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1 **Impact of nine macroalgal diets on growth and initial reproductive**
2 **investment in juvenile abalone *Haliotis tuberculata***

3

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16

17

18 **Abstract**

19 The commercial culture of *Haliotis tuberculata* has recently started in Europe. As abalone is
20 herbivorous, the use of local collected algae as feed may appear advantageous. The nutritional
21 value of eight monospecific seaweed diets was studied using *Palmaria palmata*
22 (Rhodophyta), filamentous algae, mainly *Gracilaria* sp. (Rhodophyta), *Enteromorpha* sp. and
23 *Ulva lactuca* (Chlorophyta), together with *Saccharina latissima*, *Saccorhiza polyschides*,
24 *Laminaria digitata* and *Laminaria hyperborea* (Ochrophyta, Phaeophyceae) and a mixed
25 macroalgal diet. An integrative approach consisted in monitoring the seasonal composition
26 changes of these algae in terms of protein, lipid, soluble carbohydrate, fatty acid and amino-
27 acid contents, and to relate it to seasonal growth and reproduction investment during a large-
28 scale experiment. Abalone and algae were studied for one year in commercial sea-cage
29 structures. Abalone fed with monospecific diet using either *P. palmata* or *S. latissima*, and
30 with mixed diet presented the best growth rate, muscle ratio and gonad development. Seasonal
31 daily weight gain was mainly associated with n-3/n-6 ratio, soluble carbohydrate content and
32 total protein content. In term of amino-acid contents, the daily weight gain was associated
33 with free phenylalanine as well as isoleucine levels. Moreover, 90% of 2-years old abalone
34 started gonad development but less than a quarter featured a fully matured gonad. The gonad
35 development of *H. tuberculata* was mostly associated to total valine, methionine, leucine,
36 arginine and isoleucine levels. The age of initial sexual maturity in *H. tuberculata* turned to be
37 a highly plastic trait in response to different growth rates and algal diets. Even if *P. palmata* is
38 the best option for growth performance, mixed diets should probably be preferred to a
39 monospecific diet in order to avoid too high pressure on a single algal resource.

40 Keywords: abalone; algae; reproduction; growth; protein

42 1. Introduction

43

44 Abalone farming in Europe is a small growing industry (Cook, 2014). The main advantages of
45 an ecological and profitable abalone aquaculture business are the minimization of effluents
46 and the use of a local and cheap sustainable resource as feed (Troell et al., 2006). In this
47 context, the use of algal diet to feed the local species, *Haliotis tuberculata*, may appear
48 advantageous. In addition, abalone health and condition can be improved when fed
49 macroalgal diet (Dang et al., 2011; Stone et al., 2014). Nevertheless, feeding abalone with
50 locally collected algae also presents many constraints. The main issues are about availability,
51 cost and nutritional value of feed that will allow good growth rates and survival. Costs of
52 seaweeds can vary depending on the cultivation or harvest techniques, species choice and
53 environmental factors (Kirkendale et al., 2010). A huge variability of feeding costs using
54 algae were reported, representing approximately 14% of the production costs for a land-based
55 cultivation system of Australian abalone (Kirkendale et al., 2010) and up to 60% for *Haliotis*
56 *tuberculata* farmed in sea-rearing systems (www.sudevab.com consulted 29/03/2012). When
57 comparing cost and growth between a *P. palmata* diet, which gives the best growth
58 performance for *H. tuberculata* (Mai et al., 1996), and a formulated diet, similar results were
59 found (Basuyaux, 2000). The availability of wild harvested macroalgae is a major issue for
60 the development of aquaculture production (Troell et al., 2006). France has a high potential
61 for macroalgal harvesting (Alban et al., 2011) with at least 20 species gathered, most of them
62 coming from Brittany (Mesnildrey et al., 2012). *Palmaria palmata* is characterized by a high
63 market price in Brittany (0.4 up to 2 € / kg fresh weight, Basuyaux et al. 2018, Huchette, pers.
64 comm.). The most harvested species is *Laminaria digitata* with more than 40 000 tons
65 harvested annually (0.04 € / kg up to 0.5 € /kg fresh weight, Basuyaux et al. 2018, Huchette,
66 pers. comm.), followed by *L. hyperborea* reaching 26 000 tons. In contrast, less than 300 tons

67 of species such as *P. palmata* or *Ulva* species are harvested annually. Most of the algae
68 harvested in France are used for food processing industry, chemistry and microbiology while
69 less than 25% of seaweed is used in agricultural, health and well-being sectors (Mesnildrey et
70 al., 2012).

71

72 In order to avoid the use of a single algal species by the industry, which would represent a
73 danger for the sustainability of this species, alternating feeding with various species should be
74 instituted if the European abalone industry wants to sustain its durability. In order to optimise
75 feeding, algae need to be chemically characterised, their biochemical composition being
76 highly species- (Mercer et al., 1993; Mai et al., 1994; Mai et al., 1995; Jung et al., 2013),
77 seasonal- and site- (Renaud and Luong-Van, 2006; Villares et al., 2013; Schmid et al., 2014)
78 dependent. Red algae, such as *P. palmata* and *Gracilaria* spp. are known to contain a
79 relatively large proportion of protein with a year average of 18.3 % (Jacquin et al., 2006). A
80 30 % total protein content was reported in *G. bursa-pastoris* (Valente et al., 2006) and *G.*
81 *cornea* cultivated in tanks enriched with fertiliser. Green species belonging to *Ulva* spp and
82 *Enteromorpha* spp. monitored in Japan contain respectively 26.1% and 19.5% of proteins,
83 0.7% and 0.3% of lipids and 46.1% and 58.1% of carbohydrates (Nisizawa et al., 1987). In
84 Brittany, the protein content of *P. palmata* varied from 9.7 % to 25.5 % of dry weight
85 depending of the season : the protein content was reported to be lower during summer and
86 autumn (12 – 15 %) and higher during winter and spring (22 – 25 %) (Galland-Irmouli et al.,
87 1999; Jacquin et al., 2006). In Ireland, a protein content of 13% was reported in winter and
88 spring in *Ulva lactuca* (Mercer et al., 1993). In this context, spatial and seasonal variations in
89 the biochemical compositions of specific algal species available to this industry need to be
90 studied in relation to *H. tuberculata* needs.

91

92 Only a few studies directly compared the effects of different macroalgal diets on growth
93 focusing on their seasonal composition changes on the long term (Basuyaux et al., 2018) and
94 on a commercial scale (Nelson et al., 2002). The first objective of this study was to determine
95 the nutritional value of eight monospecific seaweed diets and their respective impact on the
96 growth and reproduction of *H. tuberculata*, reared in large sea-based growing structures
97 during one year. The second objective was to evaluate the seasonal variation of algal
98 composition. The third objective was to determine the role of each major algal component in
99 relation to the seasonal growth and reproduction of farmed abalone. Because the literature
100 consistently demonstrates that growth is improved when abalone are fed with a combination
101 of macroalgal species in preference to a single species (Stuart and Brown, 1994; Simpson and
102 Cook, 1998; Qi et al., 2010a; Viera et al., 2011), a mixed diet based on algae easily collected
103 on the shore at each specific season was also studied.

104

105 **2. Materials and Methods**

106 **2.1. Animals**

107 This study was located downstream from the Aber Wrac'h river (48°36'46N; 4°33'30W),
108 Brittany, France between the beginning of February 2012 and the end of January 2013.
109 Abalone were born in summer 2010 at the local hatchery France Haliotis (Plouguerneau,
110 France). After one year spent in the land-based nursery tanks, juveniles were transferred to the
111 sea in pre-growing compartments. During this stage, they were fed a mixed algal diet based
112 essentially on *P. palmata*, *S. latissima* and *L. digitata* and received more occasionally smaller
113 quantity of the other experimental algal diets. In February 2012 (t_0), abalone were placed in 9
114 abblox farming sea cages. Each cage was made of 4 compartments of 1 m³ (1 m x 1 m x 1 m)
115 with a squared mesh size of 5 mm, each compartment containing 1000 individuals. Initial

116 abalone length ($L_{t0} = 24 \pm 0.15$ mm; mean \pm s.e.) and weight ($W_{t0} = 2.2 \pm 0.041$ g; mean \pm
117 s.e.) were balanced between the treatments at the beginning of the experiment (Table 1).
118 Eighteen rows of 30 circular black plastic oyster-seed collectors of 140 mm diameter were
119 placed into the compartments as shelter for daytime. Each compartment was identified with a
120 tag attached inside the compartment enabling to identify the seaweed diet and compartment
121 number. The cages were moored on a long-line used for anchorage.

