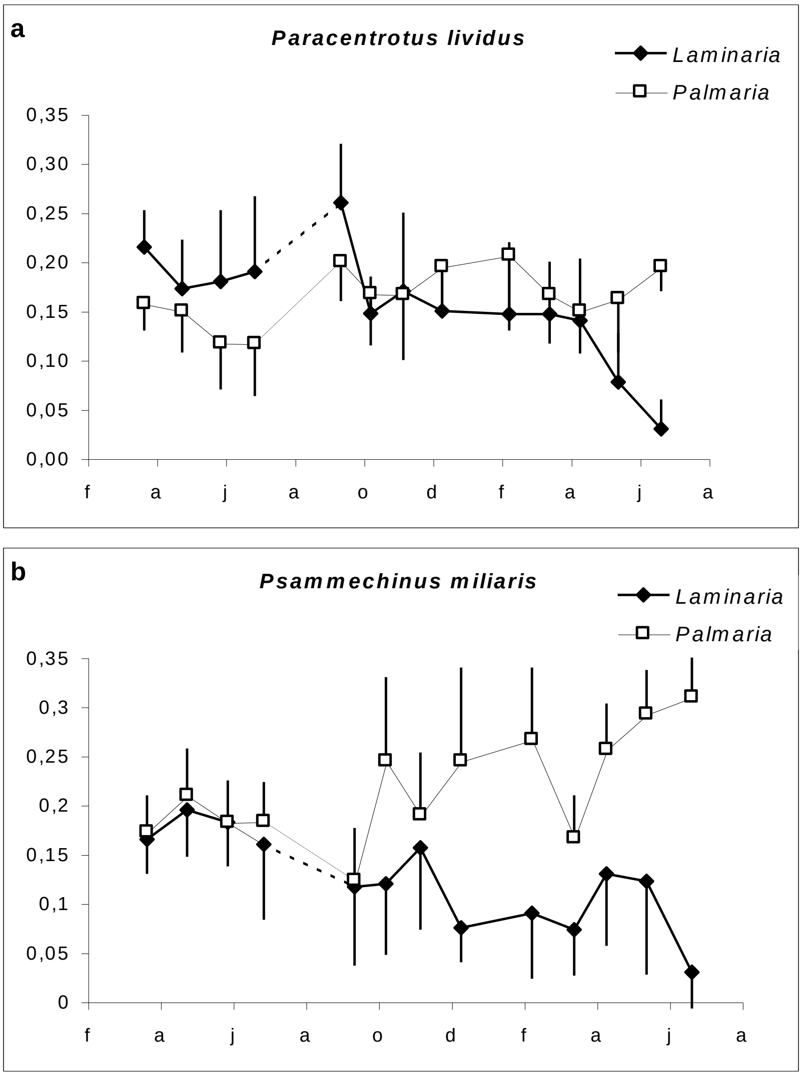

Fig. 3 Seasonal changes in the biomass of Laminaria digitata and Palmaria palmata ingested by the sea urchins (in g WW d^{-1} $urchin^{-1}$) (±SD) from March 2000 to March 2001; a : Paracentrotus lividus; b : Psammechinus miliaris.

