Aquaculture
November 2019, Volume 513, Pages 734385 (11p.)
https://doi.org/10.1016/j.aquaculture.2019.734385
https://archimer.ifremer.fr/doc/00509/62066/

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

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Abstract :

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

Highlights

► Mixed diets conferred higher fitness than the average of single-species diets, but not for the best single species ► Abalone are likely to invest more energy in muscle and gonad developments when fed on *Palmaria palmata*. ► At 2-year-old, an average of 90% of *H. tuberculata* started gonad development but <23% of the cohort had a full development ► Soluble carbohydrate, n-3/n-6 and total protein are the major algal components for reproduction and growth performance

Keywords : Abalone, Algae, Reproduction, Growth, Protein

1. Introduction

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

annually. Most of the algae harvested in France are used for food processing industry, chemistry and microbiology while less than 25% of seaweed is used in agricultural, health and well-being sectors (Mesnildrey et al., 2012).

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

Only a few studies directly compared the effects of different macroalgal diets on growth focusing on their seasonal composition changes on the long term (Basuyaux et al., 2018) and

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

2. Materials and Methods

2.1. Animals

This study was located downstream from the Aber Wrac'h river (48°36'46N; 4°33'30W), Brittany, France between the beginning of February 2012 and the end of January 2013. Abalone were born in summer 2010 at the local hatchery France Haliotis (Plouguerneau, France). After one year spent in the land-based nursery tanks, juveniles were transferred to the sea in pre-growing compartments. During this stage, they were fed a mixed algal diet based essentially on *P. palmata*, *S. latissima* and *L. digitata* and received more occasionally smaller quantity of the other experimental algal diets. In February 2012 (t_0), abalone were placed in 9 abblox farming sea cages. Each cage was made of 4 compartments of 1 m^3 (1 m x 1 m x 1 m) with a squared mesh size of 5 mm, each compartment containing 1000 individuals. Initial abalone length (L_{t0} = 24 ± 0.15 mm; mean ± s.e.) and weight (W_{t0} = 2.2 ± 0.041 g; mean ± s.e.) were balanced between the treatments at the beginning of the experiment (Table 1). Eighteen rows of 30

circular black plastic oyster-seed collectors of 140 mm diameter were placed into the compartments as shelter for daytime. Each compartment was identified with a tag attached inside the compartment enabling to identify the seaweed diet and compartment number. The cages were moored on a long-line used for anchorage.

2.2. Experimental design

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

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

2.3. Abalone measurements

2.3.1. Mortality

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

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

2.3.2. Growth

At the beginning of February 2012 (t_0), April (t_2), June (t_4), August (t_6), the end of October (t_8) and the end of January 2013 (t_{11}), 30 individuals from each compartment, i.e. 90 individuals per treatment, were randomly collected (the 2nd nearest neighbour to the first sighted chosen randomly) and brought back to France Haliotis farm. After pressing the abalone in absorbent paper to remove water from the pallial cavity, abalone were weighed to the nearest 0.01 g using a balance (Toploader balance, Kern) (W_{t0} , W_{t2} , W_{t4}, W_{t6} ,W_{t8}, W_{t11}) and shell length was measured to the nearest 0.5 mm using a Vernier calliper (L_{t0} , L_{t2} , L_{t4}, L_{t6}, L_{t8}, L_{t11}). After sampling, abalone individuals were removed from the experiment.

The following growth indices were calculated:

Final weight-to-shell length ratio (W/L_{t11}, g.mm^{-1}) = $\mathrm{Wt11/Lt11}$

Final daily weight gain ($DGW_{t0\text{-}11}$ in mg.day^{-1}) = $(\mathrm{Wt11} - \mathrm{Wt0})/\mathrm{day}$

Final daily length gain ($DGL_{t0\text{-}11}$ in µm.day^{-1}) = $(\mathrm{Lt11} - \mathrm{Lt0})/\mathrm{day}$

Specific growth rate (*SGRw*, %.day^{-1}) = 100 x ((LnWt11 − LnWt0)/day)

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

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

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

2.3.3. Gonad development

Twelve individuals per replicate were randomly sampled in July 2012 (n = 36 individuals per treatment). This corresponds to the beginning of the spawning period in *H. tuberculata* (Girard, 1972). For each individual, the muscle was separated from the gonad-digestive gland (*GDG* comprising crop, stomach, spiral cecum and gonad) and from the rest of the soft tissues (head, gills, heart, mantle, hypobranchial glands, anus, and intestine) by the same experimenter to reduce dissection variability. Total soft tissue (weighed before muscle separation), muscle, GDG, the rest of the soft tissues and shell were weighed to the nearest 0.01 g, just after dissection. Shell length was measured to the nearest 0.5 mm. Reproduction data were averaged per replicate (compartment) for analysis.

