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Evaluation of dried macrophytes as an alternative diet for the rearing of the sea urchin *Paracentrotus lividus* (Lamarck, 1816).

Running title: Alternative diets for *Paracentrotus lividus*

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Abstract

The aim of this work was to assess the potential use of different dried macroalgae as food in the rearing of *Paracentrotus lividus*. Growth, consumption and food conversions were compared in adult sea urchins fed with fresh or dried thalli of four macroalgae species. Six experimental diets were tested: 1) fresh *Palmaria palmata*, 2) fresh *Saccharina latissima*, 3) dry *P. palmata*, 4) dry *S. latissima*, 5) dry *Laminaria digitata* and 6) dry *Grateloupia turuturu*. No significant differences were found between treatments in terms of linear growth (LGR). Specific growth rate (SGR) was higher in sea urchins fed with fresh *P. palmata*, but no difference was found between animals fed with fresh *S. latissima* and those fed with dried diets. Regarding daily food consumption (DFC), sea urchins consumed the same amount of dried macroalgae as fresh but exhibited a higher food conversion efficiency (FCE) when fed with fresh *P. palmata*. However, this FCE was only significantly higher when compared to sea urchins fed

with dry *L. digitata*. Dried *G. turuturu* is not a suitable diet due to its rapid degradation after rehydration.

The results suggest that *P. lividus* adults can be reared on dried macroalgae thalli without detriment to its somatic growth, especially over short periods. The low cost of feeding sea urchins with this diet could allow small shellfish farmers to diversify their production.

Key words

Sea urchin, nutrition, *Palmaria palmata*, *Saccharina latissima*, *Laminaria digitata*, *Grateloupia turuturu*

1 Introduction

Sea urchin roe is highly regarded as a premium food: 100,000 metric tons of sea urchins are annually fished worldwide with a value of over 0.5 billion euros (FAO, 2016). In Europe, *Paracentrotus lividus* (Lamarck, 1816) is the most commonly consumed sea urchin (Kelly, 2004) and its fishing has a long tradition (Le Gall, 1987). France is the second largest consumer of sea urchins in the world with around 1,000 metric tons per year (Andrew et al., 2002) and the third major importer of sea urchins with around 200 metric tons in 2015 (FAO, 2016). In fact, since the collapse of the French *P. lividus* fisheries, demand has been satisfied by increased Irish and Spanish imports (Fernandez & Pergent, 1998). As a result of its high demand and market value (Kelly, 2004), sea urchin aquaculture has been successfully developed worldwide (Chow, Macchiavello, Cruz, Fonck, & Olivares, 2001; Cook & Kelly, 2009; Liu et al., 2007). However, in France, this activity remains anecdotal probably because farming methods are not economically sustainable for shellfish producers. *P. lividus* is described as a macroalgivore (Lawrence, 2013) preferring different species of macrophytes depending

on its habitat, from Ireland to southern Morocco and throughout the Mediterranean sea (Bayed, Quiniou, Benrha, & Guillou, 2005; Byrne, 1990; Sánchez-España, Martínez-Pita, & García, 2004). The rearing of this species necessitates regular harvesting of fresh macroalgae and their conservation in facilities, which have a relatively high cost. Moreover, fresh macrophytes currently used in echinoculture are not always available to farmers due to their seasonality (Fleurence, 1999; Lüning, 1993; Schiener, Black, Stanley, & Green, 2015) and their conservation is difficult in summer due to their rapid degradation. Although *P. lividus* can adapt to artificial diets (Fernandez & Pergent, 1998), small sized shellfish producers cannot afford the high production costs of formulated feeds.

In order to promote shellfish aquaculture diversification on the northwest French coast with echinoculture, finding alternative sources of food is needed. Dried macroalgae are usually used as attractants in artificial diets (Dworjanyn, Pirozzi, & Liu, 2007; Naidoo, Maneveldt, Ruck, & Bolton, 2006), considering the dietary preference of the reared species for fresh algae (Vadas, Beal, Dowling, & Fegley, 2000; Vadas, 1977). With the aim of reducing costs (economically viable for small enterprises), we carried out sea urchin feeding with dried macroalgae thalli. The objective was to compare growth parameters in *Paracentrotus lividus* when this species is fed with the following local fresh or dry macroalgae species: *Palmaria palmata*, *Saccharina latissima*, *Laminaria digitata* and *Grateloupia turuturu*.

