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Growth and biochemical composition of juvenile green abalone *Haliotis fulgens* fed rehydrated macroalgae

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Abstract

Under controlled laboratory conditions, juvenile green abalone *Haliotis fulgens* were fed rehydrated natural feeds to determine the effects on growth, survival, feed efficiency and biochemical composition of the digestive gland and muscle. Five macroalgae, *Ulva* sp. (Chlorophyta), *Eisenia arborea*, *Macrocystis pyrifera*, *Egregia menziesii* (Phaeophyta) and *Porphyra perforata* (Rhodophyta) were tested. The macroalgae promoted growth, although, depending on the species, there were considerable differences in growth, feed efficiency and biochemical composition of the digestive gland and muscle. Mean growth rate in length and weight and survival rate varied within the ranges 1.6–15.1 μm day^{-1}, 1.4–8.1 mg day^{-1} and 44–69%, respectively. Significantly, higher growth rates were obtained from *Egregia menziesii*, *M. pyrifera* and *P. perforata*. Feed conversion ratio ranged from 6.5 to 42.4 for *P. perforata* and *Ulva* sp. Protein, carbohydrates and lipid contents in the digestive gland ranged from 113 to 180, 98.3 to 448 and 17.2 to 23.3 mg g^{-1}, respectively. In muscle, the ranges were 66.9–123, 9.5–23.2 and 2.8–3.9 mg g^{-1}, respectively. This study shows that rehydrated *Egregia menziesii*, *M. pyrifera* and, particularly, *P. perforata* are more efficient in promoting growth than *Ulva* sp. and *E. arborea* which match results reported by other authors when using the same fresh macroalgae.

KEY WORDS: biochemical composition, growth, *Haliotis fulgens*, juvenile, macroalgae

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Introduction

The abalone fishery on the west coast of the Baja California Peninsula of Mexico has considerable social and economic importance. Green abalone *Haliotis fulgens* Philippi 1845, together with pink abalone *Haliotis corrugata* Wood 1828, constitutes 95–98% of the abalone catch in Mexico (350–400 tonne year^{-1}; Ponce-Díaz 2003). High price in the international market has stimulated aquaculture technologies of native species to repopulate natural stocks and develop commercial farming. Green abalone is the most commonly used species on abalone farms in this region.

An abundant literature describes the nutritional value of marine plants consumed by abalone, and artificial diets have been extensively tested over the past two decades, chiefly to support refinements in abalone aquaculture (Uki & Watanabe 1992; Leighton 2000). Several studies on nutrition for green abalone in Mexico have been conducted, largely focusing on evaluating different conventional and unconventional protein sources for artificial diets (Viana-Castrillón 2002; Gómez *et al.* 2003; Nava-Guerrero *et al.* 2004) and establishing the effect of formulated diets on the biochemical composition of tissues of green abalone (Durazo-Beltrán *et al.* 2003a,b, 2004).

The macroalga *M. pyrifera* is most frequently used for rearing abalone in Mexico (McBride 1998). Occasionally, and depending on availability, other macroalgae such as green algae (*Ulva* spp.), brown algae (*E. arborea*, *Egregia menziesii*, *Padina* spp. or *Pelagophycus porra*) or red algae (*Gracilaria* spp. or *Porphyra* spp.) are used as substitutes (pers. obs.). For green or pink abalone, studies of natural foods have been conducted to compare nutritive values and effects on growth (Serviere-Zaragoza *et al.* 2001; 2009) and *in vitro* protein digestibility (Serviere-Zaragoza *et al.* 1997; Picos-García *et al.* 2000; García-Carreño *et al.* 2003); however, studies of the effects that natural feed have on growth and biochemical composition in juveniles of this species are

limited. Ponce-Díaz *et al.* (2004) assessed the growth and biochemical composition of green abalone tissue under two temperature regimes and fed either of two dried brown algae diets (*M. pyrifera* and *E. arborea*), finding that diets and temperature regimes influence growth (length and weight) and that biochemical components closely match their effects on growth. Nelson *et al.* (2002) reported differences in growth and lipid composition for cultivated green abalone in California that were fed specific fresh macroalgae. One major problem in aquaculture is to satisfy the nutritional requirements of the organisms in the first stages of life. This is a fundamental factor for the development of abalone production in Baja California; yet, the nutritional value of macroalgae used to feed abalone is not well known. Our objective was to determine the nutritional value, in terms of growth and biochemical composition, of rehydrated macroalgae used in abalone hatcheries along the coast of the Baja California Peninsula. The macroalgae *Ulva* sp. (Chlorophyta); *E. arborea*, *M. pyrifera*, *Egregia menziesii* (Phaeophyta) and *P. perforata* J. Agardh (Rhodophyta) were evaluated as feed for juvenile green abalone *H. fulgens*.