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

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

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

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

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

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

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

2.4. Algal measurements

2.4.1. Algal degradation in sea-cage structure

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

2.4.2. Biochemical analysis

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

2.4.3. Soluble carbohydrate analyses

Samples were analysed at t_0, t_2, t_4, t_6 and t_8. Soluble carbohydrate contents (potentially available, *carb*) were determined by an adaptation of the phenol sulphuric acid colorimetric method of Dubois et al. (1956). This method is based on the reduction of neutral sugars, and a little part of uronic acids, in 5-hydroxymethylfurfural by phenol, giving a characteristic yellow colour. Absorbencies were measured at 492 nm with a microplate photometer (Multiskan^{TM} FC,

Thermo Scientific) and compared to glucose standard curve. Titrations were done in triplicate and averaged for each sample.

2.4.4. Lipid analyses

Due to practical constraints, samples were only analysed at t_2. Lipid extraction was conducted on 150 to 200 mg algal powder according to Folch et al.'s method (1957) in 6 mL of a chloroform/methanol mixture (2/1, v/v). Lipid extracts were then flushed with nitrogen and stored at −20 °C before analysis. Fatty acids (FA) were analysed as fatty acid methyl esters (FAME) after total lipid transesterification. Briefly, after addition of tricosanoic acid (23:0) as an internal standard and evaporation to dryness under nitrogen, 800 µL of MeOH/BF_3 (14 % by weight) was added and the transesterification reaction occurred during 10 min at 100°C, as described by Le Grand et al. (2014). Then, 0.8 mL of hexane was added and the organic phase containing FAME was washed three times with 1.5 mL of hexane-saturated distilled water. The organic phase was finally recovered for HPLC purification to isolate FAME. FAME were then analysed by a gas chromatograph (GC) VARIAN CP 3800 equipped with two automatic on column injectors and two flame ionization detectors (FID). FAME were separated using both polar (CPWAX 52 CB—30 m 9 0.25 mm i.d.; 0.25 lm thickness, Varian) and non-polar (CP-Sil 8 CB—30 m 9 0.25 mm i.d.; 0.25 lm thickness, Varian) capillary columns. Combined with the use of commercial and home-made analytical standards, this allowed to separate and identify FAME. The quantity of each fatty acid was quantified on a proportional basis relative to the peak area of the C23:0 internal standard. Quantitative fatty acid spectra obtained by GC were used to calculate the molar content of each fatty acid in the samples. Depending on the number of double bonds they displayed, FA were classified in three groups: saturated FA (SFA, no insaturation), monounsaturated (MUFA, only one insaturation) and polyunsaturated (PUFA, two or more insaturations). FA could also be differentiated by the position of the first double

bond from the terminal carbon: n-3 (omega 3) or n-6 (omega 6). The results used in the PCA were: total lipid content (*liptot*, µg FA / mg dry matter), n-3/n-6 ratio (*n3n6*), MUFA content (*lipmono*, % of total FA), PUFA content (*lippoly*, % of total FA) and SFA content (*lipsat*, % of total FA).

2.4.5. Protein and amino-acid analyses

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

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

For total and free amino acid profiling, 50 µL of each methanol–water extract were dried under vacuum. Dry residues were suspended in 50 µL of ultrapure water and 5 µL were used for the derivatization employing the AccQ-Tag Ultra derivatization kit (Waters, Milford, MA, USA). Derivatized amino acids were analyzed using an Acquity UPLC-DAD system (Waters). BABA was used as internal standard.