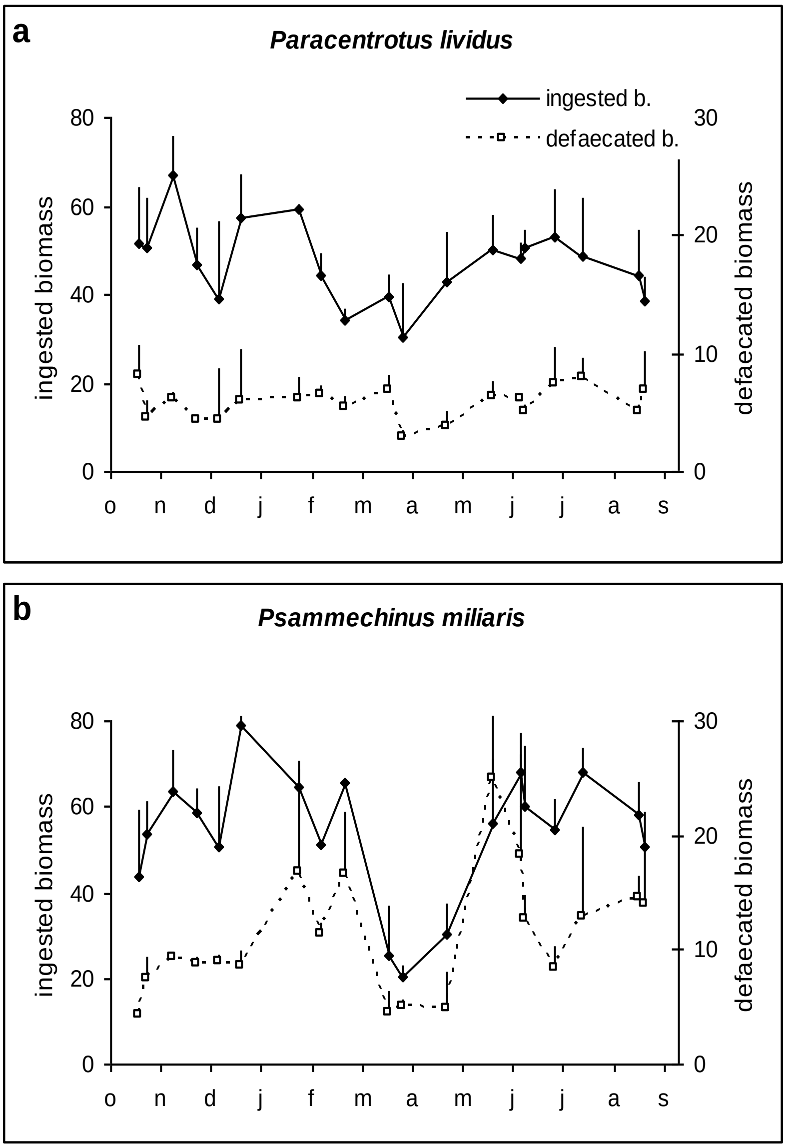

Fig. 4 Seasonal changes in the ingestion and defaecation rate of the sea urchins fed Palmaria palmata (in mg DW d^{-1} $urchin^{-1}$) (+SD) from October 2001 to August 2002. a : Paracentrotus lividus; b : Psammechinus miliaris.

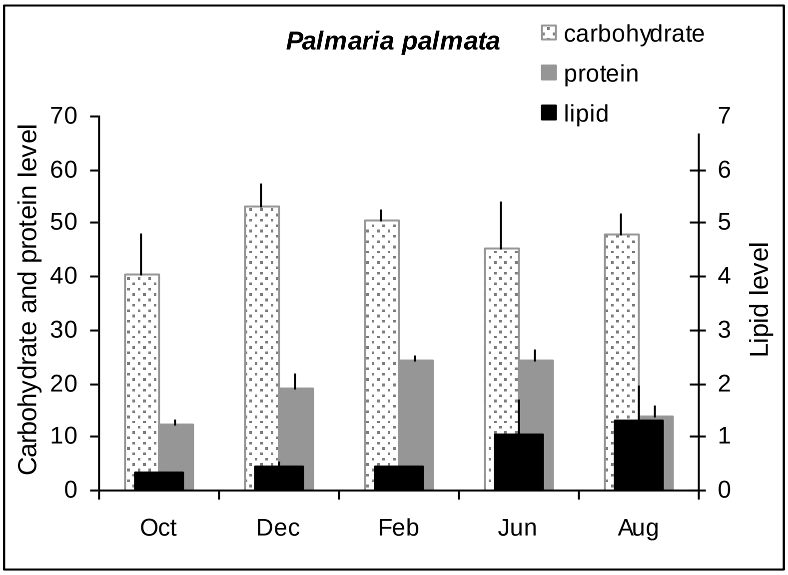

Fig. 5 Seasonal changes in the proximate organic composition of Palmaria palmata (in % of DW) (+SD)