Material and methods

Natural feeds

Ulva sp. and *E. arborea* were collected from the Pacific coast of the central part of the Baja California Peninsula, and *M. pyrifera*, *Egregia menziesii* and *P. perforata* were collected from the northern coast of the peninsula. Macroalgae were air-dried to approximately 150 g kg^{-1} fresh weight at the locations where the algae were collected and transported using cardboard boxes to the CIBNOR laboratory in La Paz, B.C.S. (southeastern coast of the Baja California Peninsula), where they were stored in the same cardboard boxes at 20 °C. The proximate composition of the dry feed was determined using the methods of AOAC (1995). Results are given in Table 1. Gross energy was determined using a bomb calorimeter (Model 1261; Parr Instrument, Moline, IL, USA).

Feeding trial

A feeding trial lasting 136 days was conducted under controlled laboratory conditions with 510 hatchery-produced juveniles that averaged 31.67 mm ± 0.11 (SE) in length and weighed 2.46 g ± 0.02 (SE). The juveniles were held in 15 16-L fibreglass tanks (50 × 30 × 35 cm). Plastic tags were glued to the shells of each specimen and placed in tanks at a density of 34 juveniles per tank, with three replicates for each dietary treatment. We used a randomized pattern of the diets among tanks. Before beginning the trial, an ANOVA was conducted to ensure homogeneity of their initial size and weight among tanks. Filtered seawater (10 μm) was vigorously aerated with air stones, and the water was exchanged at a rate of 10% per day under continuous flow. For the whole period, temperature was maintained at 20 ± 1 °C (SD) using a chiller (Ecoline RE-120; Brinkmann Instruments, Westbury, NY, USA); salinity at 37.4 g L^{-1} ± 0.5 (SD); dissolved oxygen concentration at 7.0 g L^{-1} ± 0.1 (SD) and the pH at 8 ± 0.1 (SD). Rehydrated macroalgae were fed *ad libitum* every 2 days in the afternoon. Feed were rehydrated for 5 min in seawater before feeding. Every second day, uneaten feed was removed and weighed, and faeces were removed by siphoning. Tanks were shaded to minimize extraneous microalgae growth; regular maintenance of the tanks was carried out at the same time as removing debris and microalgae growing on the inner walls of the tanks with a brush.

Data collection

Approximately, once a month during the experiment, maximum shell length of tagged individuals was measured using a vernier caliper and wet body weight using an electronic

Table 1 Proximate composition (g kg^{-1} dry matter ± SE, $n = 3$) and gross energy of macroalgae used to feed juvenile green abalone *Haliotis fulgens*

Feed[1]	Crude protein	Ether extract	Ash	Crude fibre	NFE[2]	Gross energy (kJ g^{-1})
UL	47.8 ± 4.6^{c}	35.0 ± 1.2^{a}	365 ± 2^{b}	18.2 ± 0.4^{c}	534 ± 5^{b}	8.3 ± 0.2^{d}
EA	120 ± 0^{b}	39.5 ± 0.1^{a}	288 ± 2^{d}	30.6 ± 1.5^{b}	522 ± 3^{b}	12.4 ± 0.2^{c}
MP	164 ± 1^{a}	36.3 ± 1.9^{a}	421 ± 1^{a}	29.4 ± 0.8^{b}	349 ± 2^{d}	12.3 ± 0.0^{b}
EM	119 ± 0^{b}	37.0 ± 1.8^{a}	314 ± 3^{c}	59.1 ± 0.1^{a}	469 ± 3^{c}	11.4 ± 0.1^{b}
PP	169 ± 2^{a}	14.6 ± 1.3^{b}	240 ± 0^{e}	18.4 ± 0.2^{c}	558 ± 3^{a}	4.1 ± 0.1^{a}

Mean values with different letters in each column indicate significant differences ($P < 0.05$).
[1] Feeds are *Ulva* sp. (UL), *Eisenia arborea* (EA), *Macrocystis pyrifera* (MP), *Egregia menziesii* (EM) and *Porphyra perforata* (PP).
[2] Nitrogen-free extracts are mainly carbohydrates.

