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Paracentrotus lividus (G.) and *Psammechinus miliaris*
(L.) to an hyperproteinated macrophytic diet**

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1 **The reproductive response of the sea urchins Paracentrotus**
2 **lividus (G.) and Psammechinus miliaris (L.) to an**
3 **hyperproteinated macrophytic diet.**

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15

16 **Abstract**

17

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

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

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

51

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

54

55 **1. Introduction**

56

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

71 Sea urchin biology, in general, has been well-studied all over the world,
72 however studies of urchin populations in western Brittany are rare or
73 incomplete for *P. lividus* (Allain, 1975, Dominique, 1973) and essentially
74 for *P. miliaris* (Le Gall et al., 1989, 1990). Although both species have
75 different areas of geographical distribution, they live in the Bay of Brest

76 under similar environmental conditions. Their different temperature optima
77 can lead to different patterns of reproductive cycle in the present
78 environment (Guillou, pers.obs.). Moreover, although they are inherently
79 herbivorous, they can have different diet preferences (Boudouresque and
80 Verlaque, 2001; Kelly and Cook, 2001). The purpose of this study is to use
81 these specific differences to analyze the correlation between food quality
82 and pattern of reproductive cycle in sea urchins.

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

98

99

100 **2. Materials and methods**

101

102 2.1 Sampling and maintenance

103

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

112 In the experimental study, P. lividus and P. miliaris individuals were
113 collected by dredging in March 2000 in the same site. In the laboratory, the
114 sea urchins were divided into three replicate groups consisting of 10
115 individuals of each species, to measure feeding rates. Additionnal tanks
116 maintained in the same experimental conditions were used for
117 measurements of sea urchin gonad indices and biochemical analyses on the
118 gonad tissues. A homogeneous size-class, representative of the dominant
119 size-class of each population (Guillou et al., 2002), was selected: P. lividus:
120 32-36 mm (34.3 ± 1.8) and P. miliaris 22-25 mm (24.1 ± 1.5). The sea urchin
121 groups were placed in tanks (60 × 40 × 30cm) supplied with fresh running
122 seawater from the Bay of Brest passed through on a sand-filter at
123 temperatures which ranged from 9 °C in winter up to 17 °C in summer. A
124 plastic grid of 2mm meshes on the evacuation exit of each tank prevented

125 the loss of algae or faeces. The photoperiod was adjusted weekly with a
126 timer by means of a set of neon tubes placed directly over the tanks (one 30-
127 watt tube per two tanks). Three replicate groups were used to measure
128 feeding rates.

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

140

141 2.2 Feeding rates

142

143 2.2.1 First stage 2000-2001

144 In order to select which alga (Palmaria palmata or Laminaria digitata) was
145 preferred by the two urchins, algal ingestion rates of Paracentrotus lividus
146 and Psammechinus miliaris were recorded weekly in the laboratory from
147 March 2000 to July 2000 then from September 2000 to June 2001. Each
148 group of ten sea urchins was fed 10 g (WW, dried off in blotting paper) of
149 bits of P. palmata and 10 g of bits of L. digitata which were added

150 simultaneously in the tanks. Any food remaining after three days was
151 weighed and biomass was measured to the nearest 0.01 g (WW, dried off in
152 blotting paper). The ingested biomass (in g WW per urchin per day) was
153 calculated by subtraction. The loss of algal biomass during the time period
154 between feeding and collection has been estimated prior to the experiment
155 by weighing algae in three different tanks at different temperatures. The
156 algal loss was low, 0.4 ± 0.7 % and 1.4 ± 1.3 % at 12 and 17°C
157 respectively.. The 10 g algal ration added was always in excess of the
158 amount consumed both during and between the experiments. Tanks were
159 cleaned after each feeding session.

160

161 2.2.2. Second stage 2001-2002

162 In the second part of the study, the ingestion rates and defaecation rates of
163 Paracentrotus lividus and Psammechinus miliaris, fed on the preferred alga
164 only, were recorded twice a month from October 2001 to August 2002.
165 Each group of ten sea urchins were fed with 15 g WW of the preferred alga.
166 All food offered, food remaining after 3 days and faeces collected through a
167 sieve were weighed. The faeces loss during the experiment was estimated
168 according to the procedure used for algae. This loss was 2 ± 3 % and $8.8 \pm$
169 1.2 % at 14 and 17°C respectively. For better precision, the biomasses were
170 expressed in dry weight to the nearest 1 mg. Because the offered biomass
171 was fresh and the water concentration varies seasonally in the alga, it was
172 converted to dry weight using the relationship between DW and WW
173 calculated at each feeding session. To do this, three samples of the alga were
174 first blotted dry in the paper, weighed, and then dried to constant weight

175 (48h at 60°C). The ratio of the wet weight /dry weight of these samples was
176 calculated for the conversion. Algal biomass ingested and faeces produced
177 and absorption, calculated as the difference between algal biomass ingested
178 and faeces produced, were expressed in $\text{mg DW} \cdot \text{urchin}^{-1} \cdot \text{day}^{-1}$. Absorption
179 rate was the ratio between absorption and the ingested biomass multiplied
180 by 100.

181

182 2.3 Environmental parameters

183

184 Seawater samples were collected at a station close to the seawater intake
185 that supplied the tanks in the laboratory and which was at less than 0.5
186 nautical mile from the seaweed sampling site. Samples were collected
187 weekly using the methods recommended by the French monitoring network
188 in coastal environments (SOMLIT: <http://www.obs-vlfr.fr/somlit>).

189 Seawater was collected two meters below the surface at high tide and when
190 the tide coefficient was 70 ± 10 . Temperature was measured with a
191 conductivity meter (LF 197). Seawater ammonium (NH_4^+), nitrate (NO_3^-),
192 and nitrite (NO_2^-) were measured according to the method described in
193 Strickland and Parsons (1972), and modified for a Technicon autoanalyser
194 with an accuracy of 5%.

195

196

197 2.4 Reproductive cycle

198

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

204 Five times during the second stage of the experimental feeding experiment
205 (24th October 2001, 21th December 2001, 5th February 2002, 7th June 2002,
206 19th August 2002), five urchins of each species were isolated from the
207 additional tanks to determine the gonad index according to the previous
208 experimental protocol and to analyze the biochemical composition of the
209 gonad.