2 Materials and methods

2.1 Sea urchins

The sea urchins were reared in the Benth'Ostrea Prod aquaculture farm (Bouin, Vendée, France). They were obtained following fertilization carried out in April 2015 from a

broodstock harvested in April 2013 in Port Manec'h (47°48'04.0"N 3°44'44.5"W, Brittany, France). The individuals selected were 11 ± 0.25 mm (mean $\pm$ SD) in diameter (excluding spines) and one and a half years old at the beginning of the experiment. Individuals were starved for 2 weeks prior the experiment to standardize their nutritional condition.

2.2 Macrophyte diets

Sea urchins were fed with four species of macroalgae in two different conditions (fresh and dry). Six different feeding treatments were studied: (1) fresh *Palmaria palmata* (diet FP), (2) fresh *Saccharina latissima* (diet FS), (3) dry *P. palmata* (diet DP), (4) dry *S. latissima* (diet DS), (5) dry *Laminaria digitata* (diet DL) and (6) dry *Grateloupia turuturu* (diet DG). These species are some of the dominant macrophytes in the intertidal zone of the study area. Macroalgae were collected every two weeks at low tide from the intertidal zone in Le Croisic (47°16'57.5"N 2°31'25.1"W, Pays de la Loire, France). They were rinsed, cleaned of epibionts and debris and stored in the dark at 8-10 °C in a filtered, oxygenated sea water until their use. Drying was carried out in a greenhouse over 24-48 h at a temperature from 25 to 30 °C.

2.3 Experimental design

The experiment was conducted in the dark, in a recirculating aquaculture system (Fig. 1) composed of two identical 150 L tanks equipped with a biofilter, over a period of 8 weeks. Seawater was aerated and maintained at 20°C, pH 8 and 37 mg/L. A 50% water exchange was carried out weekly.

In order to test the six feeding treatments in triplicate, sea urchins were placed in 18 PVC sieves of 30 cm depth, 20 cm diameter and 2 mm nylon-mesh pore. Twenty sea urchins were randomly distributed in each sieve. Three sieves were then assigned to

each feeding treatment. For each diet type, a control sieve, devoid of sea urchins was placed in the tanks to correct for any variation of the macroalgal biomass. All sieves were randomly distributed in the tanks and their position rotated every day to avoid possible biases due to their location in the water mass.

Feeding, removal of remaining macroalgae and biometrics of sea urchins (i.e. diameter and wet weight; WW) were performed weekly. The sea urchins were fed *ad libitum* with the same amounts of macroalgae (WW). Fresh macroalgae were drained of excess water, wet weighed and supplied to the corresponding batches. Dried macroalgae were first rehydrated in seawater for 1h, drained, wet weighed and distributed. A sample of the same WW as the feeding ration was collected for each treatment. In order to avoid biases associated to macroalgal water content variation, macroalgal biomasses (given, remaining and control) were all estimated by their dry weight (DW) after storage at -20°C and freeze-drying.

2.4 Ingestion and absorption rates

Daily food consumption rates (DFC) and food conversion efficiencies (FCE) were calculated as follows:

$$\text{DFC (g}_{\text{macroalgae}} \text{ g}^{-1} \text{ sea urchin day}^{-1}) = (Fg - Fr - Fd) / (W \times t)$$

Where Fg was the DW (g) of a given macroalgae, Fr was the DW (g) of the remaining macroalgae and Fd was the DW of the macroalgae lost by degradation. W was the WW (g) of the sea urchin and t was the time in days.

$$\text{FCE (g}_{\text{macroalgae}} \text{ g}^{-1} \text{ sea urchin weight gain}) = (Fg - Fr - Fd) / (Wt - Wi)$$

Where Wt was the whole sea urchin WW (g) at time t and Wi was the initial whole sea urchin WW (g).

During the experiment, a high degradation of *G. turuturu* was observed, so its DFC and FCE could not be evaluated.

121 2.5 Biometric parameters

122 Sea urchin diameter of each batch was determined every week by image analysis using
123 ImageJ software (Schindelin et al., 2012). The widest axis of the test (no spines) were
124 measured. Animal batch wet weight was carried out following 5 min drying on
125 absorbent paper.