balance (Ohaus Explorer, Florham Park, NJ, USA) (nearest 0.001 g). Before weighing the specimens, excess water was removed by blotting with paper towels. Growth rate on a per-day basis was calculated for shell length and wet body weight at the end of the trial (Corazani & Illanes 1998). Apparent feed conversion ratio (FCR) was determined as dry feed consumed/wet weight gain per animal per day (Britz 1996). Consumption was calculated on a dry-weight basis following Uki & Watanabe (1992), where FC = GS − *R*, where *G* is the weight of feed in mg provided each day, *S* is the correction factor for changes in weight of rehydrated macroalgae (as a percentage or proportional fraction) from the control tanks, and R is the remaining food (in grams) after each feeding (2 days). The correction factor was determined from control tanks containing only feed to estimate feed weight changes (loss or gain) in seawater (Leighton 1959). For calculating weight gain, the correction by Kitabayashi *et al*. (1971) was used to compensate for abalone mortality during the trial. Daily feed and nutrient intake were calculated on an individual weight basis. Dead specimens were removed and replaced with similar-sized, tagged abalone to maintain a standard density. Replacements were excluded from sampling.

Biochemical analysis

Five abalones were randomly selected at the beginning of the feeding and five from each replicate tank (15 abalones for each macroalga diet) at the end of the trial to assay biochemical composition of the digestive gland and the muscle. These abalones were not fed for 24 h before sampling. The digestive gland (no gonadal tissue included) and muscle were weighed, freeze-dried and pulverized. For biochemical analysis, 100 mg of each freeze-dried sample was rehydrated using saline solution and homogenized using a homogenizing apparatus (Polytron; Brinkmann Instruments). Protein content was analysed by the method of Bradford (1976) using bovine albumin standard solution for calibration. Carbohydrates were analysed according to Roe *et al*. (1961). Total lipids were analysed by the sulphophosphovanillin method (Barnes & Blackstock 1973) with a total lipid test (Merckotest® 3321; Merck & Co., Whitehorse Station, NJ, USA).

Statistical analysis

ANOVA followed by a Tukey *post hoc* mean comparison test was performed to determine whether significant differences in chemical composition of feeds, final shell length, weight, growth rates, survival, FCR and feed nutrients and energy intake occurred. Normality (Kolmogorov–Smirnov test) and variance homogeneity (Cochran's test) were applied. A multiple regression analysis, using the backward stepwise method of Draper & Smith (1980), was applied to select the nutrients in the feeds that best predict growth rate by weight. Nutrient intakes were used as independent variables for this analysis.

A Student's *t*-test determined whether differences existed between concentrations of protein, carbohydrates and lipids in the digestive gland and muscle at the beginning and the end of the tests. ANOVA determined whether differences in nutrient content in the digestive gland and muscle resulted from the diets. Linear and quadratic equations determined whether there was a relationship between the ingestion of food and concentrations of nutrients in the digestive gland and muscle. These same equations were used to test whether the concentration of the nutrients in the digestive gland and muscle was correlated. Significance level was set to $P < 0.05$. STATISTICA 6.0 (StatSoft, Tulsa, OK, USA) was used for computer statistical analyses.

Results

There were significant differences in the chemical composition of the macroalgae. *Porphyra perforata* contained the highest levels of protein and carbohydrate and the lowest levels of gross energy, lipids, and ash. *Ulva* sp. contained the lowest levels of protein and fibre. *Egregia menziesii* contained the highest per cent of fibre, and *M. pyrifera* contained the highest per cent of ash (Table 1).

No significant differences in length were detected among the diets ($F = 3.20$, $P = 0.062$; Fig. 1a), but the rate of increase in the length of the shell was significantly lower ($F = 5.99$, $P = 0.010$) for abalone fed *Ulva* sp. than the rates obtained with the other macroalgae (Table 2). Final weight was significantly lower in abalone fed *Ulva* sp. (Fig. 1b). Increase was highest in abalone fed *Egregia menziesii*, *M. pyrifera* and *P. perforata* and lowest in abalone fed *E. arborea* and *Ulva* sp. No significant differences in survival were detected among the dietary groups ($F = 1.11$, $P = 0.403$; Table 2).

