#### Research Article

# Assessing the impacts of several algae-based diets on cultured European abalone (*Haliotis tuberculata*)

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Abstract – The effects of different algal diets on the mortality, apparent ingestion, weight, length and conversion rates of the European abalone (Haliotis tuberculata) maintained in a semi-closed seawater system throughout the year were compared. Various combinations of red algae (Palmaria palmata, *Ceramium rubrum and Chondrus crispus* cultured or harvested from the natural environment, as well as Porphyra spp. collected), brown algae (Laminaria digitata) and green algae (fresh or frozen Ulva intestinalis) were tested. The results showed that P. palmata, C. rubrum and U. intestinalis administered alone were associated with significantly higher weight growth rates than the other species of algae tested. However, some combinations of algae (i.e. different proportions of L. digitata in association with P. palmata) were more favorable for weight increase when compared with the expected rates calculated for the diet based on L. digitata alone. Limiting the amount of any of these foods substantially reduced the conversion rate. Seasonal trends were apparent in both weight increase and food conversion rates, with the result that growth in weight on a diet of L. digitata was fastest in summer. Growth on P. palmata was faster in each season, and reached a maximum in early spring. The data collected allowed us to model weight increase and month-to-month food conversion rates for a diet based on P. palmata and L. digitata. The data obtained in this study were coupled with data regarding the availability of algae during the year, enabling us to suggest an optimal diet for each of the four seasons. Finally, the effects of different algae diets were investigated on hemocyte parameters, and the result suggested that *P. palmata* would reinforce the immune system of abalone.

Keywords: Abalone / rearing / aquaculture / macroalgae / diet / immune system

# 1 Introduction

The European Abalone, *Haliotis tuberculata*, is a novel candidate for aquaculture because of its high market price (Viera Toledo, 2014; Riera, 2016): 75€ per kg presently in France (www.abalonebretagne.com). Abalone have been farmed in France from the 1990s and several farms are currently in operation throughout Normandy and Brittany (Riera, 2016). Two types of diet exist for abalone in aquaculture: a formulated diet (fish meal, casein protein, seed oils and/or vegetable fiber) or fresh algae (Sales and Janssen, 2004). The main countries involved in intensive abalone

production are China, Korea, South Africa, Chile and Australia, and these traditionally utilise formulated diets. However, a fresh algae diet is thought to project an image of quality to the customer and to promote animal welfare while the formulated diets are thought to provide better results in weight (g) and length (mm) increase (Pérez-Estrada et al., 2010; Bansemer et al., 2014; Serviere-Zaragoza et al., 2015).

The European abalone is mainly phytophagous (Mottet, 1978; Clavier and Richard, 1985), and its food preferences have been well-studied (reviewed in Mgaya and Mercer, 1994; Viera Toledo, 2014). Red algae, such as *Palmaria palmata*, *Delesseria spp.* and *Griffithsia spp.* are favored, but brown and green algae, such as *Laminaria digitata*, *Ulva lactuca* and *Ulva intestinalis* are also consumed (Bossy and Culley, 1976; Koike et al., 1979; Culley and Peck 1981; Mercer et al., 1993;

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Viera et al., 2005, Viera Toledo, 2014). In addition, wild abalones are often found with hydrozoa, polyzoa, sponges, diatoms and foraminifera in their digestive system (Bossy and Culley, 1976). Mai et al. (1994) have reported that certain amino acids from algae (particularly arginine, methionine and threonine) can be the limiting factor in abalone growth, and recommend using a mixture of algae to combat this. A study by Mercer et al. (1993) showed that certain mixed diets showed better or at least similar dietary values in comparison with a single algal diet.

Despite these decades of work however. Venter et al. (2016) have suggested that this subject is still poorly understood and merits more investigation. Numerous researchers have studied the seasonal variations of the main chemical components of algae in situ (Black, 1948; Haug and Jensen, 1954; Citharel and Villeret, 1961; Chapman and Craigie, 1977; Chaumont, 1978; Morgan et al., 1980; Dion, 1983; Maita et al., 1991; Mizuta et al., 1992; Basuyaux, 1997). These authors have reported significant seasonal variations in dry matter, total nitrogen, carbon, vitamins and minerals. The two species of algae mostly used are P. palmata and L. digitata, and their composition of dry matter, total nitrogen and carbon are well known in the study area (Basuyaux (1997). Unfortunately, it is very difficult to relate increase in weight and length to only algal composition, making factors such as weight increase, feed conversion ratio, mortality, and immune parameters more appropriate measures of value for aquaculturists. The ideal diet from the perspective of an aquaculturist is one in which provides positive increases in weight and length in an economical time frame.

The nutritive quality of a given algae and its palatability to abalone varies throughout the year (Rosen et al., 2000). The abundance of different algae along the French coastline fluctuates during the year, causing seasonal changes in the cost of harvest (Le Gall et al., 2004). *Laminaria digitata* is the main species harvested on French coasts, with production varying from 40 to 60 000 metric tonnes per year, representing 90% of the total production of French algae (Mesnildrey et al., 2012). The selling price is around  $\notin$  40/metric tonnes (Arzel, 2004). *P. palmata* is known for its nutritional value to abalone, but only about 300 metric tonnes are annually harvested in France (Mesnildrey et al., 2012). The average price is about  $\notin$  400/metric tonnes. Other species (*Ascophyllum nodosum, Chondrus crispui, enteromorpha spp.*...) are also collected in smaller quantities (Mesnildrey et al., 2012).

Since 1997, the European abalone has suffered mass mortality events in both the natural environment and in aquaculture facilities due to infections by Vibrio harvevi (Nicolas et al., 2002). The abundance of this bacterial pathogen is correlated with seawater temperature and peaks during the period of sexual maturity (Travers et al., 2009). The immune system plays a critical role in resistance to this bacterium (Dubief et al., 2017), and certain algal-based diets can improve immune function; for example, Ulva spp. increases phagocytic rate in Haliotis laevigata compared to a formulated diet (Stone et al., 2014). In molluscs, the cellular immune system is composed of hemocytes, and their phagocytic immune response is considered to be the first line of defense. It is also complemented by an array of other defense mechanisms, which may include lysosomal secretion of hydrolytic enzymes involved in the degradation of foreign particles (Anderson

et al., 1995; Wootton and Pipe, 2003). Among these enzymes, non-specific esterases play an important role in the intracellular degradation of pathogenic organisms (Gagnaire et al., 2004) and their activities appear to be a sensitive indicator for ecotoxicological studies (Mottin et al., 2010; Minguez et al., 2014). In gastropods, particularly abalone, hemocytes seem to be affected by environmental factors such as dissolved organic compounds (Martello et al., 2000), infections (Wang et al., 2004) and abiotic stressors (Cheng et al., 2004a–d; Travers et al., 2008), which can result in higher susceptibility to infections and associated diseases. In order to assess the effect of different diets on the immune system of *H. tuberculata*, four parameters were measured: hemocyte density; phagocytic activity; the presence of lysosomes with a stable membrane and the activity of non-specific esterases.

Given the lack of data and the variability (both quantitative and qualitative) of algal resources for abalone, we conducted an investigation to assess (1) abalone survival and growth in relation to different algal diets and (2) the effects of seasonal variability in algal quality and quantity. The effects of these diets on abalone immune function were assessed in parallel. The ultimate aim of this study was to identify the most biologically- and financially-effective diet for farmed abalones according to the availability and nutritive qualities of algae in the English Channel throughout the year.

# 2 Materials and methods

Six experiments were carried out:

- four successive experiments (E1 to E4) of three months each investigated the influence of food quality (supplied *ad libitum*) for each season over a year;
- a fifth experiment (E5) evaluated the influence of the quantity of food supplied;
- a sixth experiment (E6) was devoted to the evaluation of each diet effect on immune parameters.

Experiment periods, initial lengths and initial weights are shown in Tables 1 and 2. The first five experiments (E1 to E5) were carried out with the same experimental design using animals that were initially similarly-sized.

# 2.1 Biological material

In the first five experiments (E1 to E5), 10-month old European abalone (*H. tuberculata*) from the Synergie Mer Et Littoral (SMEL) in Blainville sur mer (la Manche, France) were measured three times (Tab. 1). In each of the five experiments, we used 27 baskets of 50 animals between 15-20 mm in shell length with three replicates for each of the nine diets (Tab. 2) (1,350 abalones per experiment, i.e. 6,750 individuals in total).