122

123

124 **2.2. Experimental design**

125 Abalone fed on monospecific diets of eight algal species. Among the large diversity of
126 macroalgae in Brittany, we selected *Palmaria palmata* (**P**) and filamentous algae, mainly
127 *Gracilaria* sp. (**G**) (Rhodophyta), *Enteromorpha* sp. (**E**) and *Ulva lactuca* (**U**) (Chlorophyta),
128 *Saccharina latissima* (**S**), *Saccorhiza polyschides* (**B**), *Laminaria digitata* (**L**) and *Laminaria*
129 *hyperborea* (**D**) (Ochrophyta Phaeophyceae). A ninth treatment (**M**: mixed diet) corresponded
130 to the algae usually distributed to abalone in rearing facilities with different proportions
131 according to the seasonal availability on the coast (yearly average distribution in wet weight;
132 B: 15.5 %; D: 20.5%; P: 43%; S: 8%; U: 12%). Abalone were fed *ad libitum* at spring tide
133 every month with algae harvested on the coast of Plouguerneau maximum 24 hours before
134 distribution. The quantity of algae was adjusted each month in order to reach *ad libitum* algal
135 distribution. The goal was to fill the compartment with algae in excess so that not all were
136 eaten by the end of the month (at least 5% refusal). The quantity distributed ranged from 10
137 kg up to 25 kg according to the algae and the season. Each treatment was replicated three
138 times, each replicate being randomly placed in one of the nine abblox (Figure 1). An
139 additional compartment was filled only with algae in order to monitor *in situ* algal

140 degradation. Due to the difficulty to harvest *Sacchoriza polyschides* and *Enteromorpha* sp. on
141 the coast at the end of autumn period, the B and E treatments were stopped in October.

142 Multivariate integrative analysis of abalone seasonal growth and reproduction status was
143 associated to detailed seasonal biochemical composition of algal diets, based on lipid content
144 and fatty acid composition, soluble carbohydrate, protein content and amino-acid
145 composition.

146

147 **2.3. Abalone measurements**

148 2.3.1. Mortality

149 Empty shells were collected before each feeding. Mortality (%) after 11 months of diet was
150 calculated as:

$$Mortality = (\text{total number of dead abalone} / \text{initial number of abalone}) * 100$$

151

152 2.3.2. Growth

153 At the beginning of February 2012 (t_0), April (t_2), June (t_4), August (t_6), the end of October
154 (t_8) and the end of January 2013 (t_{11}), 30 individuals from each compartment, i.e. 90
155 individuals per treatment, were randomly collected (the 2nd nearest neighbour to the first
156 sighted chosen randomly) and brought back to France Haliotis farm. After pressing the
157 abalone in absorbent paper to remove water from the pallial cavity, abalone were weighed to
158 the nearest 0.01 g using a balance (Toploader balance, Kern) (W_{t_0} , W_{t_2} , W_{t_4} , W_{t_6} , W_{t_8} , $W_{t_{11}}$)
159 and shell length was measured to the nearest 0.5 mm using a Vernier calliper (L_{t_0} , L_{t_2} , L_{t_4} ,
160 L_{t_6} , L_{t_8} , $L_{t_{11}}$). After sampling, abalone individuals were removed from the experiment.

161

162 The following growth indices were calculated:

163 Final weight-to-shell length ratio (W/L_{t11} , $\text{g}\cdot\text{mm}^{-1}$) = W_{t11}/L_{t11}

164 Final daily weight gain (DGW_{t0-11} in $\text{mg}\cdot\text{day}^{-1}$) = $(W_{t11} - W_{t0})/\text{day}$

165 Final daily length gain (DGL_{t0-11} in $\mu\text{m}\cdot\text{day}^{-1}$) = $(L_{t11} - L_{t0})/\text{day}$

166 Specific growth rate (SGR_w , $\% \cdot \text{day}^{-1}$) = $100 \times ((\ln W_{t11} - \ln W_{t0})/\text{day})$

167 With W_{t0} : mean initial wet weight, W_{t11} : mean final wet weight, and day: days between initial
168 and final measures

169

170 In addition, intermediate daily weight gain (DGW) and daily length gain (DLG) were
171 calculated for each period (t_{0-2} , t_{2-4} , t_{4-6} , t_{6-8} , t_{8-11}).

172

173 The final weight-to-shell length ratio (W/L) was calculated in order to give an indication of
174 the flesh volume per unit shell length growth as recommended by Naidoo et al. (2006). The
175 value of an abalone priced by weight will depend on this ratio. In addition, this is an indicator
176 of transformation rate if abalone individuals are sold eviscerated. Growth data were averaged
177 per replicate (compartment) for analysis.

178

179 2.3.3. Gonad development

180 Twelve individuals per replicate were randomly sampled in July 2012 ($n = 36$ individuals per
181 treatment). This corresponds to the beginning of the spawning period in *H. tuberculata*
182 (Girard, 1972). For each individual, the muscle was separated from the gonad-digestive gland
183 (GDG comprising crop, stomach, spiral cecum and gonad) and from the rest of the soft tissues
184 (head, gills, heart, mantle, hypobranchial glands, anus, and intestine) by the same
185 experimenter to reduce dissection variability. Total soft tissue (weighed before muscle
186 separation), muscle, GDG, the rest of the soft tissues and shell were weighed to the nearest

187 0.01 g, just after dissection. Shell length was measured to the nearest 0.5 mm. Reproduction
188 data were averaged per replicate (compartment) for analysis.

189

190 A visual gonad index (*VGI*) ranked from 0 to 10 based on the progression of the early stages
191 of gonad development was directly scored after dissection. Photographs were taken in order to
192 verify direct observation afterward. The scores were adapted from the criteria defined by
193 McAveney et al. (2004). This fine scale *VGI* allows good estimates of the error variance in
194 the data and is adapted to distinguish between samples at the early stages of gonad
195 development. Because of the extensive reticulation with the gut and small size of the *GDG*,
196 the gonads could not be separated from the gut during dissection. In order to determine the
197 relative proportion of gonads from the conical appendage, the *GDG* was sectioned across the
198 midpoint of the conical appendage. The samples were fixed in Davidson solution for 24 h and
199 then kept in 70 % alcohol. Thereafter, samples were dehydrated using several baths of 95 %
200 and 100 % alcohol, and Claral® before being embedded in paraffin for histology. Scans were
201 done of the gonadal sections using a desktop scanner. The numbers of pixels in the total area
202 of section and the gonadal area were determined using ImageJ 1.45s (Wayne Rasband
203 National Institutes of Health, USA).

204 The gonadal index (*GI*) was calculated, adapted from Shepherd and Laws (1974), as followed:

$$GI = \text{number of pixels for the gonadal area} / \text{total number of pixels of the section}$$

205

206

207 In order to study the relative organ development of abalone from each diet, the following
208 variables were related to wet weight, with respect to the total weight (T_w = total soft tissue
209 weight + shell weight):

$$GDGr = (GDG \text{ weight} / T_w) * 100$$

$$Mr = (\text{muscle weight} / Tw) * 100$$

$$Sr = (\text{shell weight} / Tw) * 100$$

210

211 **2.4. Algal measurements**

212

213 2.4.1. Algal degradation in sea-cage structure

214 Every month, algae left in the additional compartment without abalone was collected and
215 weighed back on land with a 0.02 kg precision scale after draining of 1 hour. The degradation
216 (*Pdegraded*) corresponded to the proportion of algae left in the compartment without abalone
217 after one month. It was calculated as: $((Q_{\text{dist}} - Q_{\text{coll}}) / Q_{\text{dist}}) * 100$ with Q_{dist} : total quantity of
218 seaweed distributed and Q_{coll} : quantity of seaweed after one month in the cage without
219 abalone. *Pdegraded* was analysed for 5 periods: t_{0-2} , t_{2-4} , t_{4-6} , t_{6-8} , t_{8-11} .