Significantly higher FCRs occurred with *Ulva* sp. and *E. arborea*, which also produced the lower growth rates ($F = 13.13$, $P = 0.001$) when compared to the other diets. Significantly lower intake of feed, nutrients and energy occurred with *Ulva* sp. and *P. perforata* diets ($F = 89.74$, $P = 0.000$) when compared to *E. arborea*, *M. pyrifera* and *Egregia menziesii* (Table 3). Multiple regression analysis indicated a better prediction of growth rate in weight when

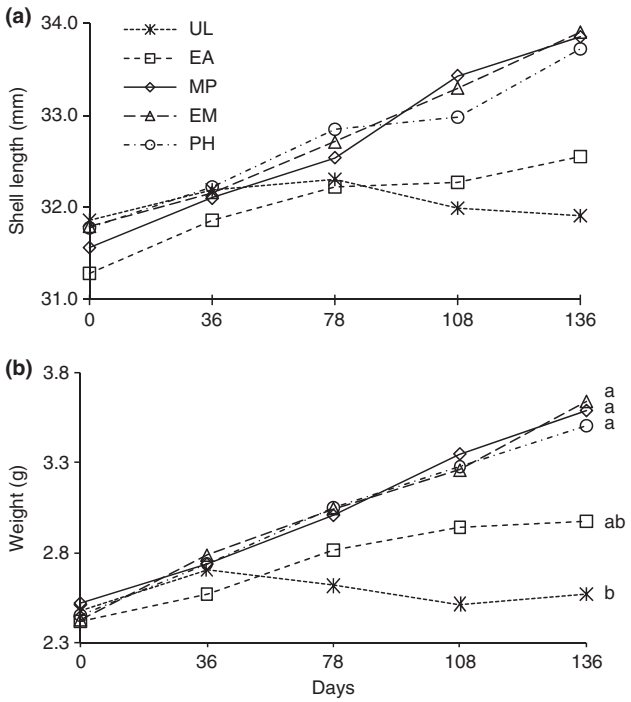

Figure 1 Growth (±SE) in length (a) and weight (b) of juvenile green abalone *Haliotis fulgens* fed the macroalgae *Ulva* sp. (UL), *Eisenia arborea* (EA), *Macrocystis pyrifera* (MP), *Egregia menziesii* (EM) and *Porphyra perforata* (PP). Different letters on each line indicate significant differences ($P < 0.05$).

using the combined intake values for protein, carbohydrates and fibre ($F = 27.3$, $P = 0.001$). Dietary protein and fibre were positively correlated with growth rate, while carbohydrates were inversely correlated.

Diets of *Ulva* sp. contained significantly less protein in the digestive gland than the others diets ($F = 6.90$, $P = 0.006$; Table 4). Also, *P. perforata* provided less protein content ($t = -4.4$, $P = 0.04$), but higher carbohydrate content ($t = 13.4$, $P = 0.005$), when compared to the initial concentration of the nutrient and when compared to other diets. The initial concentration of carbohydrate was significantly reduced when *E. arborea* ($t = -4.3$, $P = 0.04$) and *Egregia menziesii* ($t = -7.3$, $P = 0.01$) were used. Lipid content in *Egregia menziesii* and *P. perforata* was higher than in *Ulva* sp. *E. arborea* and *M. pyrifera*. When compared to initial lipid content, *Ulva* sp. ($t = -6.2$, $P = 0.02$) and *Eisenia* spp. ($t = -9.0$, $P = 0.01$) led to lower nutrient content, while *P. perforata* led to increased nutrient content ($t = 6.0$, $P = 0.02$), although this red alga had the lowest lipid content.

Diets of *Egregia menziesii* provided the highest content of protein content in muscle ($F = 6.46$, $P = 0.008$; Table 4). There were no significant differences in carbohydrates ($F = 2.85$, $P = 0.082$) and lipid content ($F = 2.53$, $P = 0.106$) in muscle between the diets. Nutrients in muscle varied significantly at the end of the experiment compared to the initial content. Protein increased with *Ulva* sp. ($t = 6.7$, $P = 0.02$), *Macrocystis* ($t = 8.3$, $P = 0.01$), *Eisenia* ($t = 17.2$, $P = 0.003$) and *Egregia* ($t = -11.0$, $P = 0.006$); reductions in carbohydrates occurred with *Eisenia* and lipids with *Ulva* sp. ($t = -6.2$, $P = 0.02$).