210

211 2.5 Biochemical composition

212

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

224 Ultra turax and this homogenate was used for the biochemical analyses.
225 Carbohydrates were analysed using the Dubois procedure (Dubois *et al.*,
226 1956). Nitrogen was determined by the total Kjeldahl method (TKN)
227 (protein content = $6.25 \times \text{TKN}$) (Indergaard and Minsaas, 1991). Total lipid
228 content was determined gravimetrically using the Bligh and Dyer method
229 (1959).

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

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

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

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

254

255 2.6 Statistics

256 Changes in ingestion and defaecation rates, gonad index, quantities of
257 ingested components (carbohydrates, proteins, lipids), of absorbed
258 components and chemical composition of the gonad, were tested for each
259 sea urchin species with a one-way analysis of variance (ANOVA) ($P <$
260 0.05) with the least significant difference test once the homogeneity of
261 variance had been tested. The gonad index of experimental and control
262 animals were arcsine-transformed.

263 All analyses were done with the statistical software STATGRAPHICS 4.

264

265

266 **3 Results**

267

268 3.1 Environmental variations

269

270 Ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) levels increased
271 beginning in October 2001 (Fig.1a). The main peak of ammonium was
272 observed at the end of October ($2.4 \mu\text{M}$) followed by a nitrite peak at the
273 end of November ($0.75 \mu\text{M}$) and a nitrate concentration peak in mid-

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

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

281

282 3.2 Reproductive cycle

283

284 Field data obtained in 1997 and 1998 on the Paracentrotus lividus
285 reproductive cycle in the Bay of Brest indicated that the time when
286 spawning started, marked by a drop in the GI, differed between years (Fig.
287 2a). In 1997, the GI reached a maximum in May (GI=7) and then decreased
288 sharply, indicating a short spawning period. In contrast during 1998, the GI
289 decrease was small during winter and spring. Each year, the minimum GI
290 values were observed in June and followed by a rapid increase. Spawning of
291 Psammechinus miliaris occurred from early March to mid-June in 1997 and
292 from mid-April to mid-June in 1998 (Fig. 2b). The GI reached maximum
293 values of 12 and 8 respectively, and a minimum value of 2. This low level
294 reflecting the resting stage remained steady during about 3 months.

295 For both species P. lividus and P. miliaris, the changes in gonad indices (GI)
296 under experimental conditions confirmed the seasonal variations (Fig. 2a
297 and b). For P. lividus, the GI increased from October 2001 (GI=2) to June
298 2002 (GI=8). In August, the GI value was still high. Comparison with the

299 field data suggested the spawning event in the experimental study would be
300 around the maximum GI value (8) observed in June. After the onset of
301 spawning, which cannot be precisely defined here, the GI might drop to a
302 low level located in mid-June in both sets of field data (1997 and 1998).
303 Spawning marks were observed visually in the laboratory tanks during this
304 time. Thus, the GI estimated in August would be during the recovery stage
305 of the gonad, as the post-spawning stage, or resting stage, was very short in
306 the field confirming the previous studies on P. lividus (Byrne, 1990; Spirlet
307 et al., 1998). For P. miliaris, GI values increased significantly from
308 December 2001 (GI=5) and reached the highest value (15) in February
309 2002, after which the GI value decreased and reached a minimum level in
310 August 2002 (GI=2.6). The high level observed in February compared to the
311 field data situated the onset of spawning event close to February. As for P.
312 lividus the two sets of 1997 and 1998 field data indicated that the end of
313 spawning took place in June. The very low value measured in August 2002,
314 and similar to the field observations, suggests the gonads were in resting
315 stage. The field and experimental observations indicate that spawning
316 occurred earlier in P. miliaris than in P. lividus .

317

318 3.3 Feeding preference

319

320 The feeding rates on Palmaria palmata and Laminaria digitata for the two
321 urchin species Paracentrotus lividus and Psammechinus miliaris from March
322 2000 to June 2001 are presented in the figure 3 using units of g WW. urchin⁻¹
323 ¹.day⁻¹. With respect to P. lividus (Fig. 3a) three feeding rate trends were

324 observed: from March 2000 to July 2000 (except for April 2000), sea
325 urchins ingested quantities significantly larger of L. digitata than P. palmata,
326 then from September 2000 to June 2001, the ingestion of L. digitata and P.
327 palmata did not differ significantly, and finally, from May 2001 to June
328 2001, the ingested biomass of P. palmata were higher than those of L.
329 digitata ones ($P < 0.05$).

330 For P. miliaris, (Fig.3b), two stages could be distinguished: from March
331 2000 to September 2000, the feeding rates on L. digitata and P. palmata
332 were not significantly different, and in the second stage, from October to
333 June 2001, more P. palmata was ingested than L. digitata ($P < 0.05$). The
334 ingestion rate of P. palmata increased significantly during this last period.
335 This increase coincided with a decreasing consumption of L. digitata over
336 the same period.

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

339

340 3.4 Ingested and defaecated biomasses during 2001-2002

341

342 Based on the previous results, Palmaria palmata was used for the second
343 part of the study. Samples were collected in Dellec Cove, near the seawater
344 sampling station. In both species, changes in ingestion and defaecation rates
345 had a similar pattern, with more pronounced variations in Psammechinus
346 miliaris than in Paracentrotus lividus (Fig. 4a and b). A decrease in
347 ingestion rate was observed from February ($60 \text{ mg DW urchin}^{-1} \text{ d}^{-1}$) to April
348 ($30 \text{ mg DW urchin}^{-1} \text{ d}^{-1}$) in P. lividus, and from January ($79 \text{ mg DW urchin}^{-1}$

349 $^1 \text{d}^{-1}$) to April (20.5 mg DW urchin $^{-1} \text{d}^{-1}$) in P. miliaris. After April, ingestion
350 rates increased through June (50 and 68 mg DW urchin $^{-1} \text{d}^{-1}$ for P. lividus
351 and P. miliaris respectively) and remained high through summer.

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

355

356 3.5 Biochemical composition of Palmaria palmata

357

358 Biochemical analyses done on Palmaria palmata five times during the year
359 showed seasonal changes in organic component levels (Fig. 5).

360 Carbohydrates increased significantly from October (40.4 % AFDW) to
361 December (53.2 %) then remained constant until August ($P < 0.05$).