220

221 2.4.2. Biochemical analysis

222 For biochemical analyses, 100 g of freshly harvested macroalgae of each treatment were
223 sampled in triplicate. Algae were immediately frozen and stored in a freezer at -20°C before
224 freeze-drying at -55°C during 96 hours. The dry matter content of each species (*drymatter*)
225 was determined weighting the samples before and after freeze-drying. The dry seaweeds were
226 then ground to pieces of about 0.5 mm with a hammer mill. For lipid analysis, algae were
227 immediately frozen with liquid nitrogen and stored in a freezer at -80°C before grinding.

228 2.4.3. Soluble carbohydrate analyses

229 Samples were analysed at t_0 , t_2 , t_4 , t_6 and t_8 . Soluble carbohydrate contents (potentially
230 available, *carb*) were determined by an adaptation of the phenol sulphuric acid colorimetric
231 method of Dubois et al. (1956). This method is based on the reduction of neutral sugars, and a

232 little part of uronic acids, in 5-hydroxymethylfurfural by phenol, giving a characteristic
233 yellow colour. Absorbencies were measured at 492 nm with a microplate photometer
234 (MultiskanTM FC, Thermo Scientific) and compared to glucose standard curve. Titrations were
235 done in triplicate and averaged for each sample.

236

237 2.4.4. Lipid analyses

238 Due to practical constraints, samples were only analysed at t_2 . Lipid extraction was conducted
239 on 150 to 200 mg algal powder according to Folch et al.'s method (1957) in 6 mL of a
240 chloroform/methanol mixture (2/1, v/v). Lipid extracts were then flushed with nitrogen and
241 stored at $-20\text{ }^{\circ}\text{C}$ before analysis. Fatty acids (FA) were analysed as fatty acid methyl esters
242 (FAME) after total lipid transesterification. Briefly, after addition of tricosanoic acid (23:0) as
243 an internal standard and evaporation to dryness under nitrogen, 800 μL of MeOH/BF_3 (14 %
244 by weight) was added and the transesterification reaction occurred during 10 min at 100°C , as
245 described by Le Grand et al. (2014). Then, 0.8 mL of hexane was added and the organic phase
246 containing FAME was washed three times with 1.5 mL of hexane-saturated distilled water.
247 The organic phase was finally recovered for HPLC purification to isolate FAME. FAME were
248 then analysed by a gas chromatograph (GC) VARIAN CP 3800 equipped with two automatic
249 on column injectors and two flame ionization detectors (FID). FAME were separated using
250 both polar (CPWAX 52 CB—30 m 9 0.25 mm i.d.; 0.25 μm thickness, Varian) and non-polar
251 (CP-Sil 8 CB—30 m 9 0.25 mm i.d.; 0.25 μm thickness, Varian) capillary columns. Combined
252 with the use of commercial and home-made analytical standards, this allowed to separate and
253 identify FAME. The quantity of each fatty acid was quantified on a proportional basis relative
254 to the peak area of the C23:0 internal standard. Quantitative fatty acid spectra obtained by GC
255 were used to calculate the molar content of each fatty acid in the samples. Depending on the
256 number of double bonds they displayed, FA were classified in three groups: saturated FA

257 (SFA, no insaturation), monounsaturated (MUFA, only one insaturation) and polyunsaturated
258 (PUFA, two or more insaturations). FA could also be differentiated by the position of the first
259 double bond from the terminal carbon: n-3 (omega 3) or n-6 (omega 6). The results used in
260 the PCA were: total lipid content (*liptot*, μg FA / mg dry matter), n-3/n-6 ratio (*n3n6*), MUFA
261 content (*lipmono*, % of total FA), PUFA content (*lippoly*, % of total FA) and SFA content
262 (*lipsat*, % of total FA).

263

264 2.4.5. Protein and amino-acid analyses

265 The protein content (*prot*) was determined for t_0 , t_4 , and t_8 based on Lowry's method (1951).
266 Titrations were done in triplicate for each sample and averaged. The results obtained were
267 used for the amino-acid quantification.

268 Three samples for a period were pooled in equivalent quantity for amino-acid content
269 analysis. A hydrolysis was performed on the samples prior to amino-acid analysis.
270 Hydrolysats were dried under vacuum, suspended in 1 mL of water containing 100 μM 3-
271 aminobutyric acid (BABA) and used for subsequent analysis. For free amino-acid content, 10
272 mg of the ground freeze-dried samples were used. A methanol–chloroform–water-based
273 extraction was performed according to the following procedure: ground samples were
274 suspended in 400 μL of methanol containing 200 μM 3-aminobutyric acid. Suspensions were
275 agitated for 15 min at room temperature. Then, 200 μL of chloroform were added, followed
276 by a 5 min agitation step. Finally, 400 μL of water were added, and samples were vortexed
277 vigorously and centrifuged at 13 000 g for 5 min to induce phase separation. The upper phase,
278 which contained amino acids, was transferred to a clean microtube and used for subsequent
279 analysis.

280 For total and free amino acid profiling, 50 μL of each methanol–water extract were dried
281 under vacuum. Dry residues were suspended in 50 μL of ultrapure water and 5 μL were used

282 for the derivatization employing the AccQ-Tag Ultra derivatization kit (Waters, Milford, MA,
283 USA). Derivatized amino acids were analyzed using an Acquity UPLC-DAD system
284 (Waters). BABA was used as internal standard.

285

286 Based on King et al. (1996) who described the essential amino acids for abalone, the
287 following amino acids were used for the PCA analysis : arginine (*F-Arg*, *T-Arg*; with F
288 indicating the free amino-acid and T total amino-acid), histidine (*F-Hist*, *T-Hist*), isoleucine
289 (*F-Ile*, *T-Ile*), leucine (*F-Leu*, *T-Leu*), lysine (*F-Lys*, *T-Lys*), methionine (*F-Met* and *T-Met*),
290 phenylalanine (*F-Phe*, *T-Phe*), threonine (*F-Thr*, *T-Thr*), tryptophan (*F-Tr*, *T-Tr*) and valine
291 (*F-Val*, *T-Val*).

292

293 **2.5. Data analysis**

294

295 Data are represented as means and standard error (SE). Statistical analysis was performed
296 with R version 3.5.1 software.

297 To study the effect of period and algae species on chemical algal composition, an analysis of
298 variance was used, with algae (B, D, E, G, H, L, P and U) and period (t_{0-2} , t_{2-4} , t_{4-6} , t_{6-8} , t_{8-11} for
299 dry matter content, degradation and carbohydrate analysis; t_0 , t_4 , t_8 , for protein analysis;
300 period was not included in the model for lipid because only one period analysis), and the
301 interaction between algae and period as fixed factors. When normal distribution of the
302 residuals and homogeneity of variances were verified and global effects were found, *post-hoc*
303 Tukey tests for multiple comparisons of means were carried out. When normal distribution of
304 the residuals or homogeneity of variances was not verified, log, square root or inverse
305 transformations were used. If analysis of variance conditions were not fulfilled, a Welch test

306 was performed to study algal effect. If significant, pairwise post-hoc comparisons were
307 carried out using Games-Howell post-hoc test based on Welch's degrees of freedom
308 correction and uses Tukey's studentized range distribution. A Friedman test was used to test
309 algal seasonal effect when conditions of analysis of variance were not fulfilled. However,
310 interaction between the seasonal and algal effect could not tested with these non-parametric
311 tests.

312

313 For initial and final growth variables as well as reproduction variables, data were averaged per
314 compartment before analysis (3 replicate per treatment, each replicate corresponding to the
315 average of the 30 abalone growth measures, and 12 abalone reproduction measures). An
316 analysis of variance was used to compare the effect of algal diets (B, D, E, G, H, L, M, P and
317 U diets) on the growth and reproduction parameters. If conditions of analysis of variance were
318 not fulfilled, a Welch test was performed. If significant, pairwise post-hoc comparisons were
319 carried out using Games-Howell post-hoc test based on Welch's degrees of freedom
320 correction and uses Tukey's studentized range distribution.

321

322 The seasonal effect of algal diet on the two-month daily weight gain and daily length gain was
323 analysed by a linear mixed effects analysis using the lmerTest package (Bates et al., 2012)
324 and using the method described by Winter (2013). Square root transformation was used to
325 homogenise variances between the treatments for DGW. The model included the period (t_{0-2} ,
326 t_{2-4} , t_{4-6} , t_{6-8} , t_{8-11}), the 6 monospecific algal diets (D, G, H, L, P and U), and an interaction
327 between period and diet as fixed effects. The cages were indicated as a random factor (18
328 cages). B and E diets could not be included in the model due to missing data for the last
329 period. In addition, because M diet was composed of algae in different proportion at each
330 season, it was not included in the model. For the post-hoc analysis, the diffMeans package

331 was used. It calculated differences of Least Squares Means for the factors of lmer mixed
332 effects model and used the Satterthwaite's approximation to degrees of freedom.