There was a significant relationship between the protein content in the digestive gland and the amount of protein ingested ($F = 11.5$, $P = 0.004$; Fig. 2). The quadratic equation produced a better fit than the linear equation and indicated that an intake of approximately 4.2 mg g ind^{-1} day^{-1} of protein maximized the concentration of the nutrient in the gland. There were significant inverse relationships between carbohydrate content in the digestive gland ($F = 9.1$, $P = 0.009$) and muscle and carbohydrate intake ($F = 7.0$, $P = 0.02$; Fig. 2). There were no significant relationships between muscle protein and protein intake ($F = 0.02$, $P = 0.88$) or between lipid content in the digestive gland ($F = 2.94$, $P = 0.11$) and muscle ($F = 0.48$, $P = 0.49$) and the intake of the corresponding nutrient. Similarly, there were no significant relationships between protein ($F = 0.94$, $P = 0.34$), carbohydrate ($F = 0.68$, $P = 0.52$) and lipid ($F = 2.3$, $P = 0.14$) concentrations in the muscle and the corresponding nutrient concentrations in the digestive gland.

Table 2 Mean values (±SE, $n = 3$) of final length and weight, growth rates and survival of juvenile green abalone *Haliotis fulgens* fed different macroalgae diets

Feed[1]	Length (mm)	Length growth rate (μm day^{-1})	Weight (g)	Weight growth rate (mg day^{-1})	Survival (%)
UL	31.9 ± 0.5	1.6 ± 0.2^{b}	2.6 ± 0.1^{b}	1.4 ± 0.0^{c}	44 ± 1.7
EA	32.6 ± 0.4	12.0 ± 2.2^{a}	3.0 ± 0.1^{ab}	4.5 ± 0.7^{bc}	65 ± 14.5
MP	33.9 ± 0.4	18.1 ± 2.9^{a}	3.6 ± 0.1^{a}	8.2 ± 0.4^{ab}	69 ± 3.5
EM	33.9 ± 0.7	17.5 ± 4.9^{a}	3.6 ± 0.3^{a}	9.2 ± 1.9^{a}	59 ± 7.4
PP	33.7 ± 0.5	15.1 ± 0.9^{a}	3.5 ± 0.1^{a}	8.1 ± 0.1^{ab}	67 ± 12.9

Mean values with different letters in each column indicate significant differences ($P < 0.05$).
[1] Feeds are *Ulva* sp. (UL), *Eisenia arborea* (EA), *Macrocystis pyrifera* (MP), *Egregia menziesii* (EM) and *Porphyra perforata* (PP).

Table 3 Mean values (±SE, $n = 3$) of apparent feed conversion ratio (FCR), feed intake and nutrient intake by juvenile green abalone *Haliotis fulgens* provided different macroalgae diets

Feed[1]	FCR	Feed intake (mg g ind^{-1} day^{-1})	Nutrient intake (mg g ind^{-1} day^{-1})					
			Crude protein	Ether extract	Ash	Crude fibre	NFE[2]	Energy (kJ g^{-1})
UL	42.4 ± 7.5^{a}	18.9 ± 0.6^{c}	0.9 ± 0.1^{d} (05)	0.7 ± 0.0^{c} (4)	6.9 ± 0.2^{c} (36)	0.3 ± 0.0^{d} (2)	10.1 ± 0.3^{cd} (53)	156 ± 05^{d}
EA	38.8 ± 6.4^{a}	47.3 ± 0.9^{a}	5.7 ± 0.1^{a} (12)	1.9 ± 0.0^{a} (4)	13.6 ± 0.3^{a} (29)	1.4 ± 0.0^{b} (3)	24.8 ± 0.5^{a} (52)	587 ± 11^{a}
MP	14.7 ± 1.5^{b}	33.0 ± 1.6^{b}	5.4 ± 0.3^{a} (16)	1.2 ± 0.1^{b} (4)	13.9 ± 0.7^{a} (42)	1.0 ± 0.0^{c} (3)	11.5 ± 0.5^{c} (35)	404 ± 19^{b}
EM	16.0 ± 3.6^{b}	34.3 ± 2.1^{b}	4.1 ± 0.3^{b} (12)	1.3 ± 0.1^{b} (4)	10.8 ± 0.7^{b} (31)	2.0 ± 0.1^{a} (6)	16.2 ± 1.0^{b} (47)	389 ± 24^{b}
PP	6.5 ± 1.0^{b}	14.0 ± 1.3^{c}	2.4 ± 0.2^{c} (17)	0.2 ± 0.0^{d} (1)	3.4 ± 0.3^{d} (24)	0.3 ± 0.0^{d} (2)	7.8 ± 0.7^{d} (56)	198 ± 18^{c}

Mean values with different letters in each column indicate significant differences ($P < 0.05$). Values within parentheses are percentages.
[1] Feeds are *Ulva* sp. (UL), *Eisenia arborea* (EA), *Macrocystis pyrifera* (MP), *Egregia menziesii* (EM) and *Porphyra perforata* (PP).
[2] Nitrogen-free extracts are mainly carbohydrates.