For the sixth experiment (E6), 15-month old abalones from Normandie Abalone in Agon Coutainville (Manche, France) were used so that a sufficient quantity of hemocytes could be extracted. Nine batches of 11 animals (3 different algae diets tested in triplicates) were assessed simultaneously (99 abalones in total).

Experiment period	Initial measurements	Midpoint measurements	Final measurements
E1 – Winter	03 November	19 December	02 February
E2 – Spring	16 February	02 April	11 May
E3 – Summer	04 June	15 July	25 August
E4 – Autumn	04 September	13 October	26 November
E5- Winter	09 December	22 January	08 March
E6- Winter	06 December	09 January 12 February	28 March

Table 1. Testing periods and measurement schedule.

Table 2. Mean initial length (mm), mean weight (g), and confidence intervals ( $\alpha = 0.05$ ) of abalone used in each experiment.

Experiment period	E1 (Winter)	E2 (Spring)	E3 (Summer)	E4 (Autumn)	E5 (Winter)	E6 (Winter)
Length (mm) Weight (g)	$\begin{array}{c} 17.6 \pm 0.3 \\ 0.82 \pm 0.04 \end{array}$	$\begin{array}{c} 19.0 \pm 0.5 \\ 1.02 \pm 0.08 \end{array}$	$18.5 \pm 0.7 \\ 1.02 \pm 0.11$	$18.3 \pm 0.7 \\ 0.96 \pm 0.11$	$\begin{array}{c} 18.9 \pm 0.5 \\ 0.97 \pm 0.09 \end{array}$	$\begin{array}{c} 30.2\pm 0.1 \\ 4.40\pm 0.10 \end{array}$

#### 2.2 Experimental structure

Abalone were placed in rectangular  $(30 \times 20 \text{ cm})$  mesh baskets (3 mm mesh size) made of polyethylene with a surface area of 600 cm<sup>2</sup> plus PVC vertical supports with a surface area of 1,572 cm<sup>2</sup>. They were arranged in a re-circulating system (8 m<sup>3</sup> h<sup>-1</sup>) described by Birais and Le Gall (1986). Basket placement was randomised within the rearing structure. The water depth was 5 cm and the rate of water renewal was 20% d<sup>-1</sup>, which was adequate for maintaining good water quality. Seawater parameters were daily monitored: temperature:  $18.5 \pm 0.5$  °C; salinity:  $33 \pm 1\%$ ; pH:  $8.3 \pm 0.1$ ; alkalinity:  $4.0 \pm 0.5 \text{ mmol } 1^{-1}$ , NH3<sup>-4</sup>:  $< 0.5 \text{ mgN } 1^{-1}$ , NO<sub>2</sub>:  $< 0.5 \text{ mgN } 1^{-11}$ , NO3:  $< 2 \text{ mgN } 1^{-1}$ . Fecal matter was daily removed by siphoning.

#### 2.3 Algae

Laminaria digitata, Palmaria palmata, Chondrus crispus, Ulva intestinalis and Porphyra umbilicalis were harvested from boulders on the coast of Blainville sur Mer (in the northwestern Manche region of France,  $49^{\circ}$  3' N/1°37' E) every two weeks, and stored in outdoor aerated tanks until use. *Chondrus crispus* and *Ceramium rubrum* were produced by the Echinoxe<sup>®</sup> company in Pirou (Manche, France). These algae were grown outdoors in a seawater pond with a surface area of 3.8 m<sup>2</sup> and a volume of 2.5 m<sup>3</sup>. These algae were held in suspension with aeration, and had continuous replenishment of dissolved nutrients.

*Ulva intestinalis* was harvested during April and June at Blainville sur Mer and frozen at -20 °C until use.

#### 2.4 Diets

Nine diets were tested in each experiment (Tab. 3). Laminaria digitata and P. palmata were tested alone or as a 25/75, 50/50 or 75/25% mixture. Other diets tested over one year depended on algae seasonal availability. The animals were fed ad libitum, except for the Pp50 and Pp75 diets which,

respectively, represented 50 and 75% of the quantities of *P. palmata* supplied in the *P. palmata* diet alone.

The impact of the quantity of food was tested in the fifth experiment (E5) using the same protocol and with algal intakes between 20 and 100% of the amount of *P. palmata* consumed by unrestricted abalone. In the 100% diet, animals always had algae available (*ad libitum*) with between 50 g and 80 g of seaweed were added once or twice a week depending on the animal apatite. When the 100% treatment group had consumed the majority (about 90%) of its algae, a treatmentappropriate amount of algae was added to all diet groups simultaneously.

In the sixth experiment, three types of algae were supplied *ad libitum* to the abalone: *P. palmata*, which is associated with optimal growth; *L. digitata*, a species with average nutritional quality; and *Fucus serratus*, an alga showing a poor nutritional value. These algae were collected from the intertidal zone in Luc-sur-Mer (Normandie, France,  $49^{\circ} 19' \text{ N/0}^{\circ} 20' \text{ E}$ ).

## 2.5 Parameters measured

#### 2.5.1 Mortality and biometrics

The animals were daily observed, and any dead individuals were removed without replacement. The mortality rate (M) was calculated at the end of the experiments as follows:

M=Number of deaths/50 (initial number of individuals in a given basket)

Individual weight ( $\pm 0.1$  g) was measured at the beginning (Tab. 2), the middle and the end of the experiment after drying each abalone for one minute on absorbent paper. Algal diets and seasonal effects were compared on the basis of specific weight growth rate (SGR in % d<sup>-1</sup>), food conversion rate (FCR without unit) and apparent ingestion rate (AIR without unit) according to the formulas described below.

$$SGR = [(fW/iW)^{1/t} - 1] \times 100$$
  
FCR = A/(n × W)  
AIR = A/(B × t)  
with

**Table 3.** Quarterly mortality rate for each diet by testing period: *Pp: Palmaria palmata, Ld: Laminaria digitata, Ui: Ulva intestinalis, Cr: Ceramnium rubrum, Pp25 – Ld75:* a diet composed of 25% *P. palmata* and 75% *L. digitata. Pp75%:* diet consisting of 75% of the amount of *P. palmata* consumed when supplied *ad libitum.* 

	Mortality rate (%)				
	E1 (Winter)	E2 (Spring)	E3 (Summer)	E4 (Autumn)	
Palmaria palmata	0	0	0	1	
Pp75 - Ld25	4	0	1	0	
Pp50 - Ld50	0	1	1	0	
Pp25 - Ld75	0	0	0	1	
Laminaria digitata	0	1	0	0	
Ulva intestinalis (frozen)	4	2			
Ulva intestinalis (fresh)		1	1		
Pp50 - Ui(frozen50)	1				
Ld50 - Ui(frozen50)	1				
Pp33 - Ld33 - Ui (frozen33)	2				
Chondrus crispus		0			
Chondruscrispus(cultivated)		2			
Pp50 - Ui(fresh50)			1		
Porphyra umbilicalis			1		
Ceramium rubrum				1	
Ceramium rubrum (cultivated)				0	
Pp50 - Cr(cultivated50)				1	
<i>Pp</i> 50			1		
<i>Pp</i> 75				1	

fW and iW: final and initial mean weights (g)

t: duration of the experiment (days)

A: cumulative amount of algae ingested (g, drained fresh weight)

n: final number of abalone

W: mean individual weight gain (g) (W = fW - iW)

B: mean abalone biomass  $(B = [iW \times 50 + fW \times n]/2)$  (g) It is noticeable that the apparent ingestion rate did not take into account the algal degradation during experiment, but this parameter was useful for aquaculturists.

#### 2.5.2 Immune parameters

#### 2.5.3 Hemocyte collection and counting

Each month, three abalones from each triplicate and each regime were randomly sampled without removal from the experimental tanks. After an incision to the foot, hemolymph was collected (10–15 ml per animal) using a 20 ml syringe fitted with a 25-gauge hypodermic needle (Terumo ). Hemolymph was transferred to a sterile tube, diluted 1:4 in cooled sterile anticoagulant modified Alsever's solution (11 mM glucose; 27 mM sodium citrate; 11.5 mM EDTA; 382 mM NaCl) (Bachère et al., 1988), and centrifuged for 10 min (300 g, 4 °C). The supernatant was then discarded and artificial sterile seawater (436 mM NaCl, 53 mM mgSO<sub>4</sub>, 20 mM HEPES, 10 mM CaCl<sub>2</sub>, 10 mM KCl, final pH 7.4) was added. Hemocytes were counted with a hemocytometer, and rapidly plated at a density of 100 000 cells per well in 24-well plates.