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

2.5. Data analysis

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

To study the effect of period and algae species on chemical algal composition, an analysis of variance was used, with algae (B, D, E, G, H, L, P and U) and period ($t_{0\text{-}2}$, $t_{2\text{-}4}$, $t_{4\text{-}6}$, $t_{6\text{-}8}$, $t_{8\text{-}11}$ for dry matter content, degradation and carbohydrate analysis; t_0, t_4, t_8, for protein analysis; period was not included in the model for lipid because only one period analysis), and the interaction between algae and period as fixed factors. When normal distribution of the residuals and homogeneity of variances were verified and global effects were found, *post-hoc* Tukey tests for multiple comparisons of means were carried out. When normal distribution of the residuals or homogeneity of variances was not verified, log, square root or inverse transformations were used. If analysis of variance conditions were not fulfilled, a Welch test was performed to study algal effect. If significant, pairwise post-hoc comparisons were carried out using Games-Howell post-hoc test based on Welch's degrees of freedom correction and uses Tukey's studentized range distribution. A Friedman test was used to test algal seasonal effect when conditions of

analysis of variance were not fulfilled. However, interaction between the seasonal and algal effect could not tested with these non-parametric tests.

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

The seasonal effect of algal diet on the two-month daily weight gain and daily length gain was analysed by a linear mixed effects analysis using the lmerTest package (Bates et al., 2012) and using the method described by Winter (2013). Square root transformation was used to homogenise variances between the treatments for DGW. The model included the period ($t_{0\text{-}2}$, $t_{2\text{-}4}$, $t_{4\text{-}6}$, $t_{6\text{-}8}$, $t_{8\text{-}11}$), the 6 monospecific algal diets (D, G, H, L, P and U), and an interaction between period and diet as fixed effects. The cages were indicated as a random factor (18 cages). B and E diets could not be included in the model due to missing data for the last period. In addition, because M diet was composed of algae in different proportion at each season, it was not included in the model. For the post-hoc analysis, the difflsmeans package was used. It calculated differences of Least Squares Means for the factors of lmer mixed effects model and used the Satterthwaite's approximation to degrees of freedom.

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

3. Results

3.1. Abalone measurements

Initial mean weight and length were not significantly different ($p > 0.05$). However, after one-year experiment, a diet treatment effect was observed on all growth parameters, i.e. on the mean final length ($p < 0.001$) and weight ($p < 0.001$), on $DGL_{t0\text{-}11}$ ($p < 0.001$), on $DGW_{t0\text{-}11}$ ($p < 0.001$), on SGRw ($p < 0.001$) and on W/L_{t11} ($p < 0.001$). No effect was reported on mortality ($p > 0.05$) (see Table 1 for p and F values).

After one-year experiment, the best growth performances in weight were observed for abalone fed the *P. palmata* diet, followed by the mixed diet. Abalone fed with *S. latissima*, *Gracilaria* sp. and *L. digitata* presented intermediate growth performances. The lowest performances were observed for abalone fed *Enteromorpha* sp., *U. lactuca*, *S. polyschides* and stipes of

L. hyperborea. This diet ranking performance was maintained for the final weight-to-shell length ratio (see Table 1 for post-hoc analysis comparison).

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

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

When studying more specifically the effect of the period and diet on abalone seasonal DGW and DGL, an important period effect (mixed model, DGW: $F_{4,60} = 25.61$, $p < 0.001$; DGL: $F_{4,60} = 10.60$, $p < 0.001$), a diet effect (mixed model, DGW: $F_{5,60} = 36.09$, $p < 0.001$; DGL: $F_{5,60} = 14.70$, $p < 0.001$) as well as an interaction between diet and period (mixed model, DGW: $F_{20,60} = 2.38$, $p < 0.01$; DGL: $F_{20,60} = 1.98$, $p < 0.05$) were observed. The periods from late spring to the end of autumn (t_{4-6} and t_{6-8}) were the best for abalone DGL and DGW compared to late winter (t_{0-2}), and spring (t_{2-4}) periods, the worst period being in winter (t_{8-11}) for DGL (Figure 2). However, the seasonal effect was different depending on algal diets. For example, abalone fed with algae such as *P. palmata* and *U. lactuca* had similar DGL and DGW from t_0 to t_8, and presented a decrease in DGL and DGW only in winter (t_{8-11}) (Figure 3). Abalone fed other diets

such as *L. digitata* presented important DGL and DGW variation depending on the period (Figure 3).