126 Linear growth rate (LGR) and specific growth rate (SGR) were calculated according to
127 the formulas (Cook & Kelly, 2007; Demetropoulos & Langdon, 2004; García-Bueno et
128 al., 2016):

$$129 \text{ LGR (mm day}^{-1}\text{)} = (L_t - L_i) / t$$

130 where LGR was the sea urchin growth in test diameter, t was the time in days, L_i was
131 the initial test diameter (mm) and L_t the test diameter (mm) at time t .

$$132 \text{ SGR (\% day}^{-1}\text{)} = 100 (\ln W_t - \ln W_i) / t$$

133 where t was the time in days, W_i was the initial whole sea urchin WW (g) and W_t was
134 the whole sea urchin WW (g) at time t .

135 2.6 Biochemical analyses

136 To characterize diet composition, samples of each fresh and rehydrated macroalgae
137 were frozen at -20°C, freeze-dried, pooled by treatment and ground to a fine powder
138 under liquid nitrogen. Aqueous crude extracts (CE) were obtained by homogenization
139 of 1 g of powder in 20 ml of a phosphate buffer solution (20 mM; pH 7) and
140 centrifugation (25 000 g, 20 min, 4 °C). Total water-soluble proteins in CE were
141 analyzed following the bicinchoninic acid (BCA) assay (Smith et al., 1985) and
142 quantified as bovine serum albumin (BSA). Total water-soluble carbohydrates in CE
143 were analyzed according to the method of (Dubois, Gilles, Hamilton, Rebers, & Smith,
144 1956).

145 For total lipid analysis, 1 g of powder was rehydrated in 4 ml of ultrapure water. Lipids
146 were then extracted with a mixture of dichloromethane/methanol (1:1 v/v) in a
147 proportion sample/solvent of 1:5 (v/v) and measured gravimetrically as described by
148 (Bligh & Dyer, 1959).

149 2.7 Statistics

150 Statistical analyses were performed using the SigmaPlot® 9.0 software. Biometrics and
151 growth parameters were compared using one-way ANOVA analysis. When normality or
152 equal variance tests failed, a Kruskal-Wallis one way analysis of variance on ranks was
153 performed. *A posteriori* multiple comparison tests were conducted between diets when
154 significant differences ($P < 0.05$) were found.

155 For each diet, linear regressions were established between sea urchins weight and
156 diameter. Slope and intercept comparisons were carried out between diets.

157 3 Results

158 3.1 Sea urchin growth

159 No mortality was observed in any of the diet treatments. Individual diameters and total
160 biomass were similar at day 0 in all batches (Fig. 2 and Fig. 3). The same pattern was
161 observed for both parameters. Significant differences between treatments were only
162 observed from the 35th day. Only the FP diet showed a significantly higher ($P < 0.001$)
163 response compared to the other diets. There was no significant difference between the
164 response with FS diet and the dry diets. However, biomass obtained with dry diets
165 showed a tendency to separate from those obtained with FS diet (Fig. 3).

166 Somatic growth observed in all treatments showed a highly significant relationship ($P <$
167 0.001) between biomass and diameter (Fig. 4; Table 1). Comparison of slopes showed

168 significantly ($P < 0.05$) higher growth in sea urchins fed with fresh vs dry diets
169 throughout the experiment.

170 No difference for mean LGR was found between the studied treatments (Fig. 5a).

171 Significant differences were observed in mean SGR ($P < 0.05$): FP diet showed a
172 significantly higher value than DP, DL and DG diets, while FS diet showed differences
173 only in comparison to DG diet (Fig. 5b).

174 3.2 *Daily food consumption and food conversion efficiency*

175 Daily food consumption showed a high variability throughout the experiment within
176 and between treatments (Fig. 6). Although not statistically supported, values tended to
177 be higher in the first three weeks.

178 No significant difference was found between sea urchins mean DFC in all treatments
179 (Fig. 5c). For mean FCE, a significant difference was observed only between FP and
180 DL diets (Fig. 5d; $P < 0.05$).