#### 2.5.4 Hemocyte analysis

Hemocyte analysis was performed using an EPICS XL 4 (Beckman Coulter) flow cytometer with a minimum of 10 000 events counted per sample. Results were expressed as cell cytograms, indicating the size (FSC value), the complexity (SSC value) and the level of fluorescence using the FL1 channel as described elsewhere (e.g. Mottin et al., 2010).

Phagocytic activity was measured by quantifying the ingestion of fluorescent beads (yellow-green carboxylatemodified FluoroSpheres<sup>®</sup> beads, diameter 1 µm, Molecular Probes<sup>®</sup>). In each culture well, beads were added (1:100 cellbead ratio), and cells were incubated at 17 °C for 60 min in darkness. We only considered the percentage of hemocytes containing three beads or more in evaluating immunoefficiency (e.g. Delaporte et al., 2003; Hégaret et al., 2003; Evariste et al., 2016). The commercial LysoTracker<sup>®</sup> kit (Green DND-26, Molecular Probes, Invitrogen") was used as a lysosomal marker. Lysotracker probe was added to each well at a final concentration of 5 µM, and cells were incubated at 17 °C for 60 min in darkness. Esterase activity was measured using the non-specific liposoluble substrate fluorescein diacetate (FDA, Molecular Probes, Invitrogen<sup>®</sup>). FDA probe was added to each well at a final concentration of  $2 \mu M$ , and cells were incubated for 60 min at 17 °C in the dark. After incubation, the wells were gently scraped, hemocyte samples were then centrifuged (10 min, 300 g, 4 °C) and finally, the supernatant was removed and the cells fixed with 3% paraformaldehyde. Samples were stored at 4 °C until analysis.

Results were expressed as the percentage of cells containing fluorescence.

**Table 4.** Mean and confidence interval ( $\alpha = 0.05$ ) of apparent ingestion rates (AIR) bytesting period: *Pp: Palmaria palmata, Ld: Laminaria digitata, Ui: Ulva intestinalis, Cr: Ceramnium rubrum, Pp25–Ld*75: a diet composed of 25% *P. palmata* and 75% *L. digitata. Pp*75%: diet consisting of 75% of the amount of *P. palmata* consumed when supplied *ad libitum*.For each season, diets that do not share a letter significantly differ (ANOVA and SNK tests, p < 0.05).

	Apparent Ingestion	a Rate (% $d^{-1}$ )		
	E1 (Winter)	E2 (Spring)	E3 (Summer)	E4 (Autumn)
Palmaria palmata	$7.1 \pm 0.3^{a}$	$12.0 \pm 0.2^{a}$	$8.3\pm0.2^{\rm a,b}$	$7.3\pm0.2^{\rm a}$
Pp75 - Ld25	$7.5 \pm 0.3^{\rm a}$	$12.2 \pm 0.4^{\rm a}$	$8.5 \pm 0.4^{a,b}$	$8.2\pm0.6^{\mathrm{a}}$
Pp50 - Ld50	$7.9 \pm 0.4^{ m a}$	$12.4 \pm 0.8^{a}$	$7.8\pm0.3^{ m b}$	$7.5\pm0.3^{\rm a}$
Pp25 - Ld75	$8.6 \pm 0.3^{a}$	$14.3\pm0.2^b$	$9.1\pm0.7^{\rm a}$	$8.3\pm0.4^{\rm a}$
Laminaria digitata	$9.9 \pm 0.9^{ m a,c}$	$15.2 \pm 0.3^{\circ}$	$7.9 \pm 0.5^{a,b}$	$9.1\pm0.3^{b}$
Ulva intestinalis (frozen)	$22.6 \pm 3.8^{b}$	$27.2 \pm 0.3^{d}$		
Ulva intestinalis (fresh)		$16.0 \pm 0.2^{\rm e}$	$16.5 \pm 0.9^{\circ}$	
Pp50 - Ui(frozen50)	$8.8 \pm 0.4^{ m a}$			
Ld50 - Ui(frozen50)	$12.3 \pm 0.3^{\circ}$			
Pp33 - Ld33 - Ui(frozen33)	$10.0 \pm 0.2^{\rm a}$			
Chondrus crispus		$8.5\pm0.4^{\rm f}$		
Chondruscrispus(cultivated)		$9.1 \pm 0.4^{ m f}$		
Pp50 - Ui(fresh50)			$12.5 \pm 0.8^{d}$	
Porphyra umbilicalis			$7.9 \pm 0.1^{b}$	
Ceramium rubrum				$7.7\pm0.9^{\mathrm{a}}$
Ceramium rubrum (cultivated)				$8.8\pm0.3^{\mathrm{b}}$
Pp50 - Cr(cultivated50)				$7.4\pm0.2^{\mathrm{a}}$
Pp50			$5.1 \pm 1^{e}$	
<i>Pp</i> 75				$6.0\pm0.4^d$

### 2.6 Statistical analyses and modeling

All the results met parametric assumptions, so data were analysed with analyses of variance (ANOVAs) followed by post-hoc tests to make pairwise comparisons between groups. When the algal diets where tested each season, two-way ANOVAs were computed in order to assess the effect of each factor (diet and season) and the interaction between them (degrees of freedom of 3, 4 and 12 for, respectively, the diets, seasons and interactions). When significant differences were detected, Tukey's tests were performed to determine homogenous groups. The results about the diets studied during a single season were tested by one-way ANOVA followed by Student Newman-Keuls (SNK) tests. The differences in mortality rates between treatments were statistically analysed using Fisher's exact tests. These analyses were conducted using the software Statview 5.0 (SAS) with an alpha-value of 0.05.

The modeling of abalone weight increase and food conversion rate was performed using TableCurve 3D (SPSS). The fitting options utilise a pre-defined equation set. The choice of equation is based not only on the coefficient of correlation but also on the best fit at the edge of the model, so that it is consistent with biological reality.

# **3 Results**

## 3.1 Mortality

For each diet, mortality was relatively low: generally less than 2% per season (Tab. 3), with no significant differences

observed between treatment groups (Fisher; p > 0.05). The maximum mortality rate (4%) occurred during the winter for two diets. No mortality was observed during the sixth experiment.

#### 3.2 Apparent ingestion rates

Apparent ingestion rates (AIR) overall showed significant differences between seasons (F = 580, p < 0.001), diets (F=46, p < 0.001) and the interaction between both factors (F=9, p<0.001) (Tab. 4). AIR values were significantly higher during spring compared to the other seasons (Tukey's tests, 0.001 ). Except for Pp50 (5.1±1.0% insummer) and Pp75 (6.0±0.4% in autumn), the lowest AIR were recorded with *Palmaria* diets  $(7.1 \pm 0.3\%)$  in winter) whereas AIR maxima were generally observed in the spring (between  $8.5 \pm 0.4$  and  $27.2 \pm 0.3$ ). The apparent ingestion rate was particularly high with an U. intestinalis-based diet, with 16 to 27.2% of total abalone biomass daily consumed. For the other diets, this rate ranged from 7 to 8%, except in the spring when it reached about 12% with *P. palmata* and 15.2% with *L*. *digitata*, probably due to the greater palatability of algae during this season.

#### 3.3 Weight growth

Weight growth showed significant differences between seasons (F=99, p < 0.001), diets (F=224, p < 0.001) and the interaction between both factors (F=14, p < 0.001) (Tab. 5). Indeed, the rates of weight growth significantly differed

**Table 5.** Mean and confidence interval ( $\alpha = 0.05$ ) of weight growth rates bytesting period: *Pp: Palmaria palmata*, *Ld: Laminaria digitata*, *Ui: Ulva intestinalis*, *Cr: Ceramnium rubrum*, *Pp25 – Ld75*: a diet composed of 25% *P. palmata* and 75% *L. digitata*. *Pp75*%: diet consisting of 75% of the amount of *P. palmata* consumed when supplied *ad libitum*. For each season, diets that do not share a letter significantly differ (ANOVA and SNK tests, p < 0.05).