333

334 Multiple linear regression analysis was not performed because normality of the residues, and
335 linearity between dependent variables and algal composition variables could not be fulfilled
336 as well as the low number of observations per predictor (Quinn and Keough, 2002). Instead, a
337 descriptive Principal Component Analysis (PCA) was performed with the package
338 FactoMineR (Lé et al., 2008). All the variables were reduced and scaled. Because the
339 proportion of algae eaten was not measured in the mixed diet, the PCA was performed only
340 on the 8 monospecific diets, using the two-month daily weight gain and daily length gain
341 (DGW and DGL for t_{0-2} , t_{2-4} , t_{4-6} , t_{6-8} , t_{8-11}), and the biochemical algal analysis corresponding to
342 the same period. Because it would have been too difficult to interpret with the large numbers
343 of variables, a second PCA was performed with the same variables to study relationship
344 between growth and amino-acid free and total composition.

345

346 **3. Results**

347

348 **3.1. *Abalone measurements***

349 Initial mean weight and length were not significantly different ($p > 0.05$). However, after one-
350 year experiment, a diet treatment effect was observed on all growth parameters, i.e. on the
351 mean final length ($p < 0.001$) and weight ($p < 0.001$), on $DGL_{t_{0-11}}$ ($p < 0.001$), on $DGW_{t_{0-11}}$
352 ($p < 0.001$), on SGR_w ($p < 0.001$) and on $W/L_{t_{11}}$ ($p < 0.001$). No effect was reported on
353 mortality ($p > 0.05$) (see Table 1 for p and F values).

354 After one-year experiment, the best growth performances in weight were observed for abalone
355 fed the *P. palmata* diet, followed by the mixed diet. Abalone fed with *S. latissima*, *Gracilaria*
356 sp. and *L. digitata* presented intermediate growth performances. The lowest performances
357 were observed for abalone fed *Enteromorpha* sp., *U. lactuca*, *S. polyschides* and stipes of
358 *L. hyperborea*. This diet ranking performance was maintained for the final weight-to-shell
359 length ratio (see Table 1 for post-hoc analysis comparison).

360 Concerning tissue and shell development of abalone after 6 months of treatment, diet effect
361 was observed for GDGr, Mr, Sr, GI and for VGI ($p < 0.001$) (See Table 2 for p and F values).
362 Abalone from *L. digitata* and *Enteromorpha* sp. diets had the highest GDGr whereas those fed
363 on *S. polyschides*, stipes of *L. hyperborea* and mixed diets had the lowest ratio. Abalone fed
364 *P. palmata* and mixed diets had the highest Mr and VGI while abalone from *Enteromorpha*
365 sp. and *L. digitata* diets had the lowest ratio. In contrast, the Sr was the most important for
366 abalone fed on stipes of *L. hyperborea* diet and the lowest for *P. palmata* and mixed diets.

367 A regression of the GDG wet weight against total wet weight gave a positive relationship ($r^2 =$
368 0.873 , $n = 295$, slope = 0.123 , intercept = -0.076 , $p < 0.001$). The regression of VGI against
369 total wet weight gave a positive relationship ($r^2 = 0.530$, $n = 295$, slope = 0.632 , intercept =
370 3.175 , $p < 0.001$): abalone with the higher VGI were also the largest.

371

372 When studying more specifically the effect of the period and diet on abalone seasonal DGW
373 and DGL, an important period effect (mixed model, DGW: $F_{4,60} = 25.61$, $p < 0.001$; DGL:
374 $F_{4,60} = 10.60$, $p < 0.001$), a diet effect (mixed model, DGW: $F_{5,60} = 36.09$, $p < 0.001$; DGL:
375 $F_{5,60} = 14.70$, $p < 0.001$) as well as an interaction between diet and period (mixed model,
376 DGW: $F_{20,60} = 2.38$, $p < 0.01$; DGL: $F_{20,60} = 1.98$, $p < 0.05$) were observed. The periods from
377 late spring to the end of autumn (t_{4-6} and t_{6-8}) were the best for abalone DGL and DGW

378 compared to late winter (t_{0-2}), and spring (t_{2-4}) periods, the worst period being in winter (t_{8-11})
379 for DGL (Figure 2). However, the seasonal effect was different depending on algal diets. For
380 example, abalone fed with algae such as *P. palmata* and *U. lactuca* had similar DGL and
381 DGW from t_0 to t_8 , and presented a decrease in DGL and DGW only in winter (t_{8-11}) (Figure
382 3). Abalone fed other diets such as *L. digitata* presented important DGL and DGW variation
383 depending on the period (Figure 3).

384

385 **3.2. Algal biochemical measurements**

386 Biochemical composition of the eight algal diets distributed to abalone during one year
387 significantly differed in term of proteins, free and total amino-acids, fatty acids and soluble
388 carbohydrates ($p < 0.01$, see Table 3 for p and F details). *P. palmata*, *S. latissima* and *L.*
389 *digitata* presented the higher yearly total carbohydrate content while *Enteromorpha* sp., *U.*
390 *lactuca*, *S. polyschides* and stipes of *L. hyperborea* were the poorest. *Enteromorpha* sp.
391 presented the highest total lipid content with 4 % of total lipids. *P. palmata* and
392 *Enteromorpha* sp. presented the highest n-3/n-6 ratio in winter period. The highest yearly
393 protein content was observed for *P. palmata* and *Enteromorpha* sp. with intermediate content
394 for *Gracilaria* sp. and *S. latissima*. The lowest protein contents were observed for *L. digitata*,
395 stipes of *L. hyperborea*, *U. lactuca*, and *S. polyschides*. An important degradation was
396 observed after one month in the sea-structure for the green algae *Enteromorpha* sp. and *U.*
397 *lactuca*, for *S. polyschides* and for *Gracilaria* sp. In contrast, more than half of the algae
398 distributed were still present after one month in sea-structure for *P. palmata*, *L. digitata* and *S.*
399 *latissima*.

400 A significant seasonal effect ($p < 0.05$) was observed for the soluble carbohydrate contents,
401 dry matter and proportion of algae degraded ($p < 0.05$) but not for protein content ($p > 0.05$).

402 Apart for histidine and tryptophan, significant differences of total amino-acid compositions
403 were observed between the eight algal diets (Table 4). The red algae *P. palmata* and
404 *Gracilaria* sp. presented the highest content for most of the total essential amino-acid. The
405 brown algae *L. digitata* and the stipes of *L. hyperborea* presented most of the time the lowest
406 total amino content while the green algae and the kelp *S. latissima* presented intermediate
407 contents. In addition, differences ($p < 0.05$) were observed between the 8 algae for free
408 amino-acid contents apart for methionine and valine. However, free essential amino acid
409 contents were variable depending of the algae and the amino-acid (Table 4).

410

411 **3.3. Relationship between growth and algal composition**

412

413 The first three components of the PCA based on the abalone variables and seasonal
414 biochemical composition of algae explained 66.8 % of the total variance (37.9 % for the first
415 component, 17.3 % for the second component and 11.5 % for the third component) (Figure 4).
416 For the abalone variables, the most important loadings on the first component were the VGI
417 ($\cos^2 = 0.80$), GI ($\cos^2 = 0.79$), DGWseason ($\cos^2 = 0.54$), inversely related to Sr ($\cos^2 =$
418 0.61). For the algal chemical composition, n3n6 ($\cos^2 = 0.74$), prot ($\cos^2 = 0.65$) and carb
419 ($\cos^2 = 0.50$) explained best the first axis. This component could represent the growth and
420 reproduction associated with the biochemical protein, n-3/n-6 ratio and soluble carbohydrate
421 richness of the algae. The most important loadings on the second component were liptot (\cos^2
422 $= 0.77$) and lippoly ($\cos^2 = 0.66$). The variable Pdegraded ($\cos^2 = 0.49$) and drymatter ($\cos^2 =$
423 0.23) were the two variables with the higher loading on the third axis, but were not related
424 with any growth variables.