Table 4 Mean values (±SE, $n = 3$) of initial and final biochemical composition of the digestive gland (DG) and muscle (M) of juvenile *Haliotis fulgens* fed different macroalgae diets

	Protein (mg g^{-1})		Carbohydrates (mg g^{-1})		Lipids (mg g^{-1})	
	DG	M	DG	M	DG	M
Initial value	184 ± 34	64.0 ± 16.0	133 ± 16	16.6 ± 3.5	20.0 ± 2.1	3.3 ± 0.6
Feed[1]						
UL	113 ± 10^{b}*	90.3 ± 12.0^{ab}*	448 ± 35^{a}*	16.5 ± 2.8	17.2 ± 0.9^{b}*	2.8 ± 0.4*
EA	167 ± 9^{a}	83.2 ± 11.2^{b}	99 ± 10^{c}*	9.5 ± 0.7*	18.1 ± 1.1^{b}*	3.8 ± 0.2
MP	169 ± 14^{a}	78.4 ± 5.5^{b}*	120 ± 20^{c}	16.8 ± 4.0	18.2 ± 0.9^{b}	3.8 ± 0.3
EM	180 ± 10^{a}	123 ± 11^{a}*	98.3 ± 7.6^{c}*	20.5 ± 4.3	21.4 ± 1.2^{ab}	3.9 ± 0.1
PP	159 ± 9^{a}*	66.9 ± 12.0^{b}	312 ± 44^{b}*	23.2 ± 0.9	23.3 ± 1.6^{a}*	3.9 ± 0.2

Mean values with different letters in each column indicate significant differences between feeds ($P < 0.05$).
[1] Feeds are *Ulva* sp. (UL), *Eisenia arborea* (EA), *Macrocystis pyrifera* (MP), *Egregia menziesii* (EM) and *Porphyra perforata* (PP).
* Significantly different from initial value.

Discussion

We found that rehydrated macroalgae promoted growth (*M. pyrifera* 18.1 μm day^{-1}, *Egregia menziesii* 17.5 μm day^{-1} and *P. perforata* 15.1 μm day^{-1}), although, depending on the diet, there were considerable differences in growth weight, feed efficiency and biochemical composition of the digestive gland and muscle.

Egregia menziesii, *M. pyrifera* and *P. perforata* provided significantly higher growth rates for abalone than did *Ulva* sp. and *E. arborea*. In Southern California, Leighton & Peterson (1998) found that the highest growth rates for juvenile and young adult green abalones occurred when fresh *Egregia menziesii* was provided. Nelson *et al.* (2002) found that young adults fed *Egregia menziesii* had a mean growth rate of 42.8 μm day^{-1}, compared with 2.9 μm day^{-1} for abalone fed *Chondracanthus canaliculatus* (= *Gigartina canaliculata*) and 2.1 μm day^{-1} for abalone fed *Ulva lobata*. To our knowledge, this is the first study to measure the growth of green abalone fed *P. perforata*. The results with *P. perforata* were comparable to those observed with *Egregia* or *Macrocystis* diets.

Rehydrated *M. pyrifera* produced a growth rate averaging 46 μm day^{-1} and 5.49 mg day^{-1}, while *E. arborea* produced 19 μm day^{-1} and 1.52 mg day^{-1} (Serviere-Zaragoza *et al.* 2001). In our trials, increased in shell length was less than the values reported by these authors, but the rate of weight gain was higher 9.2 mg day^{-1} with *Egregia menziesii* and 8.2 mg day^{-1} with *M. pyrifera* (Table 2). Body weight was apparently more sensitive to the diets than shell length. Uki (1981) attributed differences in growth rates (length and weight) to the nutrient composition of macroalgae. Leighton (1989, 2000) stated that the growth rates of juveniles fed fresh *M. pyrifera* are variable, but consistently low (<40 μm day^{-1}). Serviere-Zaragoza *et al.* (2001) and Ponce-Díaz *et al.* (2004) reported that rehydrated *M. pyrifera* produced average growth rates of 46 and 39 μm day^{-1}, respectively. Studies by Viana *et al.* (1993, 1996), where fresh *M. pyrifera* was used, reported the growth rates of 12–16 μm day^{-1} for juvenile green abalone, similar to our results.