3.2. Algal biochemical measurements

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

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

Apart for histidine and tryptophan, significant differences of total amino-acid compositions were observed between the eight algal diets (Table 4). The red algae *P. palmata* and *Gracilaria* sp. presented the highest content for most of the total essential amino-acid. The brown algae *L. digitata* and the stipes of *L. hyperborea* presented most of the time the lowest total amino

content while the green algae and the kelp *S. latissima* presented intermediate contents. In addition, differences ($p < 0.05$) were observed between the 8 algae for free amino-acid contents apart for methionine and valine. However, free essential amino acid contents were variable depending of the algae and the amino-acid (Table 4).

3.3. Relationship between growth and algal composition

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

The first three components of the PCA based on the abalone variables and amino-acid composition explained 61.4 % of the total variance (37.5 % for the first component, 14.9 % for the second component and 9.1 % for the third component) (Figure 5). For the abalone variables,

the most important loadings on the first component were the GI ($cos^2 = 0.76$), VGI ($cos^2 = 0.75$) associated with T_Val ($cos^2 = 0.88$), T_Met ($cos^2 = 0.87$), T_Leu ($cos^2 = 0.86$), T_Arg ($cos^2 = 0.85$), and T_Ile ($cos^2 = 0.84$). This component could represent the reproductive development associated with the total amino-acid composition. The most important loadings on the second component were F_Phe ($cos^2 = 0.60$), F_Ile ($cos^2 = 0.54$) associated with DGWseason ($cos^2 = 0.42$).

4. Discussion

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

Good growth rates are important to reach a marketable size within a time which is economically viable. *H. tuberculata* is a slow-growing species with an average monthly growth rate between 1 mm and 2 mm, depending on the farming conditions (Basuyaux, 1997; Lachambre, 2017). This experiment was conducted in a commercial set-up, with the same density, cage design, and feeding rhythm as France Haliotis organic certified farming practices. Our study demonstrated that abalone fed on *P. palmata*, mixed diet and *S. latissima* presented the best growth rate, muscle ratio and gonad development. *Gracilaria* sp., *L. digitata* and *Enteromorpha*

sp. gave moderate growth performances while *U. lactuca* and stipes of *L. hyperborea* presented the lowest growth performances. Previous studies on *H. tuberculata* have shown that diets based on *P. palmata* produced better growth rates than most of the algae found in Europe (Mercer et al., 1993; Mai et al., 1996; Viera et al., 2015; Basuyaux et al., 2018). In addition, the high nutritional value of *P. palmata* has been reported for other abalone species such as *H. discus hannai* (Uki et al., 1986; Mercer et al., 1993). However, other experiments found different results for algal species such as *S. latissima*, which gave one of the best growth in our one-year long experiment while it presented the lowest growth performance in other experiments (Mercer et al., 1993; Mai et al., 1996). *U. lactuca* was one of the algal diets that gave the poorer growth while it turned to be moderately good for Mercer et al. (1993). The difference of experiment duration can explain part of the difference (50 weeks for our experiment, 17 weeks for Mai et al., 1996; and 33 weeks for Mercer et al., 1993) and probably results from the high variability in algal composition according to the season and site (Renaud and Luong-Van, 2006; Villares et al., 2013; Schmid et al., 2014). In addition, these experiments were performed in controlled conditions with stable temperature and renewal of algae every 5-7 days compared to our commercial scale experiment submitted to seasonal temperature change, open-seawater conditions and a monthly feed renewal. In addition to growth performance, the degradation of algae in the sea-cage structure is an important factor to integrate in the choice of a commercial structure and varied between 33% up to 93% degradation over one month. *P. palmata*, *L. digitata* and *S. latissima* showed the least degradation over one month. However, in our experiment, growth performances of abalone were not related to degradation of algae. Another interesting finding for commercial purpose is the high variability in term of muscle on total weight ratio (%) with more than 25% more muscle in abalone fed *P. palmata* diet compared to *Enteromorpha* sp. diet. This resulted directly from diet treatment as the abalone individuals were from the same genetic stock.

In this specific context, it puts forward the importance of measuring weight in addition to length, and more specifically muscle on total weight ratio.