425

426 The first three components of the PCA based on the abalone variables and amino-acid
427 composition explained 61.4 % of the total variance (37.5 % for the first component, 14.9 %
428 for the second component and 9.1 % for the third component) (Figure 5). For the abalone
429 variables, the most important loadings on the first component were the GI ($\cos^2 = 0.76$), VGI
430 ($\cos^2 = 0.75$) associated with T_Val ($\cos^2 = 0.88$), T_Met ($\cos^2 = 0.87$), T_Leu ($\cos^2 = 0.86$),
431 T_Arg ($\cos^2 = 0.85$), and T_Ile ($\cos^2 = 0.84$). This component could represent the
432 reproductive development associated with the total amino-acid composition. The most
433 important loadings on the second component were F_Phe ($\cos^2 = 0.60$), F_Ile ($\cos^2 = 0.54$)
434 associated with DGWseason ($\cos^2 = 0.42$).

435

436

437 **4. Discussion**

438 The high survival of abalone observed for all treatments may indicate that algal diets were
439 balanced enough in term of nutrients to maintain survival although the low protein or
440 carbohydrate content for *L. hyperborea* stipe diet may not have been enough to sustain good
441 growth. Mortality during the one-year experiment was generally low, and not different
442 between the treatments, ranging from 0.8% for *S. latissima* and 1.3% for *U. lactuca* treatment
443 up to 3.3% and 3.5% for *P. palmata*, mixed diet and *Gracilaria* sp. diets.

444

445 Good growth rates are important to reach a marketable size within a time which is
446 economically viable. *H. tuberculata* is a slow-growing species with an average monthly
447 growth rate between 1 mm and 2 mm, depending on the farming conditions (Basuyaux, 1997;
448 Lachambre, 2017). This experiment was conducted in a commercial set-up, with the same

449 density, cage design, and feeding rhythm as France Haliotis organic certified farming
450 practices. Our study demonstrated that abalone fed on *P. palmata*, mixed diet and *S. latissima*
451 presented the best growth rate, muscle ratio and gonad development. *Gracilaria* sp., *L.*
452 *digitata* and *Enteromorpha* sp. gave moderate growth performances while *U. lactuca* and
453 stipes of *L. hyperborea* presented the lowest growth performances. Previous studies on
454 *H. tuberculata* have shown that diets based on *P. palmata* produced better growth rates than
455 most of the algae found in Europe (Mercer et al., 1993; Mai et al., 1996; Viera et al., 2015;
456 Basuyaux et al., 2018). In addition, the high nutritional value of *P. palmata* has been reported
457 for other abalone species such as *H. discus hannai* (Uki et al., 1986; Mercer et al., 1993).
458 However, other experiments found different results for algal species such as *S. latissima*,
459 which gave one of the best growth in our one-year long experiment while it presented the
460 lowest growth performance in other experiments (Mercer et al., 1993; Mai et al., 1996). *U.*
461 *lactuca* was one of the algal diets that gave the poorer growth while it turned to be moderately
462 good for Mercer et al. (1993). The difference of experiment duration can explain part of the
463 difference (50 weeks for our experiment, 17 weeks for Mai et al., 1996; and 33 weeks for
464 Mercer et al., 1993) and probably results from the high variability in algal composition
465 according to the season and site (Renaud and Luong-Van, 2006; Villares et al., 2013; Schmid
466 et al., 2014). In addition, these experiments were performed in controlled conditions with
467 stable temperature and renewal of algae every 5-7 days compared to our commercial scale
468 experiment submitted to seasonal temperature change, open-seawater conditions and a
469 monthly feed renewal. In addition to growth performance, the degradation of algae in the sea-
470 cage structure is an important factor to integrate in the choice of a commercial structure and
471 varied between 33% up to 93% degradation over one month. *P. palmata*, *L. digitata* and
472 *S. latissima* showed the least degradation over one month. However, in our experiment,
473 growth performances of abalone were not related to degradation of algae. Another interesting

474 finding for commercial purpose is the high variability in term of muscle on total weight ratio
475 (%) with more than 25% more muscle in abalone fed *P. palmata* diet compared to
476 *Enteromorpha* sp. diet. This resulted directly from diet treatment as the abalone
477 individuals were from the same genetic stock. In this specific context, it puts forward the
478 importance of measuring weight in addition to length, and more specifically muscle on
479 total weight ratio.

480

481 In order to reach maximum abalone growth using fresh algae, it is important to understand
482 which algal component explains best growth performances. Specific growth rate (SGR)
483 values in this study (0.2-0.7%) were similar to SGR obtained with 1-year old *H. tuberculata*
484 fed with artificial diets based on different seaweeds meals (0.2-0.3%) or fresh *G. cornea* and
485 *U. rigida* (0.56%) (Viera et al., 2015). If algal composition is clearly identified according to
486 seasonal variation, fresh algal diets can constitute high quality and efficient feeds. In this
487 experiment, the main components related to abalone growth obtained with PCA analysis were
488 the lipid n-3/n-6 ratio, the soluble carbohydrate content and total protein content. These
489 components have been often reported as important in the literature. *H. tuberculata* growth
490 seemed to be highly correlated to n-3 poly-unsaturated fatty acids (PUFA) in algal
491 composition as demonstrated by Mai et al. (1996) with a SGR of 1.31 % day⁻¹ when fed a *P.*
492 *palmata* diet containing the highest amount of n-3 PUFA (49.7% of total fatty acids)
493 compared to a SGR of 1.03 % day⁻¹ when fed *L. saccharina* diet containing the lowest n-3
494 PUFA (25.2% of total fatty acids). A lower SGR (0.30 % day⁻¹) was also observed in Jade
495 tiger hybrid abalone fed canola oil diets containing lower levels of n-3 PUFA (12.5% of total
496 fatty acids) compared to a higher SGR (0.47 % day⁻¹) when fed fish oil diet containing more n-
497 3 PUFA (27.5 % of total fatty acids) (Mateos et al., 2013). In our experiment, total PUFAs
498 were not related to growth enhancement. However, it should be highlighted lipid composition

499 was only evaluated in winter period. This might bias partly our PCA and under-evaluate lipid
500 composition importance in contrast to other biochemical algal composition evaluated at
501 different period.

502 High protein content is another main component explaining the good growth performance
503 reached for *H. tuberculata* in our experiment as observed for Mercer et al. (1993) and Viera et
504 al. (2015), in *H. asinina* (Bautista-Teruel and Millamena, 1999), *H. iris* and *H. discus hannai*
505 (Shpigel et al., 1999). In addition, the present work was designed to compare the amino acid
506 patterns of selected algae and determine which is the most related to *H. tuberculata*
507 reproduction and growth variables. Amino acid composition is usually the first parameter to
508 be considered in formulating test or commercial feeds, but it is also very expensive to
509 measure. The determination of the limiting amino-acid is based on comparisons between the
510 absolute values in the flesh diet profiles (Fleming et al., 1996). Using an ACP analysis, it was
511 observed that the growth variables were mainly associated with the free valine, leucine,
512 isoleucine as well as phenylalanine contents. Threonine, valine, methionine, isoleucine,
513 leucine, phenylalanine, tryptophan, lysine, histidine and arginine have been validated as being
514 essential amino-acid in *H. rufescens* using (U-14C) glucose (Allen and Kilgore, 1975). In
515 addition, free arginine, methionine and threonine have already been reported to be important
516 amino-acid for *H. tuberculata* growth (Mai et al., 1994).

517

518 In natural conditions, abalone consume a variety of seaweeds, trapping drift kelp or selecting
519 attached benthic algae mainly according to their abundance and availability in the surrounding
520 area, and water movement (Cornwall et al., 2009; Zeeman et al., 2012). It has been often
521 reported that growth is improved when abalone are fed on a combination of macroalgae
522 species in comparison to a single species diet (Mercer et al., 1993; Qi et al., 2010b; Viera et
523 al., 2015). In our experiment, mixed diet gave the second best growth performance, preceded

524 by *P. palmata*. This result is in accordance with the meta-analysis of Lefcheck et al. (2013)
525 performed on variety of taxa and systems showing that mixed diets conferred significantly
526 higher fitness than the average of single-species diets, but not for the best single prey species.
527 However, in order to prevent depletion of the algal resources, a mixed diet seems an optimal
528 solution for European abalone farms, with good growth performance as tested by Basuyaux et
529 al. (2018).