362 Proteins increased significantly from December (12.4 %) to February (24.4
363 %) and then decreased from June to August (13.7 %). The maximum level
364 of proteins in P. palmata was measured in February and June.

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

367

368 3.6 Quantity of ingested nutrients

369

370 The estimated ingestion of carbohydrates remained constant for
371 Paracentrotus lividus throughout the annual cycle, about 20 mg DW. urchin $^{-1}$
372 $\cdot \text{day}^{-1}$ ($P > 0.05$) (Fig. 6a). For Psammechinus miliaris, the quantity of
373 ingested carbohydrates increased significantly from October (19.6 mg DW.

374 urchin⁻¹.day⁻¹) to December (26.8 mg DW. urchin⁻¹.day⁻¹) and reached its
375 maximum level in February and June (29.5 mg DW. urchin⁻¹.day⁻¹) (Fig.
376 6b). Then it decreased from June to August (26.1 mg DW. urchin⁻¹.day⁻¹).

377 The estimated quantity of proteins ingested by P. lividus and P. miliaris,
378 increased significantly from October (6.3 mg and 6 mg DW. urchin⁻¹.day⁻¹,
379 respectively) to February (9.6 mg and 14.3 mg DW. urchin⁻¹.day⁻¹,
380 respectively). However, in P. lividus the maximum level occurred in June
381 (12.1 mg DW. urchin⁻¹.day⁻¹) ($P < 0.05$), while in P. miliaris it was
382 observed in both February (14.3 mg DW. urchin⁻¹.day⁻¹) and June samples
383 (15.7 mg DW. urchin⁻¹.day⁻¹) which were not significantly different. In both
384 species the quantity of proteins ingested decreased significantly between
385 June and August.

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

390

391 3.7 Total absorption rate and quantity of absorbed components

392

393 The total absorption rate was high for both species (Fig. 4). In
394 Psammechinus miliaris a period of low absorption occurred in May ($60.1 \pm$
395 6.36 %) between two periods of high, but significantly different, absorption
396 rates, the first from October to the end of April (82.1 ± 5.5 %) and the
397 second from the mid-June to the end of August (77.6 ± 4.9 %). In
398 Paracentrotus lividus the absorption rate was homogeneous over the year

399 (87.6 ± 3 %) and was significantly higher than even the high absorption rate
400 periods of P. miliaris ($P < 0.05$). With respect to the different components,
401 the absorption of carbohydrates was significantly higher in P. miliaris than
402 in P. lividus (97 ± 1% versus 86 ± 7% in). The protein absorption did not
403 vary significantly between the two species (78 ± 9% and 80.5 ± 7% for P.
404 miliaris and P. lividus respectively).

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

417 For both P. lividus and P. miliaris, the absorption of lipids was only
418 quantifiable in June and August (0.37 and 0.46 mg DW.urchin⁻¹.day⁻¹ in P.
419 lividus and P. miliaris, respectively). because of the scarcity of this
420 component in the alga.

421

422 3.8 Biochemical composition of the gonad

423

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

427 The protein content in Paracentrotus lividus gonads increased steadily and
428 significantly from October and reached its maximum level in June ($P <$
429 0.05); it decreased between June and August, but remained superior to
430 October and December values. For Psammechinus miliaris, the quantity of
431 proteins in the gonads increased significantly from December to February
432 then decreased steadily and significantly to August. The level in August was
433 lower than that in October ($P < 0.05$).

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

439

440

441 **4. Discussion**

442

443 4.1 Palmaria palmata as a nutritional source

444

445 One of our first objectives was to determine the preferred alga by the
446 two sea urchin species in order to use a monospecific, natural diet for
447 subsequent experiments. A previous study (Vachet, unpublished) suggested
448 the sea urchins had a preference for two algae already used commonly in

449 echiniculture: Palmaria palmata and Laminaria sp. (Basuyaux and Blin,
450 1998; Kelly, 2001, Spirlet et al., 2000). In the present study, sea urchins
451 were fed P. palmata and L. digitata for more than one year. Analysis of the
452 results showed that, in the short term (6 months), there was a variable
453 consumption rate of the two algae, by the sea urchin species. Over longer
454 time periods, there was a progressively greater consumption of P. palmata
455 by both urchin species. In this first experiment, this change in feeding
456 preference was not directly correlated to changes in alga composition or in
457 sea-urchin maturity as the feeding response during the period of intense
458 modifications in algae and in sea-urchin gonads (April-June) was
459 significantly different between 2000 and 2001. Lemire and Himmelman
460 (1996) have classified different algae according to their ability to support
461 somatic and gonadic growth (using hierarchical cluster analysis), and reported
462 that both these algae contributed strongly to the fitness of another urchin
463 species, Strongylocentrotus droebachiensis. Vadas et al. (2000) in a similar
464 study, also concluded that P. palmata among four species of preferred
465 macroalgae “induced the quickest and highest” enhancement in gonad index
466 values. The improvement in gonad yield has been credited to the high
467 protein levels measured in this alga (Fleurence, 1999 and Martinez and
468 Rico, 2002), an explanation discussed by other investigators (see review
469 Morgan et al., 1980 and Hagen Rødde et al. 2004). L. digitata contains a low
470 proportion of protein and a relatively high proportion of complex
471 carbohydrates (Otero-Villanueva et al., 2004) that can explain the poorer sea
472 urchin ingestion, absorption and assimilation efficiencies.

473 Our study showed an increase in total protein in the alga, P. palmata
474 between October and December, and maximum values were reached in
475 February and June (24.4 % AFDW). These values were close to the
476 maximal values reported from other studies in Brittany (about 25% from
477 March to May in the southern part (Galland-Irmouli et al., 1999) and 22 to
478 20.4% between February and April in the northern part (Rouxel et al.,
479 2001)) and were superior to values reported from the northern Spanish alga
480 populations (18 % between March and May (Martinez and Rico, 2002)).
481 The main difference between all these populations was the maintenance of a
482 high protein level during June in P. palmata from the Bay of Brest, while the
483 protein level decreased to 10 % in other populations along the coast of
484 Brittany and declined to 2 % at the Spanish sites. The maintenance of a high
485 protein content in P. palmata was probably related to the seawater nitrate
486 concentration. Nitrate is the most available N source and is the main
487 inorganic nutrient involved in algal nutrition (Chapman and Craige, 1977).
488 A rapid increase in protein contents of P. palmata follows high
489 concentrations of seawater nitrate (Morgan and Simpson, 1981). In our
490 study, the increase was concomitant with the increase in seawater NO_3^- and
491 NO_2^- concentrations and the maximum protein content occurred during the
492 peak of NO_3^- . The overall seawater nitrogen concentrations in the Bay of
493 Brest (maximum $\text{NO}_3^- + \text{NO}_2^-$: 24 μM) was higher compared to those on the
494 Spanish coast (9 μM).