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

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

In natural conditions, abalone consume a variety of seaweeds, trapping drift kelp or selecting attached benthic algae mainly according to their abundance and avaibility in the surrounding area, and water movement (Cornwall et al., 2009; Zeeman et al., 2012). It has been often reported that growth is improved when abalone are fed on a combination of macroalgae species in comparison to a single species diet (Mercer et al., 1993; Qi et al., 2010b; Viera et al., 2015). In our experiment, mixed diet gave the second best growth performance, preceded by *P. palmata*. This result is in accordance with the meta-analysis of Lefcheck et al. (2013) performed on variety of taxa and systems showing that mixed diets conferred significantly higher fitness than the average of single-species diets, but not for the best single prey species. However, in

order to prevent depletion of the algal resources, a mixed diet seems an optimal solution for European abalone farms, with good growth performance as tested by Basuyaux et al. (2018).

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

Due to the small size of abalone used in our study, it was not possible to separate the gonad and the digestive gland. Thus, gonad weight had been estimated using histology. This method gave a good proxy of the proportion of gonad on the gonad-digestive gland even if it does not give an exact value. Abalone fed *P. palmata* and mixed diet (yearly average; *S. polyschides*: 15.5 %; *L. digitata*: 20.5%; *P. palmata*: 43%; *S. latissima*: 8%; *U. lactuca*: 12%) developed more gonad compared to abalone fed on stipes of *L. hyperborea*, which showed higher shell ratio. In addition, visual gonad index as well as gonadal index were related to protein content and some

amino-acids (total valine, leucine, isoleucine, methionine, arginine and threonine). These results are consistent with other studies in abalone *H. iris* (Tung and Alfaro, 2012) and sea urchins *Stronglyocentrotus droebachiensis* (Lyons and Scheibling, 2007) where an effect of natural or artificial diets was demonstrated on both growth performance and gonad development by influencing a shift between somatic and gonadal depots. Crude protein content of the seaweed seems to play an important role in *H. tuberculata coccinea* reproduction, fed on *Gracilaria cornea* with different protein levels (Bilbao et al., 2012).

5. Conclusion

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

Acknowledgment

This work has benefited from the support of the French Government by National Research Agency with regards to an investment expenditure program IDEALG ANR-10-BTBR-04. The authors would like to thank the team of France Haliotis, Iain McKenzie-Sproat and Xavier Lesage for the care they provided to the animals and the assistance during the experiment.

Metabolic profiles were supported by the technical platform “P2M2” (Metabolic Profiling and Metabolomic Platform, INRA, le Rheu, France), a component of the Biogenouest metabolomics core facility “Corsaire”. Special thanks go to Solenne Berardocco and Nathalie Marnet for coordinating analyses.

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Table 1: Growth in length, weight and mortality of juvenile abalone (*H. tuberculata*) which received a monospecific diet of eight different algae (B: *Saccorhiza polyschides*, D: *Laminaria digitata*; E: *Enteromorpha* sp., G: *Gracilaria* sp., H: stipes of *Laminaria hyperborea*, P: *Palmaria 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) $^{\gamma}$	Mean final length (mm)	Final daily length gain ($\mu m.day^{-1}$)	Mortality (%)$^{\gamma}$
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

Diet	Mean initial weight (g) $^{\gamma}$	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°
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	***	***	***	***	

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

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

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

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

° rank based on growth rate in weight

$^{\gamma}$ Log transformation

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

Diet	Gonad-digestive gland on total weight ratio (%)	Muscle on total weight ratio (%) $^{\alpha}$	Shell on total weight ratio (%)	Gonadal index (%) $^{\alpha}$	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}
Ftreat	$F_{8,18}$ = 5.72	$F_{8,7.4}$ = 28.84	$F_{8,18}$ = 9.66.	$F_{8,\ 6.7}$ = 110.9	$F_{8,18}$ = 11.13
Ptreat	***	***	***	***	***