530

531 Juveniles were about 2-year-old when this study was conducted. At this age, an average of
532 90% of the animals presented an early stage of gonad development (1 to 10 on the Visual
533 Gonad Index scale) but less than 23% of the cohort featured a full gonad development (over
534 an 8 score on the Visual Gonad Index scale). These results were consistent with studies
535 conducted in the natural environment in Northern Brittany where sexual maturation was
536 observed for abalone between 2 and 3-year-old with a minimum size of 30 mm necessary for
537 reproduction (Clavier and Richard, 1985). Length and weight of individuals seemed to be the
538 most important determinants of maturation in *H. tuberculata*. Considering that all the abalone
539 were 18-month-old, and that the weight and size were similar at the beginning of the
540 experiment between treatments, we could conclude that, in a maturing population, larger
541 abalone would become reproductive at least a year before smaller abalone of the same age.
542 Our results were consistent with a study in the wild population of *H. laevisgata* (McAvaney et
543 al., 2004) which suggested a significant plasticity of the maturity age in *H. laevisgata*.

544

545 Due to the small size of abalone used in our study, it was not possible to separate the gonad
546 and the digestive gland. Thus, gonad weight had been estimated using histology. This method
547 gave a good proxy of the proportion of gonad on the gonad-digestive gland even if it does not

548 give an exact value. Abalone fed *P. palmata* and mixed diet (yearly average; *S. polyschides*:
549 15.5 %; *L. digitata*: 20.5%; *P. palmata*: 43%; *S. latissima*: 8%; *U. lactuca*: 12%) developed
550 more gonad compared to abalone fed on stipes of *L. hyperborea*, which showed higher shell
551 ratio. In addition, visual gonad index as well as gonadal index were related to protein content
552 and some amino-acids (total valine, leucine, isoleucine, methionine, arginine and threonine).
553 These results are consistent with other studies in abalone *H. iris* (Tung and Alfaro, 2012) and
554 sea urchins *Strongylocentrotus droebachiensis* (Lyons and Scheibling, 2007) where an effect
555 of natural or artificial diets was demonstrated on both growth performance and gonad
556 development by influencing a shift between somatic and gonadal depots. Crude protein
557 content of the seaweed seems to play an important role in *H. tuberculata coccinea*
558 reproduction, fed on *Gracilaria cornea* with different protein levels (Bilbao et al., 2012).

559

560 **5. Conclusion**

561 The age of initial sexual maturity in *H. tuberculata* seems to be a highly plastic trait in
562 response to different growth rate and algal diet. Abalone are likely to invest more energy in
563 muscle and gonad developments when fed on *Palmaria palmata*. According to farming
564 strategies and seaweed availability in natural environment, it may be interesting to feed
565 abalone on some specific types of algae. As demonstrated in this long-term experimental,
566 seasonal quality of algae differs and will impact growth differently. Mixed diets allowed a
567 good muscle development and growth results and should probably be preferred to a
568 monospecific diet in order to avoid lack of essential nutrients and high pressure on one algal
569 resource.

570

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580

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727 abalone *Haliotis midae* from its diet and foraging behaviour. Afr. J. Mar. Sci. 34, 205-214.

728

729

730 Table 1: Growth in length, weight and mortality of juvenile abalone (*H. tuberculata*) which received a monospecific diet of eight different algae
 731 (B: *Saccorhiza polyschides*, D: *Laminaria digitata*; E: *Enteromorpha* sp., G: *Gracilaria* sp., H: stipes of *Laminaria hyperborea*, P: *Palmaria*
 732 *palmata*, S: *Saccharina latissima*, U: *Ulva lactuca*) or a mixed diet (M) during a one-year commercial scale feeding trial.

Diet	Mean initial length (mm) ^γ	Mean final length (mm)	Final daily length gain (μm.day ⁻¹)	Mortality (%) ^γ
B	23.9 ± 0.50	n.a.	53 ± 2.1	0.7 ± 0.30
D	24.0 ± 0.40	46.4 ± 0.48 ^b	65 ± 0.3 ^b	0.8 ± 0.38
E	24.2 ± 0.41	n.a.	65 ± 3.9	1.6 ± 0.53
G	24.4 ± 0.45	46.0 ± 0.45 ^b	63 ± 5.4 ^b	3.1 ± 1.63
H	24.5 ± 0.48	33.3 ± 0.53 ^a	26 ± 3.6 ^a	0.9 ± 0.20
M	24.4 ± 0.54	51.1 ± 0.35 ^{cd}	78 ± 6.2 ^{bc}	2.7 ± 2.17
P	23.8 ± 0.42	53.8 ± 0.41 ^d	87 ± 2.3 ^c	2.9 ± 2.07
S	23.9 ± 0.49	47.3 ± 0.49 ^{bc}	68 ± 3.8 ^b	0.7 ± 0.25
U	23.9 ± 0.39	45.5 ± 0.43 ^b	63 ± 1.0 ^b	1.1 ± 0.56
Ftreat	F _{8,18} = 0.03	F _{6,14} = 66.10	F _{6,14} = 25.44	F _{6,14} = 0.44
Ptreat	NS	***	***	NS

733

Diet	Mean initial weight (g) ^γ	Mean final weight (g)	Final daily weight gain (mg.day ⁻¹)	Specific growth rate (%.day ⁻¹)	Final weight-to- shell length ratio (g.mm ⁻¹)	Performance rank ^o
B	2.2 ± 0.13	n.a.	17 ± 1.0	0.43 ± 0.027	0.17 ± 0.004	8
D	2.2 ± 0.12	14.0 ± 0.14 ^b	34 ± 0.6 ^b	0.54 ± 0.015 ^{bc}	0.30 ± 0.006 ^b	5
E	2.2 ± 0.11	n.a.	25 ± 2.2	0.53 ± 0.044	0.21 ± 0.005	6
G	2.2 ± 0.12	14.2 ± 0.14 ^b	35 ± 2.7 ^b	0.55 ± 0.053 ^{bc}	0.30 ± 0.007 ^b	4
H	2.3 ± 0.13	5.1 ± 0.13 ^a	8 ± 1.2 ^a	0.24 ± 0.033 ^a	0.15 ± 0.005 ^a	9
M	2.5 ± 0.14	19.9 ± 0.15 ^c	51 ± 1.5 ^c	0.63 ± 0.069 ^{bc}	0.39 ± 0.006 ^c	2
P	2.2 ± 0.13	24.3 ± 0.15 ^d	64 ± 3.2 ^d	0.70 ± 0.016 ^c	0.45 ± 0.008 ^c	1

S	2.1 ± 0.12	14.9 ± 0.14 ^b	37 ± 4.1 ^b	0.57 ± 0.032 ^{bc}	0.31 ± 0.007 ^b	3
U	2.2 ± 0.11	11.8 ± 0.12 ^b	28 ± 0.3 ^b	0.50 ± 0.011 ^b	0.26 ± 0.005 ^b	7
Ftreat	F _{8,18} = 0.04	F _{6,14} = 52.73	F _{6,14} = 57.22	F _{6,14} = 14.46	F _{6,14} = 54.88	
Ptreat	NS	***	***	***	***	

734 Means and SE. are presented. n = 3 replicates of 30 abalone per treatment.

735 Values in the same column with different letters are significantly different ($p < 0.05$). If not indicated: analysis of variance with post-hoc Tukey
736 contrasts.

737 n.a. : non-available, NS : non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

738 For B and E: calculated for t_8 period instead of t_{11} period. These data were not used in the statistical analysis

739 ° rank based on growth rate in weight

740 ^γ Log transformation

741

742

743 Table 2: Relative gonad-digestive gland, muscle, shell development, gonadal index and visual gonad index of 2-years old *H. tuberculata*
 744 during maturation period fed on a monospecific diet of eight different algae (B: *Saccorhiza polyschides*, D: *Laminaria digitata*; E:
 745 *Enteromorpha* sp., G: *Gracilaria* sp., H: stipes of *Laminaria hyperborea*, P: *Palmaria palmata*, S: *Saccharina latissima*, U: *Ulva lactuca*) or a
 746 mixed diet (M) during 6 months.