	Weight growth rate (% $d^{-1}$ )			
	E1 (Winter)	E2 (Spring)	E3 (Summer)	E4 (Autumn)
Palmaria palmata	$1.57 \pm 0.06^{a}$	$2.20\pm0.08^{\rm a}$	$1.52 \pm 0.08^{\rm a}$	$1.71 \pm 0.06^{a,c,d}$
Pp75 - Ld25	$1.50 \pm 0.04^{a,b}$	$2.05\pm0.07^a$	$1.52 \pm 0.11^{a}$	$1.61 \pm 0.01^{a,c}$
Pp50 - Ld50	$1.41 \pm 0.09^{b,c}$	$1.85 \pm 0.06^{\rm b}$	$1.31 \pm 0.13^{a}$	$1.45\pm0.04^{\rm a}$
Pp25 - Ld75	$1.22 \pm 0.11^{\circ}$	$1.57 \pm 0.02^{\circ}$	$1.23\pm0.12^{b}$	$1.31\pm0.05^b$
Laminaria digitata	$0.85 \pm 0.13^{d}$	$0.87 \pm 0.08^{\rm d}$	$1.04 \pm 0.07^{b,c}$	$1.16 \pm 0.05^{b}$
Ulva intestinalis (frozen)	$0.33 \pm 0.07^{\rm e}$	$0.80 \pm 0.10^{\rm d}$		
Ulva intestinalis (fresh)		$1.54 \pm 0.04^{\circ}$	$0.89 \pm 0.04^{\circ}$	
Pp50 - Ui (frozen50)	$1.27 \pm 0.03^{\circ}$			
Ld50 - Ui(frozen50)	$0.77 \pm 0.10^{ m f}$			
Pp33 - Ld33 - Ui(frozen33)	$1.29 \pm 0.04^{\circ}$			
Chondrus crispus		$0.69 \pm 0.06^{d}$		
<i>Chondruscrispus</i> (cultivated)		$0.79\pm0.20^d$		
Pp50 - Ui(fresh50)			$1.47 \pm 0.09^{\rm a}$	
Porphyra umbilicalis			$0.94 \pm 0.06^{\circ}$	
Ceramium rubrum				$0.94 \pm 0.15^{e}$
Ceramium rubrum (cultivated)				$1.88 \pm 0.10^{ m c,d}$
Pp50 - Cr(cultivated50)				$1.93 \pm 0.05^{d}$
<i>Pp</i> 50			$0.96 \pm 0.05^{\circ}$	
Pp75				$1.56 \pm 0.07^{a,c}$

(Tukey's tests, p < 0.001) between seasons except in winter vs summer (Tukey's test, p = 0.57). For each season, significant variations in weight growth occurred for each of the diets tested (Tukey's test, p < 0.001). Maximum yields were obtained for *P. palmata* (2.20% d<sup>-1</sup> in spring) and *C. rubrum* (1.88% d<sup>-1</sup> in autumn) alone or mixed (1.93% d<sup>-1</sup> in autumn). *U. intestinalis* was also associated with high weight increases; however, the nutritional quality of this alga greatly varied between spring (1.54% d<sup>-1</sup>) and summer (0.89% d<sup>-1</sup>). In addition, freezing seems to reduce the nutritional value of this seaweed: when harvested in the spring and then frozen, it resulted in a growth rate of only 0.80% d<sup>-1</sup> vs 1.54% d<sup>-1</sup> with fresh alguae. *C. crispus* cultured or harvested from nature was associated with a weight increase rate of about 0.75% d<sup>-1</sup>. *P. umbilicalis* harvested during summer resulted in weight increase rates of 0.94% d<sup>-1</sup>, which is comparable to that obtained with *L. digitata* alone.

The rate of weight gain significantly varied according to the season (SNK, P < 0.001). For *P. palmata*, the maximum rate of weight increase occurred in spring while the maximum growth for *L. digitata* was observed in autumn. In spring, the rate of the weight gain observed with *L. digitata* alone was 0.87% d<sup>-1</sup>, and 2.20% d<sup>-1</sup> for *P. palmata* alone. Based on these data, a mixture of 75% *L. digitata* and 25% *P. palmata* should allow a weight increase of 1.20% d<sup>-1</sup> ( $0.87 \times 75\%$  $+ 2.2 \times 25\%$ ), but instead the rate was measured at 1.57%: a gain of 30%. A 50/50 mixture of *C. rubrum* and *P. palmata* was associated with greater or equal weight increase than the growth obtained with either of these algae alone. It therefore appeared that a mixture of several algae species could increase abalone weight gain in some cases.



**Fig. 1.** Mean and confidence interval of the percentage relative weight gain  $[RWG = 100*SGR_{ration}/SGR_{100})$  for both periods according to the amount of food (*P. palmaria*). Continuous line: 1st period (9 December to 22 January), discontinuous line: 2nd period (22 January to 8 March), gray line: theoretical curve (Experimentation E5).

A decrease in food intake led to a significant decrease in weight (SNK, P < 0.001) (Tab. 6). The gains relative to the unrestricted weight increase calculated with *P. palmata* showed that there were no significant differences between the two periods. An intake of 20% of the maximum ration resulted in a 20% increase in weight in both periods. However, the correlation was not linear, with a 60% ration resulting in weight gain of about 70% and an 80% ration associated with growth of almost 85% (Fig. 1).

P. palmaria percentage	Weight growth rate (%.	Food conversion rate	
	First period	Second period	Overall
100	$2.26 \pm 0.05^{a}$	$1.83 \pm 0.06^{\mathrm{a}}$	$5.60 \pm 0.06^{\rm a}$
90	$2.11 \pm 0.01^{b}$	$1.81 \pm 0.05^{a}$	$5.46 \pm 0.27^{a,c}$
80	$1.96 \pm 0.09^{b,c}$	$1.79 \pm 0.19^{a,b}$	$5.24 \pm 0.18a^{a,d}$
70	$1.88 \pm 0.05^{ m c,d}$	$1.67 \pm 0.04^{b,c}$	$5.19 \pm 0.08a^{a,c,d}$
60	$1.82 \pm 0.07^{ m d}$	$1.55 \pm 0.13^{c,d}$	$5.02 \pm 0.28^{b,d}$
50	$1.47 \pm 0.05^{e}$	$1.53\pm0.05^d$	$4.91 \pm 0.10^{b,d}$
40	$1.24\pm0.09^{\rm f}$	$1.39 \pm 0.04^{\rm e}$	$5.18 \pm 0.05^{a,c,d}$
30	$1.06 \pm 0.12^{ m g}$	$1.20 \pm 0.06^{ m f}$	$4.93 \pm 0.06^{b,d}$
20	$0.66\pm0.13^{\rm h}$	$0.90\pm0.08^g$	$5.64 \pm 0.81^{a,c,d}$

**Table 6.** Mean and confidence intervals ( $\alpha = 0.05$ ) of the rate of increase for weight (% d<sup>-1</sup>) bytesting period according to the food ration (*P. palmaria*) (experimentation E5). For each period, diets that do not share a letter significantly differ (ANOVA and SNK, p < 0.05).

**Table 7.** Mean and confidence interval of food conversionrates ( $\alpha = 0.05$ ) by testing period *Pp*: *Palmaria palmata*, *Ld*: *Laminaria digitata*, *Ui*: *Ulva intestinalis*, *Cr*: *Ceramnium rubrum*, *Pp*25 – *Ld*75: a diet composed of 25% *P. palmata* and 75% *L. digitata*. *Pp*75%: diet consisting of 75% of the amount of *P. palmata* consumed when supplied *ad libitum*. For each season, diets that do not share a letter significantly differ (ANOVA and SNK tests, p < 0.05).