495 P. palmata in our study remained very rich in proteins even in June.
496 These proteins serve as a reserve source used for growth, maintenance and
497 reproduction by the alga. In Brittany, the reproductive stage of P. palmata

498 occurs during winter and the maximum growth rate, during winter and
499 spring (Le Gall, 2002). Thus in June the protein content should have been
500 low in the alga as it is the case in the Spanish coast, except if a nitrogen
501 source was still present in the seawater., Two indices suggests the higher
502 level of nitrogen in the Bay of Brest; the first is the presence of low but not
503 insignificant concentrations of NH_4^+ which can also be utilized by the algae
504 to contribute to the maintenance of growth (Martinez and Rico, 2002). The
505 second is the occurrence of successive peaks of chlorophyll *a*,
506 corresponding to phytoplankton blooms, from May to the end of August.
507 These summer peaks of low intensity typical of the Bay of Brest ecosystem
508 (<http://www.obs-vlfr.fr/somlit>) suggest sufficient nutrients were present to
509 support bloom conditions, which could benefit the macroalgae also.

510

511 4.2 Changes in ingestion and defaecation rates

512

513 In the two sea urchin species, monthly variations were observed for
514 both ingestion and defaecation rates. The possible loss in alga and faeces
515 biomass during the experiment was too low to explain the main changes.
516 The difference in timing for the start of an ingestion rate decrease (in
517 January for *P. miliaris* and in February for *P. lividus*) may also be related to
518 the relative stage of maturity in each species. During 2002, the highest GI
519 reported here, and corroborated by the earlier field data (Fig. 2), showed that
520 the maturity stage occurred earlier in *P. miliaris* than in *P. lividus* with the
521 bay of Brest environmental conditions. Some previous studies have shown
522 that echinoid feeding rates decrease before spawning (Fuji, 1967, De Ridder

523 and Lawrence, 1982). The reason for this phenomenon may be
524 physiological or due to the gonad size increase into the coelomic space
525 during the gametogenesis. The first hypothesis is plausible for both species,
526 but the second only concerns P. miliaris, since the P. lividus GI was high in
527 April when feeding activity increased again. In both species, the increase in
528 food consumption was concomitant with a water temperature increase in
529 mid-April, suggesting temperature can control the sea urchin feeding rates
530 also (see review Lares and Mc Clintock, 1991).

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

541

542 4.3 Changes in nutrient absorption rate: connection with the proximate
543 composition of food and gonad

544

545 Absolute changes in absorption rate differed for each nutrient, but the
546 patterns were very similar for both species. The carbohydrates were
547 absorbed uniformly throughout the year, in contrast to the absorption of

548 proteins and lipids, which changed seasonally. The absorption of proteins
549 significantly increased from October to June, and then decreased from June
550 to August when the absorption of lipids increased. These changes in sea
551 urchin nutrient absorption were linked to several factors: the total
552 concentration of the nutrient in the food, the specific composition of lipids,
553 carbohydrates and proteins, the physiological requirements of the sea urchin
554 for a particular nutrient, and the digestive characteristics of the sea urchin,
555 (especially its enzymatic equipment). Without data on changes in specific
556 composition of the nutrients and their digestibility in the sea urchins, this
557 discussion was only based on the relationship between the proximate
558 organic composition of the alga and its absorption by the sea urchins with a
559 particular attention to the gonad production.

560 For the two sea urchin species in our study, carbohydrate absorption was not
561 affected by diet as has been previously described in Watts et al., (1998) for
562 Lytechinus variegatus (L). In our study, the carbohydrate absorption did not
563 vary during the year-long experiment, even though this component
564 increased significantly in Palmaria palmata from October to December. The
565 carbohydrate absorption rate strongly suggest that these sea urchins were
566 efficient in digesting the available carbohydrates. However, overall lower
567 carbohydrate absorption recorded for *P. miliaris* suggest that *P. lividus* has
568 better enzymatic conditions for digesting the insoluble carbohydrate fraction
569 (which can represent about 55% of the dry weight of P. palmata) (Lahaye,
570 1991; Hagen Rødde et al., 2004). Total carbohydrate absorption was
571 probably not affected by physiological demand for reproduction because the
572 maximum need in this component (essentially as glycogen, Monteiro-

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

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

584 The protein level in P. palmata increased from October to February. This
585 increase was followed by the increase of ingested proteins from October to
586 February for P. miliaris and from October to June for P. lividus. In both
587 species, the maximum level of absorbed protein was observed in June. From
588 February, the quantity of absorbed protein was significantly higher in P.
589 miliaris than in P. lividus. These observations attest to a physiological
590 relationship between the increase in the protein absorption and reproduction,
591 the gonad growth phase being earlier in P. miliaris than in P. lividus. Protein
592 is the major component of P. lividus and P. miliaris gonads (Monteiro-
593 Torreiro and Garcia-Martinez, 2003) and the need for this nutrient increases
594 strongly before spawning (Fenaux et al., 1977; Fernandez, 1998, Monteiro-
595 Torreiro and Garcia-Martinez, 2003). In our study, this requirement was
596 highest in February for P. miliaris and in June for P. lividus and would have
597 been supported by the high protein content in P. palmata production during

598 the same period. The protein conversion from ingested food to gonad
599 biomass is known to be rapid (Fernandez, 1996) and suggests that gonadal
600 growth cannot be effective when only protein reserves are available. The
601 organism needs the protein-rich food also.