Diet	Gonad-digestive gland on total weight ratio (%)	Muscle on total weight ratio (%) ^α	Shell on total weight ratio (%)	Gonadal index (%) ^α	Visual gonad index
B	9.4 ± 0.35 ^a	35.4 ± 0.52 ^a	23.8 ± 1.93 ^b	7.7 ± 0.09 ^a	2.4 ± 0.35 ^{abc}
D	11.8 ± 0.34 ^b	34.1 ± 0.63 ^a	24.1 ± 1.87 ^b	2.4 ± 2.37 ^a	1.8 ± 0.30 ^{ab}
E	12.5 ± 0.44 ^b	32.2 ± 0.50 ^a	24.8 ± 2.12 ^b	8.9 ± 4.95 ^a	3.0 ± 0.38 ^{bc}
G	10.7 ± 0.35 ^{ab}	37.6 ± 0.90 ^{ab}	23.7 ± 3.46 ^b	44.8 ± 12.95 ^{ab}	4.3 ± 0.41 ^{cd}
H	8.0 ± 0.34 ^a	35.0 ± 1.05 ^a	28.3 ± 3.86 ^c	0.8 ± 0.79 ^a	0.8 ± 0.27 ^a
M	9.6 ± 0.47 ^a	39.5 ± 0.58 ^{bc}	21.3 ± 2.47 ^a	50.8 ± 7.21 ^{ab}	7.4 ± 0.50 ^{de}
P	11.3 ± 0.33 ^{ab}	40.2 ± 0.47 ^b	20.8 ± 1.68 ^a	69.4 ± 1.68 ^b	8.1 ± 0.29 ^e
S	10.9 ± 0.36 ^{ab}	36.7 ± 0.63 ^{ac}	24.1 ± 2.08 ^b	20.9 ± 4.43 ^a	3.8 ± 0.46 ^{bc}
U	11.4 ± 0.28 ^b	35.6 ± 0.46 ^a	24.4 ± 2.26 ^b	19.2 ± 7.21 ^{ab}	3.8 ± 0.49 ^{bc}
F _{treat}	F _{8,18} = 5.72	F _{8,7.4} = 28.84	F _{8,18} = 9.66.	F _{8, 6.7} = 110.9	F _{8,18} = 11.13
P _{treat}	***	***	***	***	***

747 Means ± SE are presented. n = 3 replicate of 12 abalone per treatment. *** p < 0.001. Values in the same column with different letters are
 748 significantly different (p < 0.05). If not indicated: analysis of variance with post-hoc Tukey contrasts.

749 ^α non-parametric Welch test with Games-Howell post-hoc analysis

750 ^γ Log transformation

752

753 Table 3: Yearly average biochemical composition and degradation of eight different algae (B: *Saccorhiza polyschides*, D: *Laminaria digitata*; E:
 754 *Enteromorpha* sp., G: *Gracilaria* sp., H: stipes of *Laminaria hyperborea*, P: *Palmaria palmata*, S: *Saccharina latissima*, U: *Ulva lactuca*)
 755 distributed to *H. tuberculata* during a one-year commercial scale feeding trial (mean \pm SE, n = 3 samples / algae / period, with 5 periods for dry
 756 matter content, degradation and carbohydrate analysis, 3 periods for protein analysis, and 1 period for lipid analysis)

	Dry matter (%) ^α	Soluble carbohydrate content (% dry matter) ^α	Total lipid content ($\mu\text{g FA}\cdot\text{mg}^{-1}$ dry matter).	n-3/n-6 ratio	Mono- unsaturated fatty acid content (mol %)	Poly-unsaturated fatty acids content (mol %)	Saturated fatty acid content (mol %)	Protein content (% dry matter) ^α	Proportion of algae degraded after 1 month in sea-structure (%) ^α
B	9.7 \pm 0.52 ^a	5.1 \pm 2.30 ^a	8.2 \pm 1.51 ^a	1.5 \pm 0.27 ^{ab}	21.2 \pm 0.08 ^{bc}	48.9 \pm 0.99 ^{bcd}	29.9 \pm 0.91 ^a	6.4 \pm 0.30 ^{ab}	85.2 \pm 6.11 ^a
D	18.8 \pm 1.33 ^b	13.6 \pm 17.13 ^{ab}	10.7 \pm 0.83 ^a	1.5 \pm 0.43 ^{ab}	22.1 \pm 1.29 ^{ab}	47.1 \pm 2.84 ^{bcd}	30.9 \pm 1.55 ^a	5.0 \pm 0.57 ^a	44.7 \pm 4.51 ^b
E	13.4 \pm 1.25 ^{ab}	5.2 \pm 2.98 ^a	35.5 \pm 0.27 ^b	8.6 \pm 2.03 ^b	11.8 \pm 1.01 ^{ab}	64.4 \pm 3.69 ^d	23.8 \pm 2.70 ^a	14.0 \pm 0.93 ^{bc}	92.9 \pm 2.14 ^a
G	14.9 \pm 1.72 ^{ab}	6.4 \pm 2.24 ^{ab}	8.04 \pm 1.72 ^a	1.4 \pm 0.08 ^{ab}	9.8 \pm 1.88 ^{ab}	8.7 \pm 0.70 ^a	81.5 \pm 1.18 ^b	11.6 \pm 1.23 ^{bc}	84.0 \pm 4.72 ^a
H	14.4 \pm 0.44 ^{ab}	4.0 \pm 1.47 ^a	5.7 \pm 2.45 ^a	0.7 \pm 0.37 ^a	29.0 \pm 3.38 ^c	29.3 \pm 7.76 ^{ab}	41.7 \pm 4.38 ^a	4.8 \pm 0.49 ^a	37.4 \pm 7.13 ^b
P	15.0 \pm 0.52 ^{ab}	37.5 \pm 11.38 ^c	12.4 \pm 0.08 ^a	38.0 \pm 0.45 ^b	6.8 \pm 0.46 ^a	61.6 \pm 3.60 ^{cd}	31.5 \pm 3.14 ^a	19.6 \pm 0.97 ^c	33.4 \pm 6.51 ^b
S	18.8 \pm 0.90 ^b	23.1 \pm 18.64 ^{bc}	4.7 \pm 0.41 ^a	0.5 \pm 0.09 ^a	28.1 \pm 3.24 ^c	33.7 \pm 4.17 ^{ac}	38.3 \pm 0.93 ^a	9.7 \pm 1.27 ^{ab}	48.5 \pm 2.66 ^b
U	19.7 \pm 1.56 ^b	4.4 \pm 2.49 ^a	9.5 \pm 2.37 ^a	7.7 \pm 1.86 ^a	19.1 \pm 3.53 ^{ac}	39.4 \pm 9.93 ^{bcd}	41.5 \pm 6.39 ^a	6.6 \pm 0.31 ^{ab}	80.5 \pm 3.82 ^a
F algae	F _{7,12,9} = 5.29	F _{7,12,9} = 5.80	F _{7,8} = 43.74	F _{7,8} = 158.70	F _{7,8} = 13.42	F _{7,8} = 12.40	F _{7,8} = 31.11	F _{7,6,6} = 10.04	F _{7,13,5} = 26.39
P algae	**	**	***	***	***	***	***	**	***
F period	Q = 12.1	Q = 12.5	n.a	n.a	n.a	n.a	n.a	Q = 3.25	Q = 12.4
P period	*	*	n.a.	n.a.	n.a.	n.a.	n.a.	NS	*

757 n.a. : non-available, NS : non-significant, * p < 0.05, ** p < 0.01, *** p < 0.001. If not indicated: analysis of variance with post-hoc Tukey
 758 contrasts.

759 ^α. Welch test for algae effect with Games-Howell post-hoc analysis. Friedman test for period effects.
 760 Table 4: Amino-acid composition of eight different algae (B: *Saccorhiza polyschides*, D: *Laminaria digitata*; E: *Enteromorpha* sp., G: *Gracilaria*
 761 sp., H: stipes of *Laminaria hyperborea*, P: *Palmaria palmata*, S: *Saccharina latissima*, U: *Ulva lactuca*) distributed to *H. tuberculata* during a
 762 one-year commercial scale feeding trial (mean ± SE, n = 3 replicate corresponding to t₀, t₄, and t₈ period per algae).