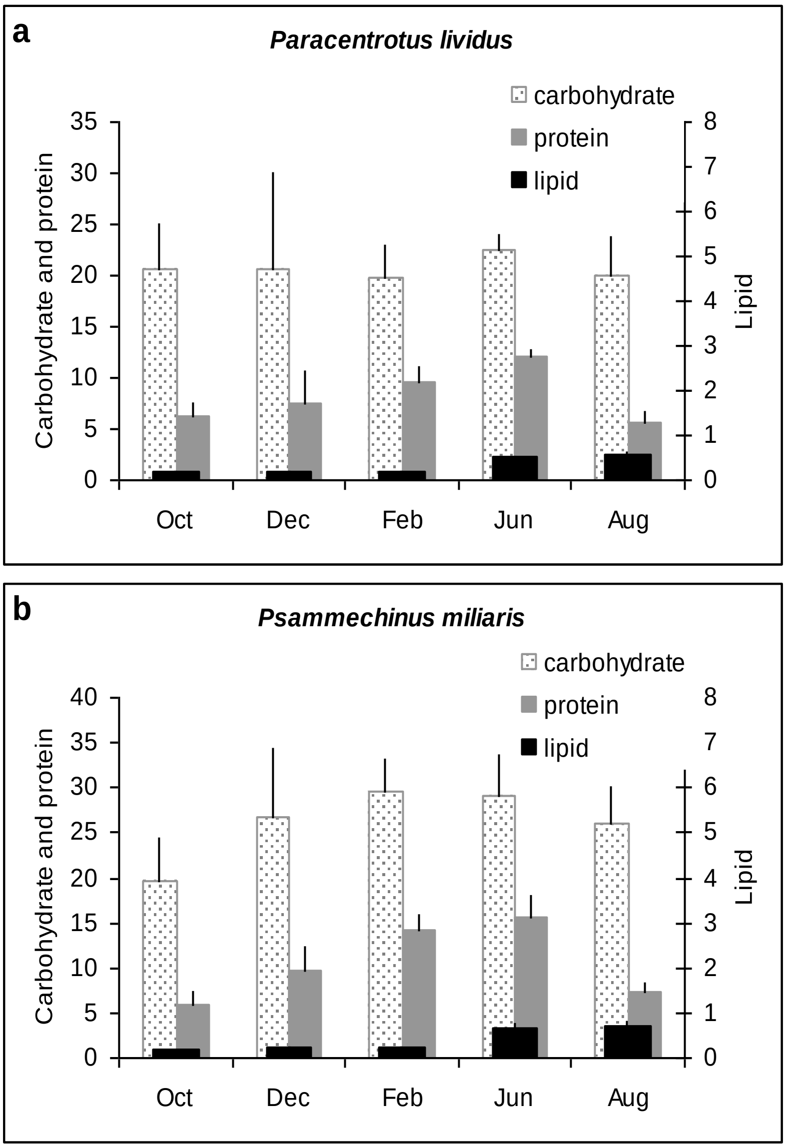

Fig. 6 Seasonal changes in the ingestion rate of the sea urchins fed Palmaria palmata in term of proteins, carbohydrates and lipids (in mg DW d^{-1} $urchin^{-}$) (+SD). a : Paracentrotus lividus; b : Psammechinus miliaris.