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

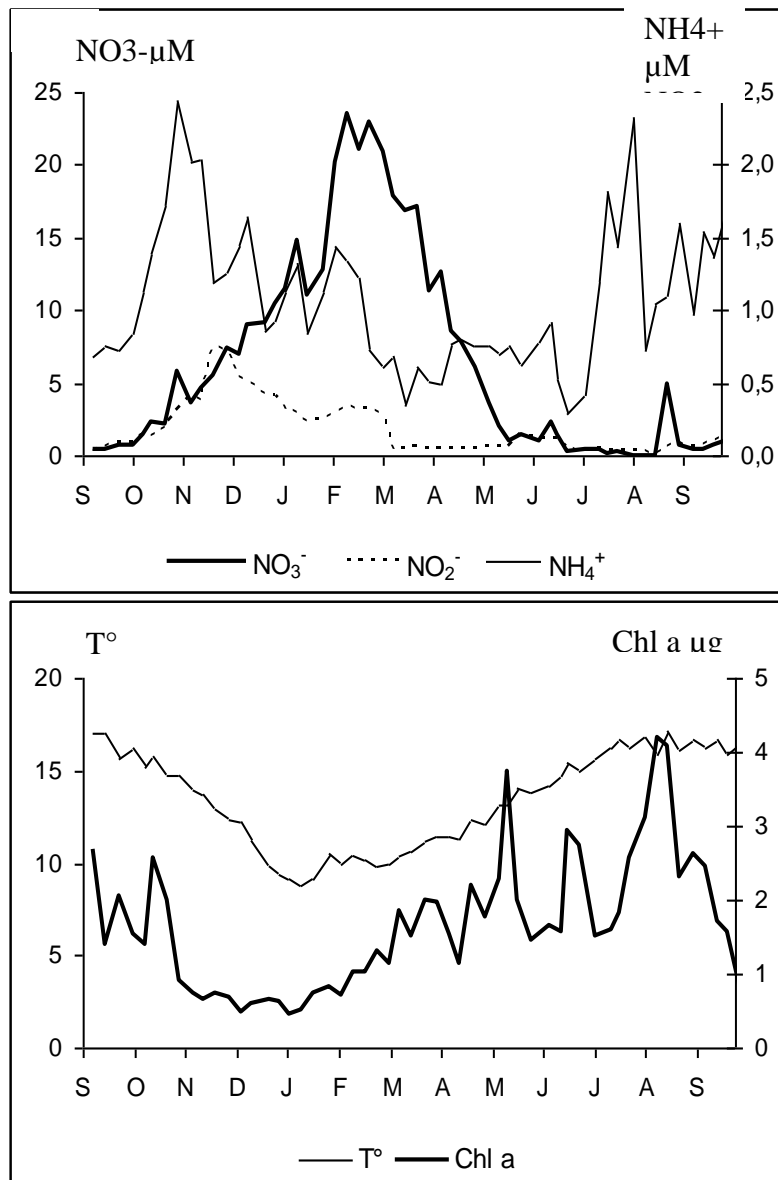
612 Our experimental results showing the stronger preference of P. miliaris for
613 the more protein-rich alga P. palmata (as compared to P. lividus) is
614 consistent with the possibility that P. miliaris has a higher protein
615 requirement. Higher protein ingestion may also explain the higher in situ P.
616 miliaris GI values as compared to those of P. lividus (Le Gall, 1989; Kelly,
617 2000; this study). The enhanced gonad index in P. lividus when fed a
618 monospecific high protein diet suggests that the optimum protein level
619 (Akiyama et al., 2001) to maximize P. lividus gonad production is not
620 reached under natural conditions, compared to P. miliaris. A protein-rich
621 algal diet, atypical for P. lividus, could favour gonad growth in this species,
622 whereas P. miliaris can utilise food of animal origin under natural

623 conditions. The quantity of ingested and absorbed nutriments per urchin per
624 day related to the sea urchin test biomass was higher in P. miliaris than in P.
625 lividus. However, the maximum gonad biomass recorded in 2002 from P.
626 miliaris (0.45g DWW) remained low compared to the maximum gonad
627 biomass from P. lividus (0.70g DWW). In the same way, the conversion
628 efficiency of food to gonadal production at a mature stage (ratio of ingestion
629 rate to gonad growth rate) is better for *P. lividus* than for P. miliaris (20%
630 and 9% respectively). Under echiniculture conditions, gonad production
631 enhancement by protein input from natural food sources is likely to be more
632 productive for P. lividus than P. miliaris.

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635 LEGENDS

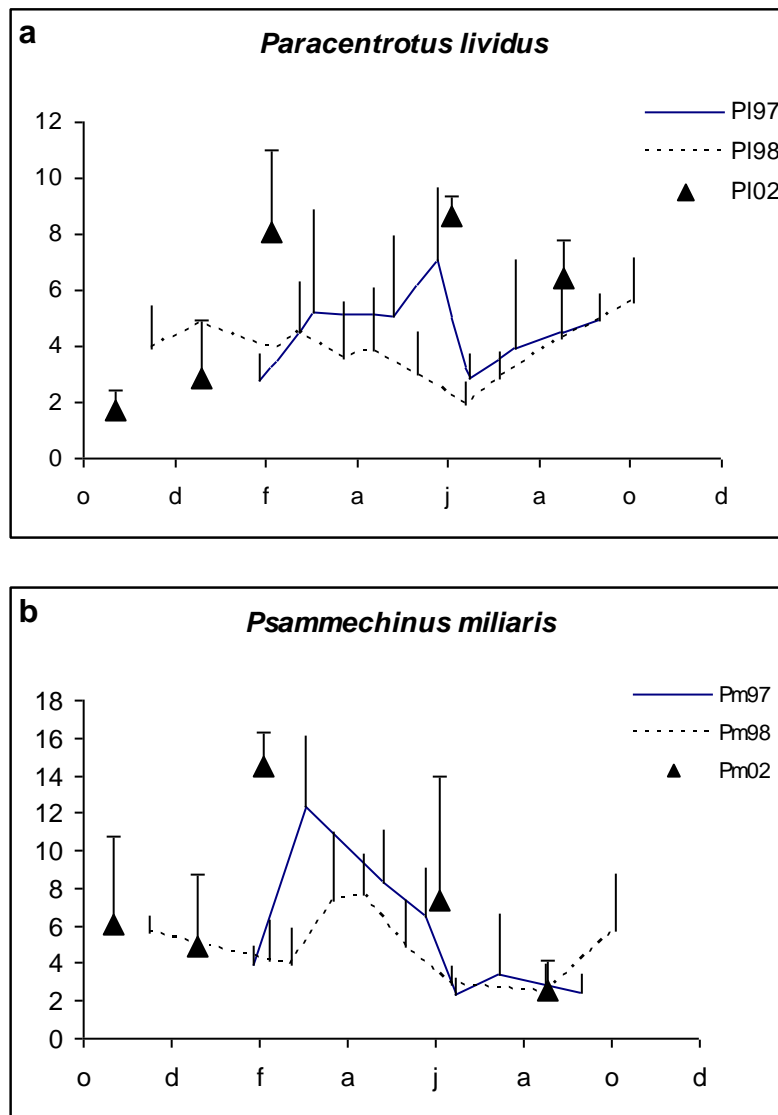


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637 Fig.1. Seasonal changes in the seawater parameters in the Bay of Brest. from