High growth rates are normally accompanied by low FCR (Mercer *et al.* 1993). Macroalgae yielding high growth

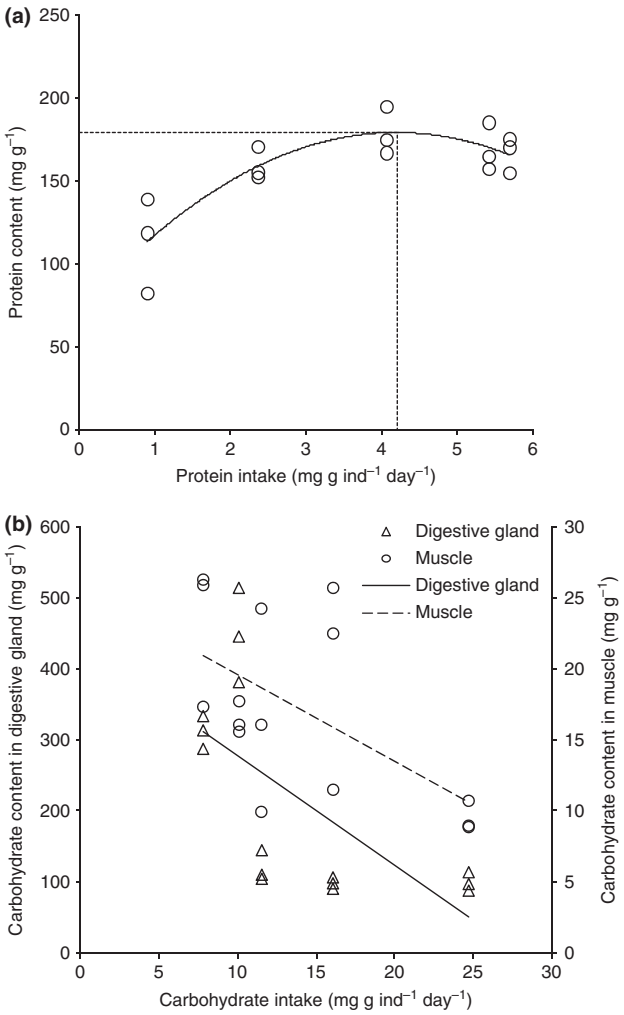

Figure 2 Relationship between protein (a) and carbohydrate content (b) in the digestive gland and foot muscle of green abalone *Haliotis fulgens* and nutrient intake from different macroalgae.

rates tended to produce lower FCR, (*M. pyrifera*, *Egregia menziesii* and *P. perforata*). *Porphyra perforata* is an efficient feed, compared to the other macroalgae, because it had the lowest ingestion rate. Although *Ulva* sp. and *P. perforata* had similar ingestion and nutrient intakes, *P. perforata* was clearly better at promoting growth; *Ulva* sp. produced the poorest results in growth rates and FCR. Intake of protein, carbohydrates and fibre was statistically significant for the prediction of growth rate (weight), indicating no clear dominance of a specific nutrient to explain differences in growth. Nutrient composition of the macroalgae corresponded to reports of 150 g kg^{-1} protein, 30–50 g kg^{-1} lipids and 200–300 g kg^{-1} carbohydrates of macroalgae consumed by herbivorous marine organisms (Leighton 1968; Santelices 1986), except for *Ulva* sp., with lower concentrations of protein, and *P. perforata*, with lower concentrations of lipids.

Many algae are available to abalone. In the northern hemisphere, there is a preponderance of brown algae in the abalone diet, and in the southern hemisphere, abalone feed mostly on abundant red algae (Shepherd & Steinberg 1992). Brown algae produce better results than red algae (Leighton & Booloottian 1963; Hirose 1974; Uki *et al.* 1986; Mai *et al.* 1995). We found that the red alga (*P. perforata*) produced growth rates that were not significantly different than brown algae. Moreover, the feed efficiency of this alga is superior to brown algae. Good results using a red macroalgae for *Haliotis iris* and *Haliotis australis* were obtained by Poore (1972); D.L. Leighton (pers. comm.) reports that *Gracilaria* sp. supports a growth rate that is comparable to *Egregia* at an abalone farm in San Diego County, California.