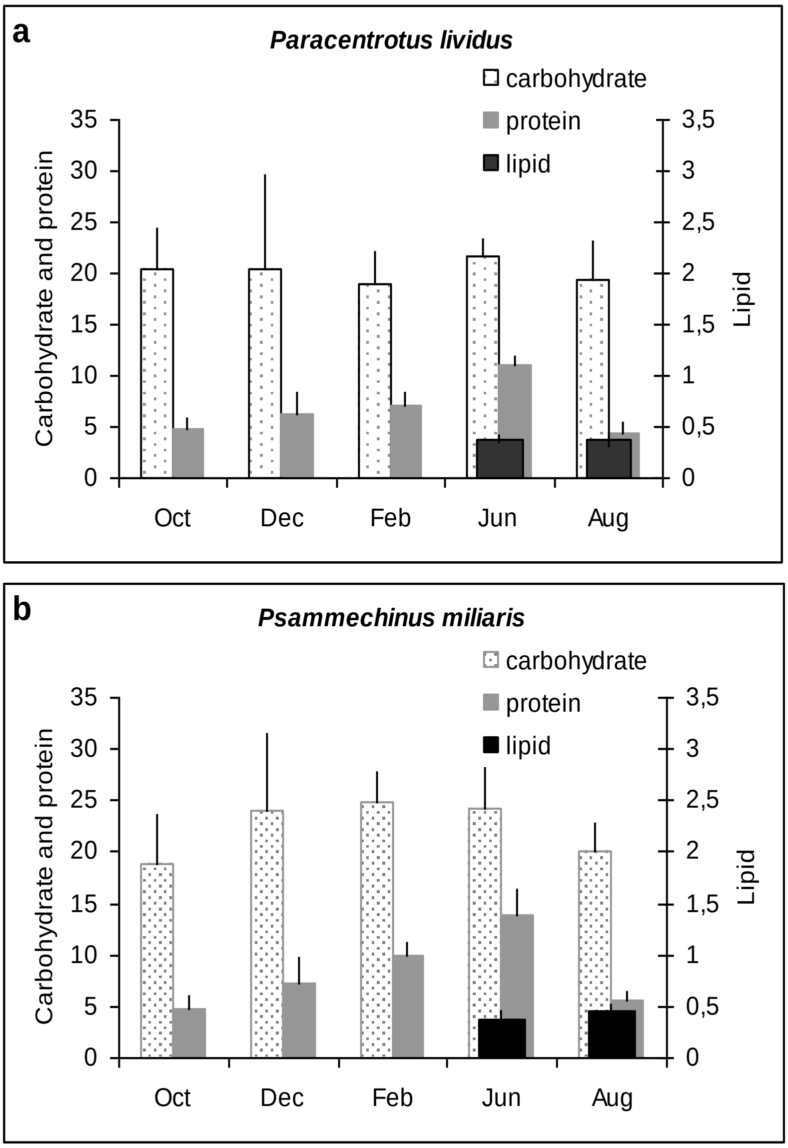

Fig. 7 Seasonal changes in the absorption rate of the sea urchins fed Palmaria palmata in term of proteins, carbohydrates and lipids (in mg DW d^{-1} $urchin^{-1}$) (+SD). a : Paracentrotus lividus; b : Psammechinus miliaris.

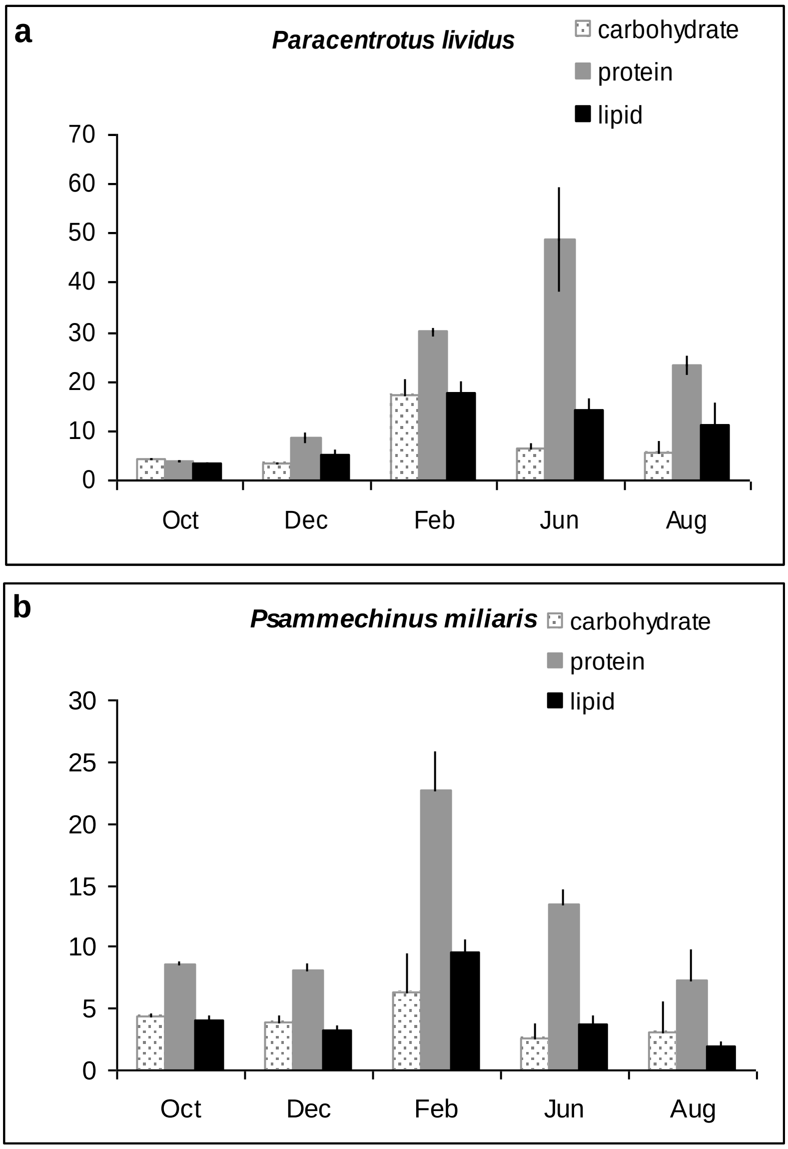

Fig. 8 Seasonal changes in the estimated composition of the gonad of the sea urchins fed Palmaria palmata (in mg DW) (+SD). a : Paracentrotus lividus; b : Psammechinus miliaris.

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