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

$^{\alpha}$ non-parametric Welch test with Games-Howell post-hoc analysis

$^{\gamma}$ Log transformation

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

	Dry matter (%) $^{\alpha}$.	Soluble carbohydrate content (% dry matter) $^{\alpha}$	Total lipid content (µg FA.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) $^{\alpha}$	Proportion of algae degraded after 1 month in sea-structure (%) $^{\alpha}$
B	9.7 ± 0.52 ^{a}	5.1 ± 2.30 ^{a}	8.2 ± 1.51 ^{a}	1.5 ± 0.27 ^{ab}	21.2 ± 0.08 ^{bc}	48.9 ± 0.99 ^{bcd}	29.9 ± 0.91 ^{a}	6.4 ± 0.30 ^{ab}	85.2 ± 6.11 ^{a}
D	18.8 ± 1.33 ^{b}	13.6 ± 17.13 ^{ab}	10.7 ± 0.83 ^{a}	1.5 ± 0.43 ^{ab}	22.1 ± 1.29 ^{ab}	47.1 ± 2.84 ^{bcd}	30.9 ± 1.55 ^{a}	5.0 ± 0.57 ^{a}	44.7 ± 4.51 ^{b}
E	13.4 ± 1.25 ^{ab}	5.2 ± 2.98 ^{a}	35.5 ± 0.27 ^{b}	8.6 ± 2.03 ^{b}	11.8 ± 1.01 ^{ab}	64.4 ± 3.69 ^{d}	23.8 ± 2.70 ^{a}	14.0 ± 0.93 ^{bc}	92.9 ± 2.14 ^{a}
G	14.9 ± 1.72 ^{ab}	6.4 ± 2.24 ^{ab}	8.04 ± 1.72 ^{a}	1.4 ± 0.08 ^{ab}	9.8 ±1.88 ^{ab}	8.7 ± 0.70 ^{a}	81.5 ± 1.18 ^{b}	11.6 ± 1.23 ^{bc}	84.0 ± 4.72 ^{a}
H	14.4 ± 0.44 ^{ab}	4.0 ± 1.47 ^{a}	5.7 ± 2.45 ^{a}	0.7 ± 0.37 ^{a}	29.0 ± 3.38 ^{c}	29.3 ± 7.76 ^{ab}	41.7 ± 4.38 ^{a}	4.8 ± 0.49 ^{a}	37.4 ± 7.13 ^{b}
P	15.0 ± 0.52 ^{ab}	37.5 ± 11.38 ^{c}	12.4 ± 0.08 ^{a}	38.0 ± 0.45 ^{b}	6.8 ± 0.46 ^{a}	61.6 ± 3.60 ^{cd}	31.5 ± 3.14 ^{a}	19.6 ± 0.97 ^{c}	33.4 ± 6.51 ^{b}
S	18.8 ± 0.90 ^{b}	23.1 ± 18.64 ^{bc}	4.7 ± 0.41 ^{a}	0.5 ± 0.09 ^{a}	28.1 ± 3.24 ^{c}	33.7 ± 4.17 ^{ac}	38.3 ± 0.93 ^{a}	9.7 ± 1.27 ^{ab}	48.5 ± 2.66 ^{b}
U	19.7 ± 1.56 ^{b}	4.4 ± 2.49 ^{a}	9.5 ± 2.37 ^{a}	7.7 ± 1.86 ^{a}	19.1 ± 3.53 ^{ac}	39.4 ± 9.93 ^{bcd}	41.5 ± 6.39 ^{a}	6.6 ± 0.31 ^{ab}	80.5 ± 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	*

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 contrasts.
$^{\alpha}$. Welch test for algae effect with Games-Howell post-hoc analysis. Friedman test for period effects.

Table 4: Amino-acid composition of eight different algae (B: *Saccorhiza polyschides*, D: *Laminaria digitata*; E: *Enteromorpha* sp., G: *Gracilaria* sp., H: stipes of *Laminaria hyperborea*, P: *Palmaria palmata*, S: *Saccharina latissima*, U: *Ulva lactuca*) distributed to *H. tuberculata* during a one-year commercial scale feeding trial (mean ± SE, n = 3 replicate corresponding to t_0, t_4, and t_8 period per algae).

	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 $^{\alpha}$	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 $^{\alpha}$	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 $^{\alpha}$	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 $^{\gamma}$	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 $^{\alpha}$	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 $^{\gamma}$	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 $^{\beta}$	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 $^{\gamma}$	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

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

$^{\gamma}$. Log transformation

$^{\beta}$ Square root transformation

$^{\alpha}$. 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 abblox 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 ± 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 ± 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

Highlights of the manuscript:

1. Mixed diets conferred significantly higher fitness than the average of single-species diets, but not for the best single *P. palmata* species
2. Abalone are likely to invest more energy in muscle and gonad developments when fed on *Palmaria palmata*.
3. At 2-year-old, an average of 90% of *H. tuberculata* started gonad development but less than 23% of the cohort featured a full gonad development
4. Soluble carbohydrate content, n-3/n-6 ratio, total protein content and phenylalanine -isoleucine free amino-acids are the major algal components implicated in initial gonad development and growth performance