763

	B	D	E	G	H	P	S	U	F _{7,16} F _{7,6.0} ^α	P algae
Total essential amino acids (μmol.mg ⁻¹ dry matter)										
Arginine	0.7 ± 0.05 ^a	0.3 ± 0.07 ^a	1.3 ± 0.31 ^{ab}	1.9 ± 0.25 ^b	0.3 ± 0.04 ^a	2.0 ± 0.26 ^b	0.8 ± 0.06 ^a	1.2 ± 0.26 ^{ab}	10.72	***
Histidine	0.20 ± 0.100	0.24 ± 0.035	0.40 ± 0.083	0.38 ± 0.178	0.26 ± 0.013	0.41 ± 0.185	0.25 ± 0.094	0.49 ± 0.133	0.79	NS
Isoleucine	0.9 ± 0.21 ^{ab}	0.4 ± 0.07 ^a	1.2 ± 0.31 ^{ab}	1.7 ± 0.27 ^b	0.5 ± 0.06 ^a	1.8 ± 0.17 ^b	0.9 ± 0.11 ^{ab}	1.4 ± 0.29 ^{ab}	5.90	**
Leucine	1.7 ± 0.40 ^{ab}	0.8 ± 0.13 ^a	2.3 ± 0.59 ^{ab}	2.9 ± 0.36 ^b	0.8 ± 0.15 ^a	3.2 ± 0.25 ^b	1.9 ± 0.21 ^{ab}	2.7 ± 0.55 ^b	6.12	**
Lysine	1.0 ± 0.39 ^{ab}	0.2 ± 0.07 ^a	1.3 ± 0.49 ^{ab}	2.0 ± 0.09 ^{bc}	1.2 ± 0.03 ^{ab}	3.3 ± 0.46 ^c	1.2 ± 0.26 ^{ab}	1.8 ± 0.36 ^{ac}	7.69	***
Methionine	0.45 ± 0.069 ^{ac}	0.26 ± 0.037 ^{ab}	0.57 ± 0.118 ^{bcd}	0.63 ± 0.083 ^{cd}	0.18 ± 0.018 ^a	0.87 ± 0.072 ^d	0.55 ± 0.037 ^{bcd}	0.50 ± 0.095 ^{ac}	8.43	***
Phenylalanine	0.9 ± 0.14 ^{ab}	0.6 ± 0.08 ^{ab}	1.4 ± 0.24 ^b	1.5 ± 0.24 ^b	0.4 ± 0.06 ^a	1.6 ± 0.14 ^b	1.0 ± 0.04 ^{ab}	1.5 ± 0.38 ^b	5.11	**
Threonine	1.1 ± 0.22 ^{ab}	0.6 ± 0.10 ^a	2.0 ± 0.51 ^{ac}	2.2 ± 0.21 ^{bc}	1.2 ± 0.01 ^{ac}	2.6 ± 0.24 ^c	1.5 ± 0.20 ^{ac}	2.0 ± 0.44 ^{bc}	5.24	**
Tryptophan	0.003 ± 0.0033	0.000 ± 0.0000	0.013 ± 0.0033	0.006 ± 0.0033	0.003 ± 0.0033	0.016 ± 0.0033	0.006 ± 0.0033	0.013 ± 0.0133	1.14	NS
Valine	1.4 ± 0.34 ^{ab}	0.6 ± 0.11 ^a	2.2 ± 0.55 ^{ac}	2.5 ± 0.27 ^{bc}	1.0 ± 0.11 ^{ab}	3.2 ± 0.34 ^c	1.6 ± 0.17 ^{ac}	2.4 ± 0.51 ^{bc}	6.43	**
Free essential amino acids (nmol.mg ⁻¹ dry matter)										
Arginine ^α	0.14 ± 0.091	0.13 ± 0.041	0.61 ± 0.083	0.10 ± 0.096	0.03 ± 0.016	0.00 ± 0.000	0.12 ± 0.036	0.07 ± 0.071	7.16	*
Histidine ^α	0.07 ± 0.037	0.05 ± 0.011	0.00 ± 0.000	0.42 ± 0.271	0.08 ± 0.044	0.25 ± 0.034	0.32 ± 0.285	15.30 ± 6.205	7.37	*
Isoleucine	0.22 ± 0.067	0.12 ± 0.027	0.22 ± 0.022	0.07 ± 0.017	0.10 ± 0.022	0.36 ± 0.141	0.34 ± 0.082	0.34 ± 0.046	3.19	*
Leucine ^α	0.28 ± 0.087	0.09 ± 0.015	0.26 ± 0.052	0.02 ± 0.004	0.08 ± 0.025	0.65 ± 0.201	0.22 ± 0.051	0.45 ± 0.173	7.37	*
Lysine ^γ	0.30 ± 0.040 ^{ab}	0.11 ± 0.047 ^a	0.30 ± 0.060 ^{ab}	0.17 ± 0.052 ^{ab}	0.07 ± 0.029 ^a	0.20 ± 0.051 ^{ab}	0.20 ± 0.004 ^{ab}	0.52 ± 0.194 ^b	3.49	*
Methionine ^α	0.019 ± 0.006	0.012 ± 0.006	0.016 ± 0.000	0.018 ± 0.007	0.023 ± 0.012	0.019 ± 0.004	0.044 ± 0.016	0.065 ± 0.040	0.52	NS
Phenylalanine	0.21 ± 0.059 ^{ab}	0.10 ± 0.018 ^a	0.11 ± 0.007 ^a	0.06 ± 0.042 ^a	0.09 ± 0.024 ^a	0.35 ± 0.079 ^b	0.26 ± 0.008 ^{ab}	0.17 ± 0.057 ^{ab}	5.06	*
Threonine ^γ	0.54 ± 0.063 ^a	1.19 ± 0.368 ^a	0.30 ± 0.065 ^a	0.56 ± 0.185 ^a	0.89 ± 0.265 ^a	0.63 ± 0.121 ^a	4.85 ± 1.604 ^b	0.23 ± 0.054 ^a	8.71	***
Tryptophan ^β	0.13 ± 0.042 ^a	0.06 ± 0.013 ^{ab}	0.05 ± 0.012 ^b	0.01 ± 0.004 ^b	0.04 ± 0.005 ^b	0.06 ± 0.013 ^{ab}	0.05 ± 0.001 ^{ab}	0.05 ± 0.010 ^{ab}	4.80	**
Valine ^γ	0.64 ± 0.208	0.46 ± 0.109	0.47 ± 0.026	0.25 ± 0.045	0.67 ± 0.235	0.68 ± 0.293	1.46 ± 0.445	0.60 ± 0.114	2.28	NS

764 * p < 0.05, ** p < 0.01, *** p < 0.001. If not indicated, analysis of variance with post-hoc Tukey contrasts.

765 ^γ. Log transformation

766 ^β Square root transformation

767 ^α. Welch test for algae effect with Games-Howell post-hoc analysis test.

Figure 1: Treatment distribution (B: *Saccorhiza polyschides*, D: *Laminaria digitata*; E: *Enteromorpha sp*, G: red filamentous algae, H: stipes of *Laminaria hyperborea*, M: mixed diet, P: *Palmaria palmata*, S: *Saccharina latissima*, U: *Ulva lactuca*). Numbers 1, 2 and 3 correspond to triplicates with abalone and 0 to compartments without abalone to study algal degradation. C corresponds to the abbox sea-cage number.

Figure 2: Seasonal daily weight gain (DGW) and daily length gain (DGL) of *H. tuberculata* receiving 6 monospecific diets during a one-year commercial scale feeding trial (n= 3 replicate per diet). Five periods were studied (t0-2, t2-4, t4-6, t6-8, t8-11). Means \pm SE are presented. Different letters indicate significant period differences (Linear mixed-effects model with post-hoc analysis).

Figure 3: Seasonal daily length gain (DGL) of abalone receiving *Laminaria digitata*, *Palmaria palmata* and *Ulva lactuca* monospecific diets during a one-year commercial scale feeding trial (n= 3 replicate per diet). Five periods were studied (t0-2, t2-4, t4-6, t6-8, t8-11). Means \pm SE are presented. Different letters indicate significant period differences (Linear mixed-effects model with post-hoc analysis).

Figure 4: Principal component analysis plot of the algal biochemical composition, and abalone growth and morphological parameters

Figure 5: Principal component analysis plot of algal free and total amino-acid composition, and abalone growth and morphological parameters

C2			
1	2	3	4
M1	E0	H1	G1

C1			
5	6	7	8
D1	B0	M2	P1

C3			
9	10	11	12
M3	U0	H2	D2

C4			
13	14	15	16
U1	P0	B1	E1

C5			
17	18	19	20
P2	S0	G2	U2

C6			
21	22	23	24
B2	M0	E2	U3

C7			
25	26	27	28
H3	D0	D3	S1

C8			
29	30	31	32
S2	H0	P3	G3

C9			
33	34	35	36
B3	G0	S3	E3