638 September 2001 to October 2002 : a : ammonium, nitrite and nitrate; b :

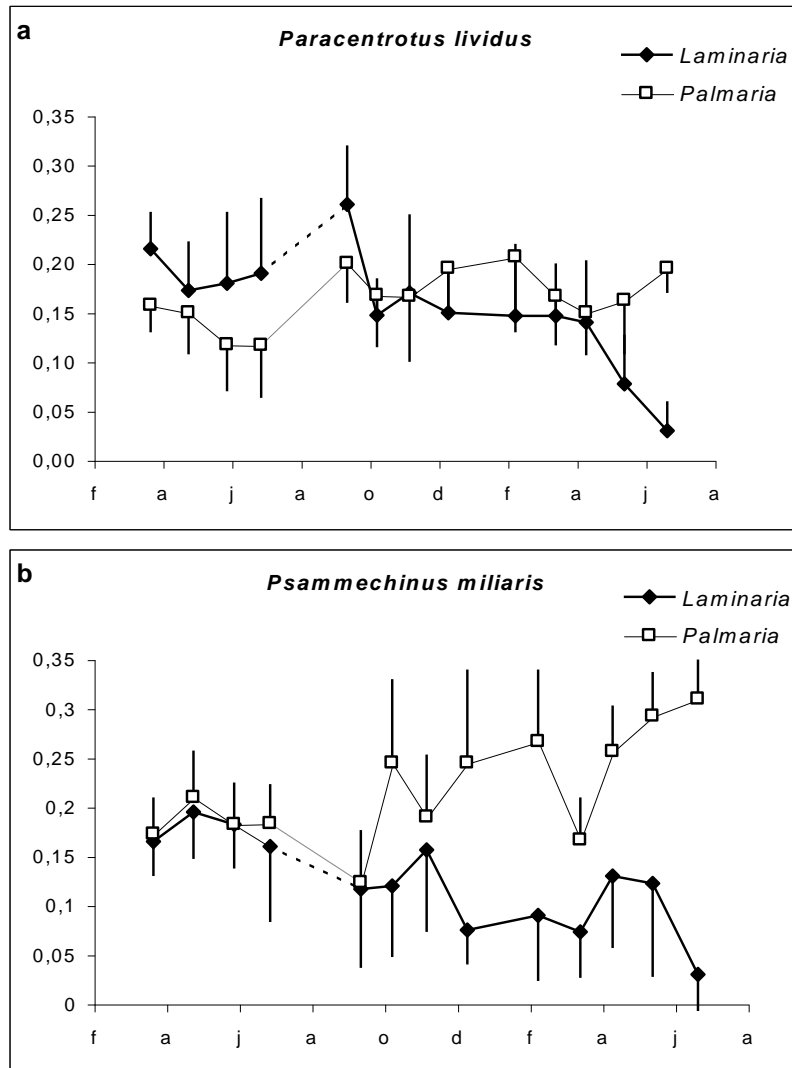
639 temperature and chlorophyll *a*



640

641 Fig. 2 Gonad indices (in % of dry weight) during the experiment (black
 642 triangle +SD) compared to the IG seasonal changes recorded in 1997 and
 643 1998 from *in situ* populations

644



645

646 Fig. 3 Seasonal changes in the biomass of *Laminaria digitata* and *Palmaria*647 *palmata* ingested by the sea urchins (in g WW d⁻¹ urchin⁻¹) (\pm SD) from648 March 2000 to March 2001; a : *Paracentrotus lividus*; b : *Psammechinus*649 *miliaris*.