181 3.3 *Biochemical composition of diets*

182 The same pattern was observed for water-soluble protein and carbohydrate contents:
183 very low contents in dry diets and values for FS diet were higher compared to FP (Table
184 2). Regarding dried diets, DP and DG had double protein content compared to DS and
185 DL. Carbohydrate content was twice as high in DG and three times less in DL
186 compared to DP and DS diets.

187 FP and DP diets presented similar lipid contents and were half that of FS, DL and DG
188 diets.

189 **4 Discussion**

190 This study is the first to test dried macroalgae thalli in sea urchin nutrition. Few studies
191 on the use of dried macroalgae to feed abalones (Naidoo et al., 2006) or to constitute
192 formulated diets for fishes (Valente et al., 2006) and sea urchins (Dworjanyn et al.,
193 2007; Jong-Westman, March, & Carefoot, 1995) have been performed.

194 In our experiment, sea urchins consumed the same amounts of dried and fresh
195 macroalgae. The weekly consumption assessment showed a high temporal variability
196 with higher values in the first three weeks. This may be attributed to the two week
197 starvation period prior to the experiment, indicating the ability to consume more food
198 when it became available (Bandolon, 2014; Cook & Kelly, 2007). Along the duration of
199 the experiment, consumption was, on average, similar in all treatments despite clear
200 differences in the protein and carbohydrate contents between fresh and dried thalli due
201 to rehydration of the latter. This is contradictory with previous works which found that
202 consumption was negatively correlated to the protein content (Cook & Kelly, 2007;
203 Fernandez & Boudouresque, 2000; Frantzis & Grémare, 1992). As the lipid content was
204 similar for all the diets, we hypothesize that this is also a factor regulating consumption.
205 Moreover, despite the loss of water-soluble compounds in dried diets, higher growth
206 were observed for all the experimental diets compared to previous studies (Cook &
207 Kelly, 2007; Fernandez & Pergent, 1998; Frantzis & Grémare, 1992; McCarron,
208 Burnell, & Mouzakitis, 2009). In Cook & Kelly (2007), the difference could be
209 explained by their use of older and larger animals, according to allometry relationships.
210 In the other studies, using animals of comparable size to ours, the use of lower
211 nutritional value diets could be responsible of their lower growth rates. This indicates
212 the great potential of natural diets composed of *P. palmata*, *S. latissima*, *L. digitata* and

213 *G. turuturu* compared to artificial food (Fernandez & Pergent, 1998) and other
214 macrophytes (Frantzis & Grémare, 1992; McCarron et al., 2009).
215 Fresh macrophytes, specifically *P. palmata*, allowed the best growth performance, as
216 illustrated by the biometric and SGR assessment. Their biochemical composition was in
217 accordance with that previously published (García-Bueno, 2015; Harnedy & FitzGerald,
218 2013; Jard et al., 2013; Schiener et al., 2015) and are known to best fit *P. lividus*
219 nutritional requirements (Lawrence, 2013; Vadas et al., 2000).
220 Sea urchins fed with dried diets presented similar diameters and biomass to those fed
221 with fresh *S. latissima*. They also showed a mean LGR similar to sea urchins fed with
222 fresh *P. palmata*. Conversion efficiencies were the same in both dried (except for *L.*
223 *digitata*) and fresh diets. These results suggest that all the experimental diets could be
224 used without detriment to somatic growth. They also indicate that their lipid content
225 remained high enough after rehydration to promote growth.
226 Concerning *G. turuturu*, (García-Bueno et al., 2016) highlighted the difficulties in
227 using fresh *G. turuturu* thalli in abalone aquaculture and concluded that it might be
228 more appropriate to use in dry or powder form. In the present study, we could not
229 evaluate the use of dried *G. turuturu* due to its high degradation, making its sampling
230 and analysis impossible. However, it would be interesting, given its availability, to
231 valorize this invasive algae (Gavio & Fredericq, 2002) in the aquaculture industry. Its
232 use as an ingredient for artificial foods has not yet been investigated.

233 **5 Conclusion**

234 We confirmed the suitability of *P. palmata* and fresh diets for *P. lividus* nutrition, but
235 without denying the great growth performances of sea urchins when these diets are
236 replaced with dried macroalgae. These feeds could therefore be used by farmers without

detriment to production, especially during short periods when, for example, the algal biomass is less abundant.