	Food conversion rate (FCR)			
	E1 (Winter)	E2 (Spring)	E3 (Summer)	E4 (Autumn)
Palmaria palmata	$4.83 \pm 0.32^{a}$	$6.63 \pm 0.36^{a}$	$6.32 \pm 0.52^{\rm a}$	$4.71 \pm 0.34^{a,b,c}$
Pp75 - Ld25	$5.26 \pm 0.09^{a}$	$7.03\pm0.38^{\rm a}$	$6.40 \pm 0.71^{a}$	$5.22 \pm 0.25^{a,b,c}$
Pp50 - Ld50	$5.64 \pm 0.35^{a}$	$7.77 \pm 0.64^{\rm a}$	$6.48\pm0.57^{\rm a}$	$5.67 \pm 0.30^{a,b,c}$
Pp25 - Ld75	$6.35\pm1.25^{\rm a}$	$10.25 \pm 0.11^{a}$	$8.02 \pm 1.18^{\rm b}$	$6.46 \pm 0.02^{a,c}$
Laminaria digitata	$11.43 \pm 1.88^{b}$	$17.95 \pm 1.95^{\rm b}$	$7.83\pm0.04^{\rm b}$	$8.06\pm0.34^a$
Ulva intestinalis (frozen)	$60.64 \pm 11.26^{\circ}$	$35.31 \pm 4.87^{\circ}$		
Ulva intestinalis (fresh)		$11.52 \pm 0.33^{a}$	$19.41 \pm 1.14^{\circ}$	
Pp50 - Ui(frozen50)	$7.51\pm0.27^a$			
Ld50 - Ui(frozen50)	$16.10 \pm 1.99^{\circ}$			
Pp33 - Ld33 - Ui(frozen 33)	$7.71 \pm 0.51^{a}$			
Chondrus crispus		$12.56 \pm 1.27^{\mathrm{a}}$		
Chondruscrispus(cultivated)		$12.28 \pm 4.16^{a}$		
Pp50 - Ui(fresh50)			$9.40\pm0.40^{\rm b}$	
Porphyra umbilicalis			$8.97\pm0.99^{\rm b}$	
Ceramium rubrum				$9.94 \pm 1.63^{d}$
Ceramiumrubrum (cultivated)				$5.04 \pm 0.42^{\circ}$
Pp50 - Cr(cultivated50)				$4.22\pm0.08^b$
<i>Pp</i> 50			$5.57 \pm 0.63^{\rm a}$	
Pp75				$4.19 \pm 0.39^{b}$

#### 3.4 Food conversion rate

Food conversion rates significantly differed between seasons (F=65, p < 0.001), diets (F=89, p < 0.001) and the interaction between both factors (F=17, p < 0.001) (Tab. 7). Indeed, the food conversion rate was significantly different (Tukey's tests, p < 0.001) between seasons except in the winter vs autumn (Tukey's test, p=0.07) and winter vs summer (Tukey's tests, p=0.31). Conversion rates were highest in spring (6.6 for *P. palmata* and 18 for *L. digitata*), except in the case of frozen *U. intestinalis* in winter, which was associated with a growth rate of more than 60% due to the rapid degradation of algae in seawater.

The lowest food conversion rates were obtained with *C.* rubrum and *P. palmata*  $(4.2\pm0.1)$  in autumn and with *P. palmata* when feeding was limited to 75% of maximum  $(4.2\pm0.4)$ . Similarly, the mixture of several species of algae substantially reduced food conversion rate. For example, the spring conversion rate for a 50/50 mix of *P. palmata* and *L. digitata* was  $7.77\pm0.64$ , while the mean food conversion rate for *P. palmata* alone  $(6.63\pm0.36)$  and *L. digitata* alone  $(17.95\pm01.95)$  was 12.29. The conversion rate significantly varied with the amount of algae consumed (SNK, p < 0.001); it was  $5.6\pm0.1$  when abalone were fed *P. palmata ad libitum* and reached a minimum of  $4.9\pm0.1$  when the abalone was limited to 50% of its maximum consumption (Tab. 6).



**Fig. 2.** Concentration of circulating hemocytes (in millions cells  $ml^{-1}$ ), after one, two and three months of experimentation, as a function of the diet: *P. palmata, L. digitata* and *F. serratus* (Experimentation E6). Results are expressed as mean ± SEM (*n*=9). At each date, diets that do not share a letter differ significantly (ANOVA and SNK tests, *p* < 0.05).

#### 3.5 Immune parameters

The number of circulating hemocytes in hemolymph was measured in order to evaluate the immune response of abalone (Fig. 2). The concentration of hemocytes in hemolymph increased between the beginning of the experiment and after one month of experiment but no statistical differences were calculated whatever the diet. The number of circulating hemocytes varied significantly according to diet during the second month (ANOVA, p < 0.001). The hemolymph of animals fed *P. palmata* contained significantly more circulating hemocytes compared to abalone fed *L. digitata* (SNK, p < 0.05) and *F. serratus* (SNK; p < 0.001). By contrast, after three months, only *P. palmata* was associated with a significantly higher hemocyte density (SNK, p < 0.001). This increase was especially marked ( $4.39 \pm 0.33$  million cells ml<sup>-1</sup> at T0).

Using flow cytometry, the effects of the algal diets on several other immune parameters (phagocytic activity, non-specific esterase activity and the presence of lysosomes) were analysed after three months (Fig. 3). The results demonstrate that the hemocytes of abalone fed *P. palmata* showed significantly greater phagocytic activity than those of animals fed with the two other species of algae (ANOVA, p < 0.01). This difference was +32.18% ( $21.09\pm2.11\%$  vs.  $14.30\pm1.28\%$  of fluorescent cells) and +39.30% ( $21.09\pm2.11\%$  vs.  $12.80\pm2.25\%$ ) for animals fed *L. digitata* and *F. serratus*, respectively.

The hemocytes with the highest non-specific esterase activity were those from the abalone fed *F. serratus* (ANOVA, p < 0.001), while the activity associated with the two other species did not significantly differ (SNK, p = 0.94). The percentage of fluorescent cells were + 17.28% (87.05 ± 1.25% vs. 72.01 ± 1.29% fluorescent cells) and 20.47% (87.05 ± 1.25% vs. 72.26 ± 1.96% fluorescent cells) in comparison with *L. digitata* and *P. palmata*.

The presence of lysosomes inside the hemocytes was measured with a Lysotracker  $^{\textcircled{R}}$  probe. As with the activity of



**Fig. 3.** Effect of diet on three immune parameters of abalone after three months of experimentation: Phagocytosis: phagocytic efficacy; Fluorescein Diacetate (FDA): activity of non-specific esterases; Lysotracker: lysosomal marker (Experimentation E6). Results are expressed as mean  $\pm$  SEM (n=9). Diets that do not share a letter differ significantly (ANOVA and SNK tests, p < 0.05).

non-specific esterases, hemocytes from the abalone fed *F.* serratus contained the highest number of lysosomes (Fig. 3). However, the difference between diets was not significant (ANOVA, p=0.26), possibly due to the small number of animals tested (n=9).

# 4 Discussion

The purpose of this study was to investigate the weight increase and health of the European abalone in relation to several diets and seasons. The identification of the most biologically- and financially-efficient diet for abalone rearing according to the availability and palatability of algae throughout the year would greatly benefit the commercial aquaculture of this species.

There were important differences in the growth rate of H. tuberculata associated with the various algae tested. According to the literature (Mottet, 1978; Mercer et al., 1993; Fleming, 1995), abalone grow more rapidly with red algae (e.g. P. palmata and C. rubrum except C. crispus) than with brown algae (e.g. L. digitata, and Porphyra sp.). With green algae (e.g. *Ulva intestinalis*), growth was satisfactory but its nutritive quality varied according to the season. Based on the proportions of each alga and by comparison with the expected growth, some mixtures of multiple algae species yielded better results than any single species alone. Indeed, in the spring, weight gain with P. palmata and L. digitata alone was 2.2 and 0.87% d<sup>-1</sup> respectively. The expected growth with the different diets were 1.87 (i.e. 1.65 for 75% P. palmata+0.22 for 25% L. digitata), 1.54 and 1.20 % d<sup>-1</sup> for, repectively, 75, 50 and 25% P. palmata whereas the observed growth rates for these mixtures were 2.05, 1.85 and 1.57%  $d^{-1}$  that corresponded to gains of 10, 20 and 30%, respectively. The mixture of frozen U. intestinalis and P. palmata appeared even more beneficial: the two algae alone produced a growth increase of 0.33 and 1.57%  $d^{-1}$  whereas a 50/50 mixture of the two allowed an increase of weight of 1.27%  $d^{-1}$ , a weight gain of 35%.



**Fig. 4.** Model of the weight growth rate  $(\%.d^{-1})$  by *P. palmata/L. digitata* ratio and the time of year  $[r^2=0.95, \text{ F-stat} = 23.70; \text{SGR} = a + b \quad \ln x + \text{cy} + d(\ln x)^2 + \text{ey}^2 + \text{fylnx} + g(\ln x)^3 + \text{hy}^3 + \text{iy}^2\ln x + \text{jy}(\ln x)^2$  (with *x* = month; *y* = *P. palmaria/L. laminaria* (%); *a*=3,5798; *b*=-6,6031; *c*=0,0470; *d*=4,5827; *e*=-0,0004; *f*=-0,0096; *g*=-0,9478; *h*=9,73E-07; *i*=9,16E-05; *j*=-0,0017)].