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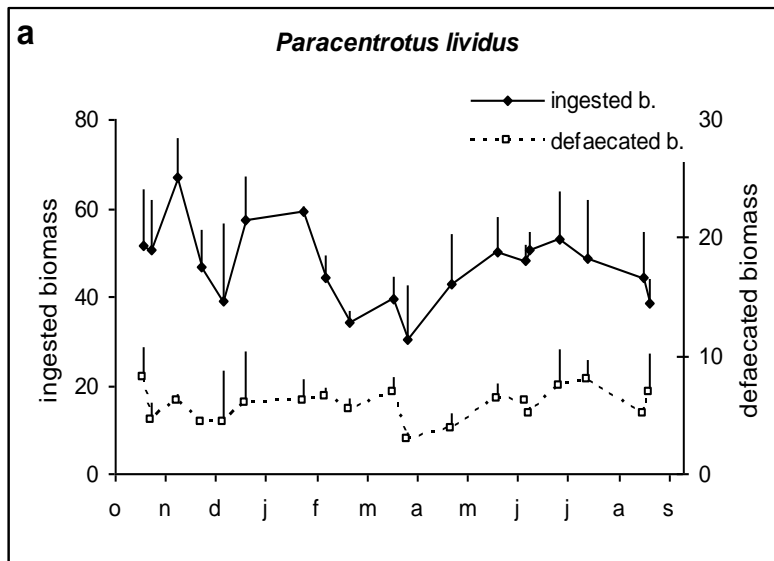
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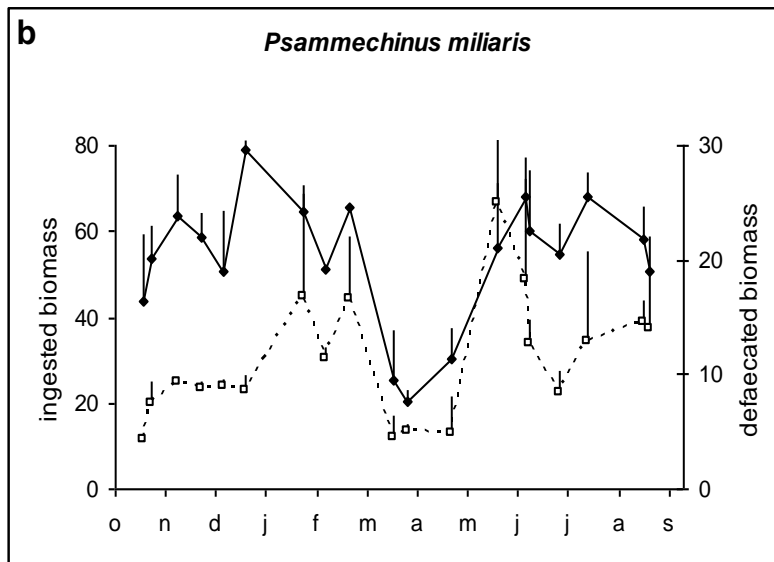
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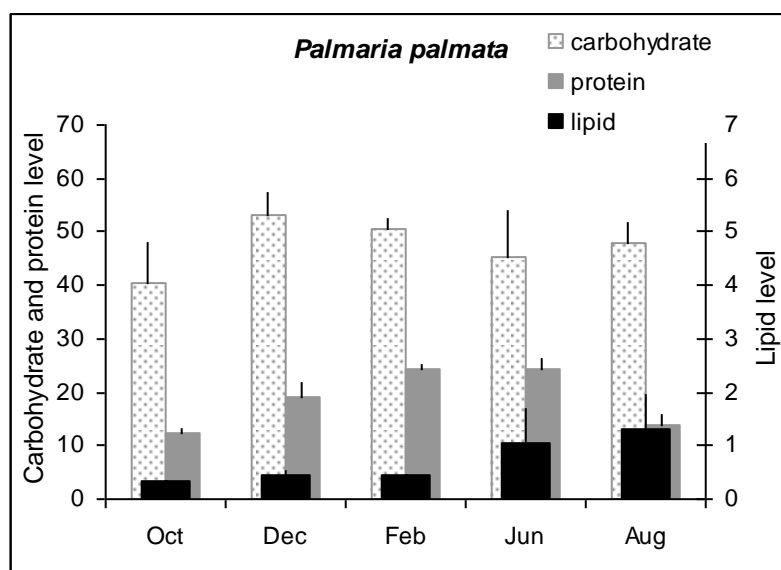
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667 Fig. 4 Seasonal changes in the ingestion and defaecation rate of the sea

668 urchins fed *Palmaria palmata* (in mg DW d⁻¹ urchin⁻¹) (+SD) from October669 2001 to August 2002. a : *Paracentrotus lividus*; b : *Psammechinus miliaris*.

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671

672 Fig. 5 Seasonal changes in the proximate organic composition of Palmaria673 palmata (in % of DW) (+SD)

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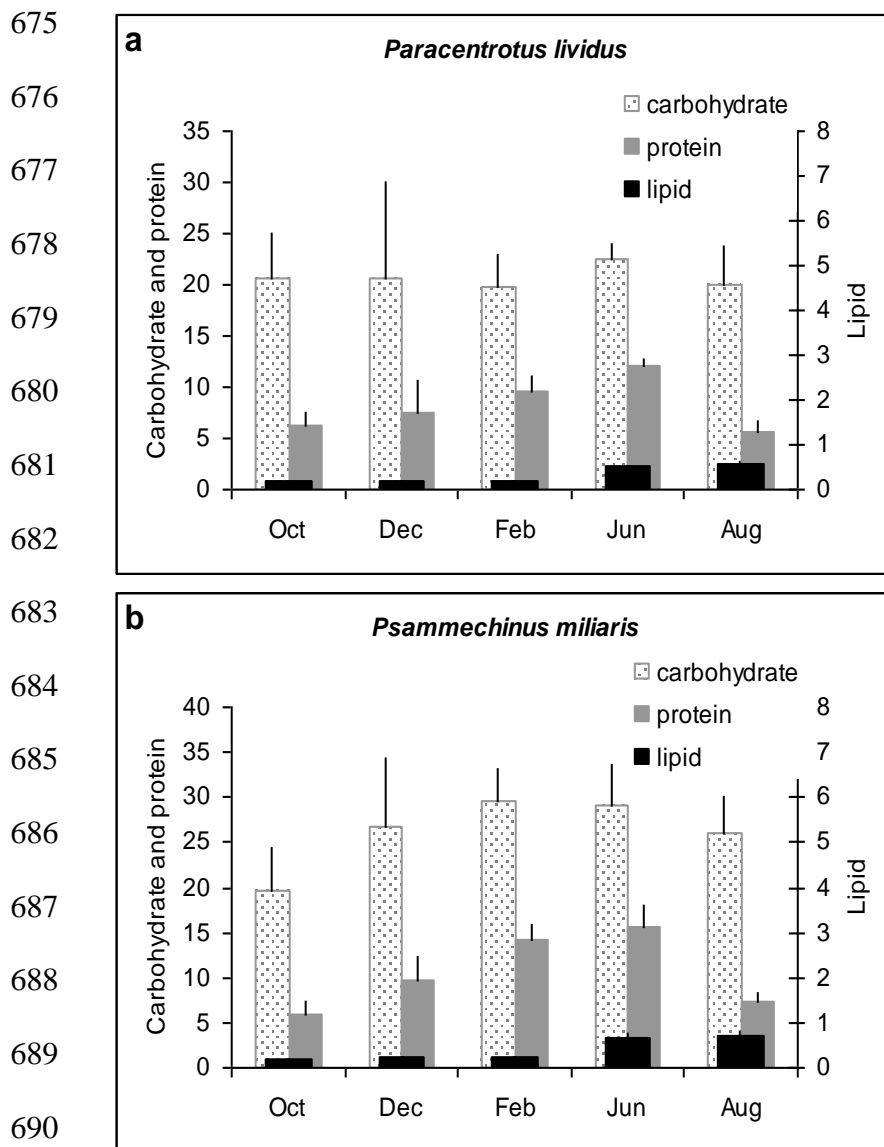


Fig. 6 Seasonal changes in the ingestion rate of the sea urchins fed *Palmaria palmata* in term of proteins, carbohydrates and lipids (in mg DW d⁻¹ urchin⁻¹) (+SD). a : *Paracentrotus lividus*; b : *Psammechinus miliaris*.