Further investigations could be done on the effect of these diets on the sea urchin gonad enhancement and quality. However, for a fattening phase of the production cycle, the lower cost of feeding with natural dried algae compared to the cost of formulated diets could allow small producers to diversify their activities.

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Table 1 Equations describing relationships between weight and diameter of sea urchins *P. lividus* fed different experimental diets. FP = fresh *P. palmata*; FS = fresh *S. latissima*; DP = dry *P. palmata*; DS = dry *S. latissima*; DL = dry *L. digitata*; DG = dry *G. turuturu*.

	Equation	R ²	F	P
FP diet	y = 0.21x – 1.78	0.9911	671	< 0.001
FS diet	y = 0.23x – 2.01	0.9874	472	< 0.001
DP diet	y = 0.19x – 1.50	0.9873	470	< 0.001
DS diet	y = 0.19x – 1.47	0.9962	1615	< 0.001
DL diet	y = 0.19x – 1.51	0.9918	729	< 0.001
DG diet	y = 0.17x – 1.29	0.9892	550	< 0.001

Table 2 Biochemical composition (% DW) of the six experimental diets for sea urchins. FP = fresh *P. palmata*; FS = fresh *S. latissima*; DP = dry *P. palmata*; DS = dry *S. latissima*; DL = dry *L. digitata*; DG = dry *G. turuturu*.

	FP	FS	DP	DS	DL	DG
Proteins	4.12	7.82	0.65	0.27	0.31	0.73
Carbohydrates	6.80	10.29	0.78	0.71	0.25	1.35
Lipids	1.77	3.36	1.69	2.22	3.53	3.19

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378 **Fig. 1** Experimental design. Dotted lines represent control sieves devoid of sea urchins.
 379 Red arrows indicate the direction of the sieves rotation. FP = fresh *P. palmata*, DP = dry
 380 *P. palmata*, DG = dry *G. turuturu*, FS = fresh *S. latissima*, DS = dry *S. latissima*, DL =
 381 dry *L. digitata*

382 **Fig. 2** Mean diameter of sea urchins *P. lividus* fed with six different fresh or dry
 383 macroalgae diets. FP = fresh *P. palmata*; FS = fresh *S. latissima*; DP = dry *P. palmata*;
 384 DS = dry *S. latissima*; DL = dry *L. digitata*; DG = dry *G. turuturu*. Error bars represent
 385 confidence intervals (95%).

386 **Fig. 3** Mean biomass of sea urchins *P. lividus* batches fed with six different fresh or dry
 387 macroalgae diets. FP = fresh *P. palmata*; FS = fresh *S. latissima*; DP = dry *P. palmata*;
 388 DS = dry *S. latissima*; DL = dry *L. digitata*; DG = dry *G. turuturu*. Error bars represent
 389 confidence intervals (95%).

390 **Fig. 4** Sea urchins *P. lividus* biomass/diameter relationship depending on the type of
 391 experimental diet. FP = fresh *P. palmata*; FS = fresh *S. latissima*; DP = dry *P. palmata*;
 392 DS = dry *S. latissima*; DL = dry *L. digitata*; DG = dry *G. turuturu*.

393 **Fig. 5** Mean (a) linear growth rate, (b) specific growth rate, (c) daily food consumption
 394 and (d) food conversion efficiency of sea urchins *P. lividus* fed with different diets
 395 throughout the experiment. FP = fresh *P. palmata*; FS = fresh *S. latissima*; DP = dry *P.*
 396 *palmata*; DS = dry *S. latissima*; DL = dry *L. digitata*; DG = dry *G. turuturu*. Error bars
 397 represent confidence intervals (95%).

398 **Fig. 6** Daily food consumption of sea urchins *P. lividus* fed with six different
 399 experimental diets throughout the experiment. FP = fresh *P. palmata*; FS = fresh *S.*

400 *latissima*; DP = dry *P. palmata*; DS = dry *S. latissima*; DL = dry *L. digitata*. Error bars
401 represent confidence intervals (95%).

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For Review Only

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3 **Fig. 1**

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6 **Fig. 2**

Fig. 3

Fig. 4

Fig. 5

Fig. 6