In the wild, the choice of macroalgae by abalone appears to be related to the nutritional content of the species and is also influenced by the morphology and the presence of noxious metabolites in the macroalgae (Shepherd & Steinberg 1992; Leighton 2000). Growth of juvenile green abalone was poor when fed *E. arborea*, although its protein content was similar to algae producing the highest growth rates. The thallus of *E. arborea* is rigid, while thalli of other macroalgae are soft. Reduced palatability is unlikely, because abalone consume large amounts of this alga. Leighton (1966) discussed the secondary metabolites with distasteful components, such as terpenoids and bromophenols, which have been found in *Eisenia bicyclis* (Freile-Pelegrin 2001) and other macroalgae (Shepherd 1975). Such ectometabolites would negatively affect the resource as food. Rehydated macroalgae fragments may release soluble carbohydrates; *Macrocystis* spp. are well known to lose fucoidan, laminarin, mannitol and alginates when stressed (D.L. Leighton, pers. comm.). These components could adversely affect abalone, if sufficiently concentrated, by coating delicate gill membranes.

Abalone shows a moderate preference for the genus *Ulva* (Mercer *et al.* 1993). Good growth was reported for *Haliotis tuberculata* fed *Ulva lactuca* (Uki & Watanabe 1992) and *Haliotis discus hannai* fed *Ulva* sp. (Mercer *et al.* 1993). These species of *Ulva* contained approximately 131 g kg^{-1} protein, which was higher than the species of *Ulva* used in our study. Higher consumption of this alga by juveniles did not compensate for its low protein content, which resulted in the lowest protein intake among the diets.

Biochemical composition of the digestive gland was considered, because the content of this gland should reflect recently consumed food constituents compared with the slower turnover of constituents in the muscle tissue, which

should reflect retained nutrients. Most concentrations of nutrients in abalone tissue tended to remain unaltered compared to initial concentrations. In cases where the concentrations changed, decreases rather than increases occurred. Simultaneous decreases in the concentrations of nutrients in the digestive gland and muscle occurred in *Ulva* sp. and *E. arborea* diets, which was also reflected by lower growth rates. In the other diets, reductions occurred in only one nutrient in the digestive gland or the muscle. Increases in the concentration of carbohydrates and lipids in the digestive gland indicated higher feed efficiency with *P. perforata*. Among the diets, nutrients in the digestive gland showed greater variability than in muscle, the latter differed only in protein content. Higher or lower protein and lipid levels in the digestive gland tended to be related to higher or lower rates of increase in weight. There was no apparent relationship between the growth rate (weight) of juveniles fed *Ulva* sp. or *M. pyrifera*, yet the highest protein concentration in muscle occurred with these two diets. There was no relationship between weight gain and higher carbohydrate content in the digestive gland with *Ulva* sp. and *P. perforata*.

According to Mercer *et al.* (1993), Nelson *et al.* (2002) and Ponce-Díaz *et al.* (2004), abalone does not use lipids as the main energy source, but it constitutes an essential nutrient for growth and reproduction. In *P. perforata* and *Egregia menziesii* diets, abalone had higher lipid levels in the digestive gland, indicating better physiological and metabolic condition. Ostensibly, the high nutritive value of *P. perforata* provides a lipid reserve in the gland, similar to *Palmaria palmata* fed to *H. tuberculata* and *H. discus hannai* (Mercer *et al.* 1993) and with *M. pyrifera* fed to *H. fulgens* (Ponce-Díaz *et al.* 2004). Weber (1970), working with *Haliotis cracherodii*, and Ponce-Díaz *et al.* (2004), working with green abalone, found carbohydrates (mainly glycogen) stored in abalone muscle. In our study, our initial and final carbohydrates concentration was similar.

Protein content in the digestive gland was related to protein intake. The intake rate that maximized the accumulation of protein was close to the intake rate of *Egregia menziesii* (Fig. 2). Reduction in carbohydrate content of the digestive gland and muscle as carbohydrate intake increased suggests that higher mobilization and utilization of carbohydrates is the primary energy source. However, there was no direct evidence of nutrient transfers between the digestive gland and foot muscle.

This study showed that differences in chemical composition of macroalgae result in different growth responses, feed efficiencies and biochemical composition of the digestive gland and muscle of juvenile *H. fulgens*. Rehydrated *Egregia menziesii*, *M. pyrifera* and, particularly, *P. perforata* are more efficient than *Ulva sp.* and *E. arborea* in promoting growth. *Porphyra perforata* was more efficiently used by abalone than other macroalgae, as indicated by the low FCR.

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