These trends also applied to conversion rates. The conversion was close to 18 for *L. digitata* and 6.6 for *P. palmata* alone, whereas it was 10 with 75% *L. digitata* and 25% *P. palmata*. These results support previous work suggesting that a varied diet greatly improves nutrient availability (Mercer et al., 1993), circumvents problems associated with nutrient deficiencies, and makes higher growth rates possible (Simpson and Cook, 1998). Models of growth and food conversion rates are presented in Figures 4 and 5. *Laminaria. digitata* was associated with sinusoidal variations in growth as a function of the season (Fig. 4). Weight increase was greater in autumn than in spring despite higher food intake during spring. However, the nitrogen content in *L. digitata* is 0.2% N of fresh weight in October vs. 0.6% N of fresh weight in April (Basuyaux, 1997).

By contrast, with P. palmata growth was greatest in the spring when the nitrogen level was high and lowest in winter when nitrogen is low. The total nitrogen/protein conversion factor of 6.25 is probably an overestimation, and does not accurately reflect the true protein content (Wells et al., 2017). High protein levels are not always correlated with good growth, although at least one study seems to indicate that protein-enriched algae allow higher growth rates (Naidoo et al., 2006). Therefore, factors other than protein content must be involved. It is now well known that the nutritional value is related to other molecules such as lipids, fatty acids, sterols, polysaccharides, amino acids vitamins and defensive compounds (e.g. Wells et al., 2017). According to McShane et al. (1994), the tenderness of the algae is the most influential factor in food selection by abalone. Softer algae allow abalone to ingest more. In this study however, the appetence (ingestion rate) and growth rate appear to be unrelated. Recent investigations of formulated foods support this finding: even with diets specially-formulated to the specific needs of abalone, health may decline during the breeding season (summer) if they do not have an adequate supply of algae



**Fig. 5.** Model of the food conversion rate according to *P. palmata/L. digitata* ratio and the time of year  $[r^2=0.97;$  F-stat=39.70;  $z=(a+bx+cx^2+dy+ey^2+fy^3)/(1+gx+hx^2+ix^3+jy)$  (with x= month; y=P.palmaria/L.laminaria (%); a=5,4714; b=1,1188; c=-0,1143; d=0,0407; e=0,0015; f=-9,25E-06; g=-0,5330; h=0,1232; i=-0,0072, j=0,02216)].

(Bansemer et al., 2014; Venter et al., 2016). Our data did not provide evidence to this issue, and thus further investigation is necessary.

The annual growth rates with *P. palmata*, steadily decreased from March to December, likely due to the annual growth cycle of *P. palmata*. Indeed, many young *P. palmata* can be easily harvested in March, but the algae's thallus progressively degenerates during the year before disappearing almost completely during the winter (Aidara, 1997; Le Gall et al., 2004). In many cases, winter storms dislodge these older algae, allowing younger ones to establish themselves in their place. This means that in each season, several generations cohabitate in varying proportions, and since the harvest of algae is random, it is the seasonal age profile of this species that determines abalone growth.

Weight growth and conversion rates for different combinations of *L. digitata* and *P. palmata* were modeled (Figs. 4 and 5, respectively). In winter, *Pp*75-*Ld*25 and *Pp*50-*Ld*50 weight gain rates varied between 1.41 and 1.50% d<sup>-1</sup>, while abalone fed with only *P. palmata* showed an even higher growth rate of 1.57 % d<sup>-1</sup> and food conversion rate of 4.83%. However, the availability of *P. palmara* during this period is extremely limited, increasing the cost of the algae, so mixing it with *L. digitata* seems more cost-effective. Another solution can be to add thawed *U. intestinalis* (harvested during spring and frozen) to the *P. palmata* and *L. digitata* but the conversion rate of this mixture was very high.

During the spring, *P. palmata* was associated with considerably higher weight increase rates than the other algae, and on the French coast this species is abundant and therefore inexpensive. Fresh *U. intestinalis* should be used to supplement *P. palmata* if necessary. In the summer, the *Pp*75-*Ld*25 and *Pp*50-*Ui*50 diets were associated with similar growth rates to each other, but *P. palmata* density is generally quite

low and a mixture of *P. palmata, U. intestinalis* and *L. digitata* is more cost-effective. In autumn, resurgence of the *P. palmata* population enables greater quantities to be harvested, whereas the density of *U. intestinalis* is fairly low. Thus, during autumn, a mixture of *P. palmata* and *L. digitata* seems to be the ideal diet.

The conversion rates measured in this study can be used to estimate the costs of each diet. The current price of P. *palmaria* ( $\in$  400/metric tonnes) is 10 times greater than that of L. digitata ( $\notin$  40/metric tonnes) (Arzel, 2004; Pien, pers. comm.), while our data show that the average conversion rate is about 2 times greater for L. digitata (12.2) than P. palmata (5.7). Thus, the overall cost of feeding abalone with P. *palmata* is five times higher ( $\notin 2.4/kg$  per abalone to 1 year) than with L. digitata ( $\notin 0.5$ /kg per abalone to 1 year). At the same time, formulated diets show variable growth performance as a function of composition and abalone species (FitzGerald, 2008). One formulated diet, made by Adam & Amos Abalone Foods Pty Ltd<sup>®</sup>, is associated with abalone growth comparable to that with P. palmata (FitzGerald, 2008; Adam and Amos, pers. comm.). With a price of about 2.2  $\epsilon$ /kg of feed (including the cost of transportation) and a food conversion rate of 1.3, the total cost of feeding with this formulated diet (€ 2.6/kg per abalone to 1 year) is equivalent to the cost of feeding with *P. palmaria*. With a market price of  $\notin$  69/kg (France Haliotis<sup>®</sup>), the cost of feeding is a relatively low proportion of the total price of abalone rearing (estimated at less than 5% over a production cycle). Other production costs (e.g. electricity, labor, etc.), and especially high fixed costs make it necessary to reduce the length of the rearing period in order to significantly reduce the cost of production, and thus, maximal growth rates are optimal. Furthermore, the impact of seaweed harvesting on the environment must also be taken into account.  $40\,\overline{000}$ -60000 metric tonnes of L. digitata must be harvested annually in order to feed abalone stocks. Arzel (2004) has reported that the biodiversity and density of an area harvested of L. digitata is replenished within two years. This is not the case for *P. palmata*. The total biomass of this species in France is unknown; Aidara (1997) estimated that 10.5 metric tonnes of P. palmata were harvested at Gouville-sur-mer (Manche, France) from a surface area of 27.7 ha. Therefore, intensive harvesting of this species can result in its local disappearance for several years (2002-2015) (S. Pien, pers. comm.). Indeed, since spore dispersal is very limited in this species, it is necessary to leave some algae in place in order to ensure its reproduction (Philippe, 2013). Diversifying the species of algae utilised and selecting them according to their availability and quality constitute more sustainable management practices for abalone rearing.

In addition to diets based on algae harvested from the natural environment, certain algae, such as *C. rubrum*, can be grown to feed abalone and may possibly result in higher growth rates than with *P. palmata*. However, Bazès et al. (2006) have reported that a defensive substance isolated from another species of the same genus (*Ceramium botryocarpum*) shows anti-fouling properties that have been found to cause mass mortality of abalone in aquaculture facilities. Thus, *P. palmata* appears to be the best species to use throughout the year, though this should be supplemented with *L. digitata* (25–50%) in winter, summer and autumn and fresh

*U. intestinalis* in the spring. Up to 25% frozen *U. intestinalis* may also be mixed with *P. palmata*.

From an aquaculturist's point of view, a low food conversion rate is especially interesting. In the present study, it is possible to significantly reduce (25%) this rate by feeding abalone only 75% of what they would eat in an unrestricted regime. Such a restricted diet in *P. palmata* should induce reduced costs, but also a decrease in weight gain by about 8%. However, Francis et al. (2008) showed that animals fed *ad libitum* for long periods have better conversion rates than animals fed *ad libitum* for shorter periods. Thus, a reduction in food quantity may be a suitable, and possibly even beneficial, response to periodic shortage of available algae.