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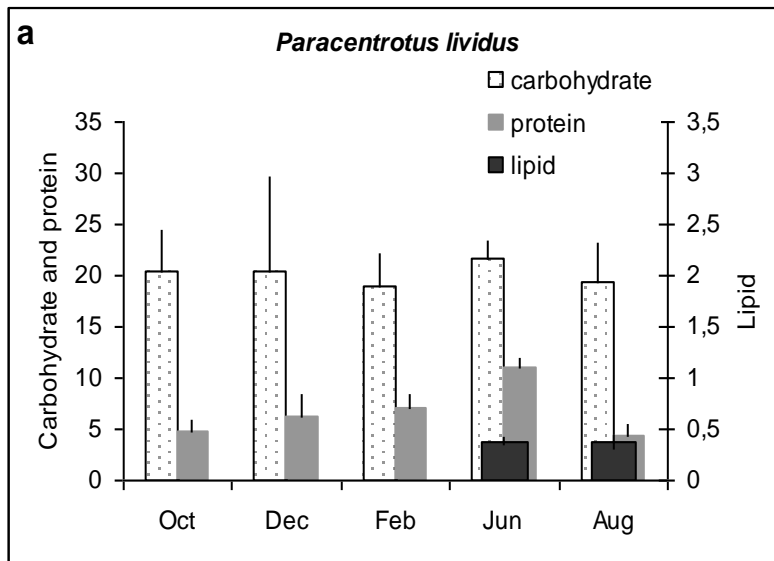
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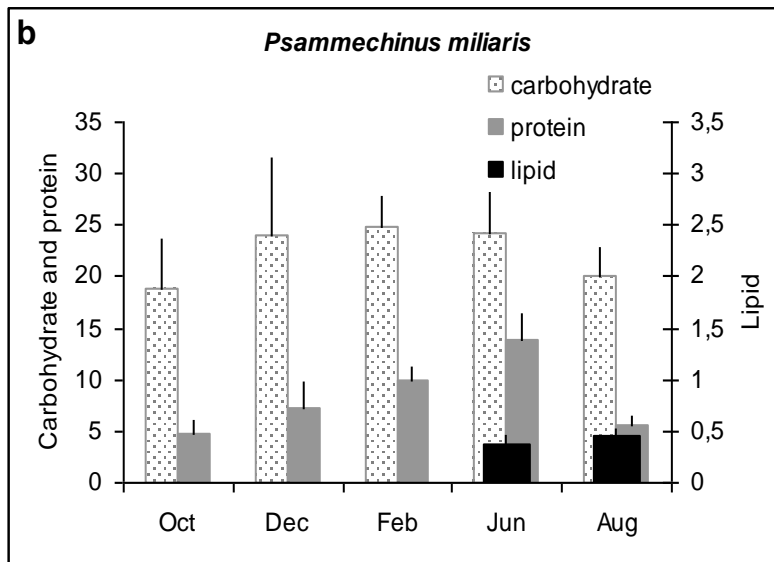
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712 Fig. 7 Seasonal changes in the absorption rate of the sea urchins fed

713 *Palmaria palmata* in term of proteins, carbohydrates and lipids (in mg DW d⁻¹714 urchin⁻¹) (+SD). a : *Paracentrotus lividus*; b : *Psammechinus miliaris*.

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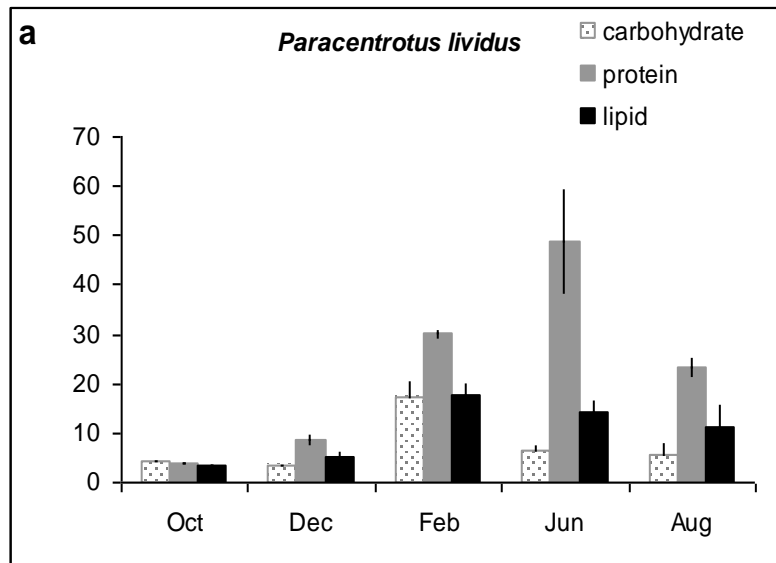
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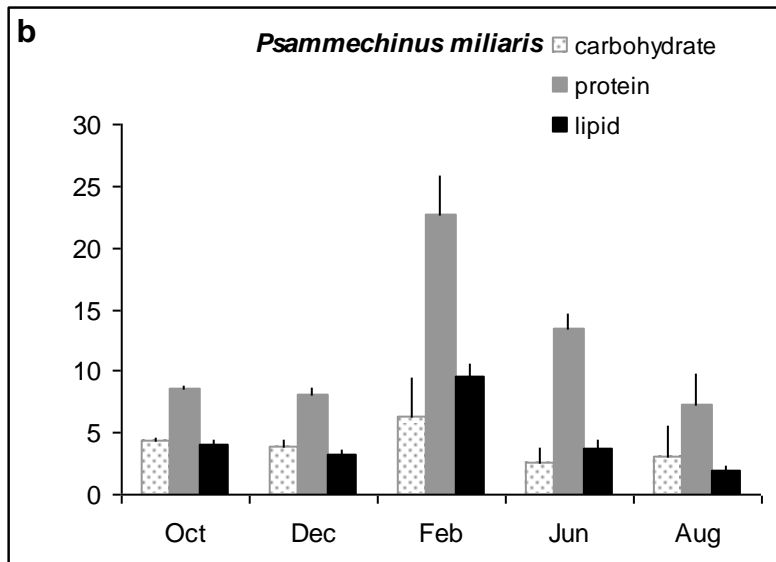
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733 Fig. 8 Seasonal changes in the estimated composition of the gonad of the

734 sea urchins fed *Palmaria palmata* (in mg DW) (+SD). a : *Paracentrotus*735 *lividus*; b : *Psammechinus miliaris*.

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