Our results regarding immune parameters showed that the abalone fed a diet of P. palmata alone possess more circulating hemocytes with higher phagocytic activity than abalone fed L. digitata or F. serratus. Experiments carried out on another abalone species (Haliotis diversicolor supertexta) showed that "stressful" variations in abiotic factors such as ammonium or nitrite levels, salinity, or water temperature result in decreased immune capacity (Cheng et al., 2004a, 2004b, 2004c, 2004d). For example, Cheng et al. (2004b) have reported that after 72 h of rearing in the presence of  $10.34 \text{ mg L}^{-1}$  ammonium, the number of circulating hemocytes and the phagocytic activity of H. diversicolor supertexta decreased by 34% and 64%, respectively, as compared to controls. When exposed to Vibrio parahaemolyticus, the animals appeared much more vulnerable to a bacterial infection: 73% mortality was recorded in animals reared with a high ammonium concentration as compared to only 26% in control animals. The immunological results obtained in this study (the number of circulating hemocytes and phagocytic activity) suggest a strengthening of the abalone immune system in response to a diet based on P. palmata, which could result in better resistance to bacterial infections in crowded rearing conditions.

In addition to more hemocytes and higher phagocytic activity, abalone fed P. palmata exhibited lower non-specific esterase activity and lysosomal labeling compared to abalone fed other algal diets. Data from the literature have suggested that increases in these biomarkers indicate stressful abiotic conditions. For instance, Mottin et al. (2010) found a 69% increase in the activity of nonspecific esterases when European abalone hemocytes were grown in the presence of zinc chloride (1000 µM) for 24 h. Similarly, an increase in fluorescence intensity (indicating higher lysosome labelling) was demonstrated in the hemocytes of the oyster C. gigas exposed in vitro to Dibenz [a, h] anthracene (Bado-Nilles et al., 2008) and in vivo to diuron (Bouilly et al., 2007). Our observed reduction in biomarkers of stress with the P. palmata diet therefore suggests that is the least stressful to abalone compared to the other diets tested in our experimental conditions.

# 5 Conclusion

In conclusion, our results regarding weight gain showed that a diet based on *P. palmata* or a mixture of *P. palmata* and *L. digitata* (75–25%, respectively) is optimal in late summer whereas *P. palmata* alone is best in spring. *P. palmata* has the additional advantage of fortifying the immune system of the

abalone. Besides these biological parameters, the cost of food and the impact of algae harvest on the environment must be taken into account.

Aquaculture of many marine species, including the European abalone is increasing worldwide. Despite substantial financial efforts, abalone aquaculture in Europe remains limited to a few tens of metric tonnes and large-scale development seems unlikely. However, the guidelines derived from this study make it possible to envision the development of several small, sustainable abalone culture installations using fresh algae and yielding high profit margins. The information presented in this manuscript provides abalone aquaculturists with the necessary information to make the best decision according to their particular circumstances (regulation, local seaweed harvest, cost...)

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# References

- Aidara C. 1997. Action des conditions éco-physiologiques in-situ et en culture sur le développement et la composition chimique de la Rhodophycée Palmaria palmata (L.). Doctorat de l'Université de Caen, Université de Caen, France, p. 132.
- Anderson RS, Burreson EM, Paynter KT. 1995. Defense responses of hemocytes withdrawn from *Crassostrea virginica* infected with *Perkinsus marinus*. J Invertebr Pathol 66: 82–89.
- Arzel P. Les laminaires sur les côtes bretonnes. Evolution de l'exploitation et de la flottille de pêche, état actuel et perspectives. Edited by Ifremer, France, 2004 p. 140
- Bachère E, Chargot D, Grizel H. 1988. Separation of *Crassostrea gigas* hemocytes by density gradient centrifugation and counter-flow centrifugal elutriation. *Dev Comp Immunol* 12: 549–559.
- Bado-Nilles A, Gagnaire B, Thomas-Guyon H, Le Floch S, Renault T. 2008. Effects of 16 pure hydrocarbons and oil on haemocyte and Haemolymphatic parameters in the Pacific oyster, *Crassostrea* gigas (Thunberg). *Toxicol Vitr* 22: 1610–1617.
- Bansemer M, Qin J, Harris J, Howarth G, Pierre D. 2014. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Aquaculture* 8: 121–135.
- Basuyaux O. Etude et modélisation des paramètres physicochimiques sur la croissance de l'ormeau (*Haliotis tuberculata*) en élevage en circuit semi-fermé, Doctorat de l'Université de Caen, France, 1997 p. 237.
- Bazès A, Silkina A, Defer D, Bernède-Bauduin C, Quéméner E, Braud JP, Bourgougnon N. 2006. Active substances from *Ceramium botryocarpum* used as antifouling products in aquaculture. *Aquaculture* 258: 664–674.
- Birais D, Le Gall P. 1986. 200 mètres de toboggan pour oursins. *Aqua revue* 5: 31–35.
- Black WAP. 1948. The seasonal variation in chemical constitution of some of the sublittoral seaweeds common to scotland. Part II. *Laminaria digitata. J Soc Chem Ind* 67: 169–172.

- Bouilly K, Bonnard M, Gagnaire B, Renault T, Lapègue S. 2007. Impact of diuron on aneuploidy and hemocyte parameters in Pacific oyster, *Crassostrea gigas*. Arch Environ Contam Toxicol 52: 58–63.
- Bossy SF, Culley MB. 1976. Abalone fishing in Britain's Channel Island. *Fish News Int* 15: 55–62.
- Chapman ARO, Craigie JS. 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar Biol* 40: 197–205.
- Chaumont JP. 1978. Variations de la teneur en composés azotés du *Rhodymenia palmata* Grev. *Bot Mar* 21: 23–29.
- Cheng W, Juang FM, Chen JC. 2004a. The immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its succeptibility to vibrio parahaemolyticus at different salinity levels. *Fish Shellfish Immunol* 16: 295–306.
- Cheng W, Hsiao IS, Chen JC. 2004b. Effect of ammonia on the immune response of Taiwan abalone *Haliotis diversicolor* supertexta and its succeptibility to vibrio parahaemolyticus. Fish. Shellfish Immunol 17: 193–202.
- Cheng W, Hsiao IS, Hsu CH, Chen JC. 2004c. Change in water temperature on the immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its succeptibility to *vibrio parahaemolyticus*. *Fish Shellfish Immunol* 17: 235–243.
- Cheng W, Hsiao IS, Chen JC. 2004d. Effect of nitrite on immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its succeptibility to *vibrio parahaemolyticus*. *Dis Aquat Organ* 60: 157–164.
- Citharel J, Villeret S. Recherche sur le métabolisme azoté de quelques algues marines des côtes Bretonnes, in: IV Congres International des Algues Marines. Edité par Pergamon Press, Oxford-London-New York-Paris, 1961 pp. 291–300.
- Clavier J, Richard O. Etude sur les ormeaux dans la région de Saint-Malo. Edited by par l'Association pour la mise en valeur du littoral de la côte d'émeraude, Dinard, France, 1985 p. 285
- Culley MB, Peck LS. 1981. The feeding preferences of the ormer, Haliotis tuberculata L. Kiel Meer Sonder 5: 570–572.
- Delaporte M, Soudant P, Moal J, Lambert C, Quere C, Miner P, Choquet G, Paillard C, Samain JF. 2003. Effect of a mono-specific algal diet on immune functions in two bivalves species *Crassostrea gigas* and *Ruditapes Philippinarum*. J Exp Biol 206: 3053–3064.
- Dion P. Stratégies écologiques chez trois algues marines rhodophycées se distinguant par l'importance relative de leurs thalles rampant et dréssé: Palmaria Palmata, Mastocarpus stellatus et Petrocelis cruenta. Doctorat de l'Université Pierre & Marie Curie, Paris, 1983 p. 100
- Dubief B, Nunes FLD, Basuyaux O, Paillard C. 2017. Immune priming and portal of entry effectors improve response to vibrio infection in a resistant population of the European abalone. *Fish Shellfish Immunol* 60: 255–264.
- Evariste L, Auffret M, Audonnet S, Geffard A, David E, Brousseau P, Betoulle S. 2016. Functional features of hemocyte subpopulations of the invasive mollusc species *Dreissena polymorpha*. *Fish Shellfish Immunol* 56: 144–154.
- FitzGerald A. Abalone Feed Requirements. Final Report for Seafish edited by South west abalone growers association, United Kingdom, 2008 p. 34
- Fleming AE. 1995. Growth, intake, feed conversion efficiency and chemosensory preference of the Australian abalone, *Haliotis rubra*. Aquaculture 132: 297–311.

- Francis TL, Maneveldt GW, Venter J. 2008. Determining the most appropriate feeding regime for the South African abalone *Haliotis midae* Linnaeus grown on kelp. J Appl Phycol 20: 597–602.
- Gagnaire B, Thomas-Guyon H, Renault T. 2004. In vitro effects of cadmium and mercury on Pacific oyster, *Crassostrea gigas* (Thunberg), haemocytes. *Fish Shellfish Immunol* 16: 501–512.
- Haug A, Jensen A. 1954. Seasonal variations in the chemical composition of *Alaria esculenta*, *Laminaria saccharina*, *Laminaria hyperborea* and *Laminaria digitata* from Northern Norway. *Rep Norw Inst Seaweed Res* 4: 1–14.
- Hégaret H, Wikfors GH, Soudant P. 2003. Flow cytometric analysis of haemocytes from eastern oysters, *Crassostrea virginica*, subjected to a sudden temperature elevation: II. Haemocyte functions: aggregation, viability, phagocytosis, and respiratory burst. *J Exp Mar Biol Ecol* 293: 249–265.
- Koike Y, Flassch J, Mazurié J. 1979. Biological and ecological studies on the propagation of the ormer, *Haliotis tuberculata* Linnaeus. II: influence of food and density on the growth of juveniles. *La Mer* (Bull. de la Soc. Franco-Japonaise d'Océano) 17: 43–52.
- Le Gall L, Deniaud E, Rusig AM. 2004. Etude de la phénologie de la reproduction de la Rhodophyceae *Palmaria palmata* le long des côtes françaises de la Manche. *C Biol Mar* 45: 269–275.
- Mai K, Mercer JP, Donlon J. 1994. Comparative studies on the nutrition of two species abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. II Amino acid composition of abalone and six species of macroalgae with an assessment of their nutritional value. *Aquaculture* 128: 115–130.
- Maita Y, Mizuta H, Yanada M. 1991. Nutrient environment in natural and cultivated grounds of *Laminaria japonica*. *Bull Fac Fish Hokkaido Univ* 42: 98–106.
- Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire ME, Brawley SH. 2017. Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol* 29: 949–982.
- Martello LB, Friedman CS, Tjeerdema RS. 2000. Combined effects of pentachlorophenol and salinity stress on phagocytic and chemotactic function in two species of abalone. *Aquat Toxicol* 49: 213– 225.
- McShane PE, Gorfine HK, Knuckey IA. 1994. Factors affecting food selection in the abalone *Haliotis rubra* (Mollusca: Gastropoda). J Exp Mar Biol Ecol 176: 27–37.
- Mesnildrey L, Jacob C, Frangourdes K, Reunavot M, Lesueur M. La filière des macro-algues en France. Rapport d'étude. NETALGAE
  InterregIVb. Les publicaons du Pôle halieutique AGRO-CAMPUS OUEST n 9, 2012 p. 38
- Mercer JP, Mai KS, Donlon J. 1993. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. I Effects of algal diets on growth and biochemical composition. *Invert Reprod devel* 23: 75–88.
- Mgaya YD, Mercer JP. 1994. A review of the biology, ecology, fisheries and mariculture of the european abalone *Haliotis tuberculata* Linnaeus 1758 (Gasteropoda: Haliotidae). *Biol environ:Proc Roy Irish ac* 94: 285–304.
- Minguez L, Halm-Lemeille MP, Costil K, Burea R, Lebel JM, Serpentini A. 2014. Assessment of cytotoxic and immunomodulatory properties of four antidepressants on primary cultures of abalone hemocytes (*Haliotis tuberculata*). *Aquat Toxicol* 153: 3–11.
- Mizuta H, Maita Y, Yanada M. 1992. Seasonal changes of nitrogen metabolism in the sporophyte of *Laminaria japonica* (Phaeophyceae). *Nippon Suisan Gakk* 58: 2345–2350.
- Morgan KC, Wright JLC, Simpson FJ. 1980. Review of chemical constituents of the red alga *Palmaria palmata*. *Econ Bot* 34: 27–50.

- Mottin E, Caplat C, Mahaut ML, Costil K, Barillier D, Lebel JM, Serpentini A. 2010. Effect of in vitro exposures to zinc on immunological parameters of hemocytes from the marine gastropod, *Haliotis tuberculata*. *Fish Shellfish Immunol* 29: 846–853.
- Mottet MG. A review of the fishery biology of abalones. Edited by Washington department of fisheries, Technical report 37, 1978 p. 81
- Naidoo K, Maneveldt G, Ruck K, Bolton JJ. 2006. A comparison of various seaweed-based diets and artificial feed on growth rate of abalone in land-based aquaculture systems. *J Appl Phycol* 18: 437–443.
- Nicolas JL, Basuyaux O, Mazurie J, Thebault A. 2002. Vibrio carchariae, a pathogen of the abalone *Haliotis tuberculata*. *Dis Aquat Organ* 50: 35–43.
- Pérez-Estrada CJ, Civera-Cerecedo R, Hernandez-Llamas A, Serviere-Zaragoza E. 2010. Growth and biochemical composition of juvenile green abalone *Haliotis fulgens* fed rehydrated macroalgae. *Aquaculture Nutr* 17: e62–e69.
- Philippe E. Récolte des algues de rive. Guide de bonnes pratiques. Edited by Inter Bio Bretagne – projet Algmarbio, France, 2013 p. 48
- Riera F. Le goÛt de l'ormeau ou comment un gastéropode a gagné ses titres de noblesse. Edited by CTHS, France. Actes des congrès nationaux des sociétés historiques et scientifiques (édition électronique), 2016 pp. 85–100
- Rosen G, Langdon C, Ford E. 2000. The nutritional value of *Palmaria mollis* cultured under different light intensities and water exchange rates for juvenile red abalone Haliotis rufescens. *Aquaculture* 185: 121–136.
- Sales J, Janssens GPJ. 2004. Use of feed ingredients in artificial diets for abalone: a brief update. *Nutrition B* 74:13N–21N.
- Serviere-Zaragosa E, Pérez-Estrada CJ, Aldana Aranda D. 2015. Status of the digestive gland and feed index in juvenile green abalone *Haliotis fulgens* fed rehydrated macroalgae. *Aquaculture Nutr* 22: 767–775.
- Simpson B, Cook PA. 1998. Rotation diets: a method of improving growth of cultured abalone using natural algal diets. J Shellfish Res 17: 635–640.
- Stone D, Bansemer M, Lange B, Schaefer E, Howarth G, Harris J. 2014. A dietary intervention temperature challenge model to investigate summer mortality in cultured greenlip abalone (*Haliotis laevigata* Donovan). Aquaculture 430: 230–240.
- Travers MA, Le Goïc N, Huchette S, Koken M, Paillard C. 2008. Summer immune depression associated with increased susceptibility of the European abalone *Haliotis tuberculata* to *Vibrio harveyi* infection. *Fish Shellfish Immunol* 25, 800–808.
- Travers M-A, Basuyaux O, Le Goïc N, Huchette S, Nicolas JL, Koken M. et al., 2009. Influence of temperature and spawning effort on *Haliotis tuberculata* mortalities caused by *Vibrio harveyi*: an example of emerging vibriosis linked to global warming. *Glob Chang Biol* 15: 1365–1376.
- Venter L, Loots DT, Vosloo A, Jansen van Rensburg P, Zander Lindeque J. 2016. Abalone growth and associated aspects: now from a metabolic perspective. *Aquaculture* 10: 1–23.
- Viera MP, Gomez-Pinchetti J, de Vicose GC, Bilbao A, Suarez S, Haroun RJ, Izquierdo MS. 2005. Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculate* coccinea Reeve. *Aquaculture* 248: 75–82.
- Viera Toledo MP. Development of sustainable grow-out technology for abalone *Haliotis tuberculata* coccinea (Reeve) as a new species for diversification in the Canary Islands. Thesis doctoral of Las Palmas De Gran Canaria University, 2014

Wells M, Potin P, Craigiec J, Raven J, Merchant S, Helliwell K, Smith A, Ellen CM, Brawley S. 2017. Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol* 29: 949–982.

Wang SH, Wang YL, Zhang ZX, Ralph J, Weng ZH, Zou ZH, et al., 2004. Response of innate immune factors in abalone *Haliotis* 

*diversicolor* supertexta to pathogenic or non-pathogenic infection. *J Shellfish Res* 23: 1173–1177.

Wootton EC, Pipe RK. 2003. Structural and functional characterization of the blood cells of the bivalve mollusc, *Scrobicularia plana*. *Fish Shellfish Immunol* 15, 249–262.

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