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Comparative performances of juvenile abalone (*Haliotis tuberculata coccinea* Reeve) fed enriched *vs* non-enriched macroalgae: Effect on growth and body composition

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ABSTRACT

Abalone *Haliotis tuberculata coccinea* Reeve (1846), is a target species for diversification of European aquaculture production. Taking into account that sustainable, eco-friendly production methods are to be a part of future expansion of the abalone industry, growth performance of juvenile abalone reared in an integrated culture system was evaluated and compared with that of abalone fed non-enriched macroalgae. Four macroalgae treatments, three monospecific: *Ulva rigida* (UN), *Hypnea spinella* (HN) and *Gracilaria cornea* (GN) and a composite one (MN), were produced out of fishpond wastewater effluents, while other four control treatments consisted of the same species reared in fresh seawater (U; H; G; M). Seaweeds reared in fishpond wastewater effluents increased their protein content from 11–17% to 29–34%. Lipids consisted mainly of saturated fatty acids (SFA) (43–60%), palmitic acid being the most abundant fatty acid (40–47%). Highest EPA percentage was found in red algae *H. spinella* (6.9%), being ten times higher than that of *U. rigida* (0.7%). All the algae tested contained very low levels of arachinodic acid (0.1–1.6%) and docosahexaenoic acid (0.5–3%). Protein levels in foot muscle (74–76%) did not differ significantly ($P<0.05$) among treatments. Survival was generally high, ranging from 85 to 100%. Weight gain (17–561%) and SGR (0.2–2.3%) were positively related to protein content; whereas, protein efficiency ratio (PER) (0.5–3.7) was negative correlated. PE ratios increased by 82–159% (DW) as a function of the enrichment among the different diets. Food conversion ratio (FCR) (7–188) improved according to the increase in PER. Overall, biofilter-produced macroalgae showed a significantly higher dietary value compared to the control treatments. Similarly, animals fed the mixed diets performed significantly better than those fed a single algal diet. Feeding *G. cornea* led to the lowest growth performance probably due to the lowest feed intake. The results clearly indicate that *H. tuberculata coccinea* growout can efficiently take place in an integrated-culture system suggesting that on-farm seaweed-abalone production could be part of future development of the abalone industry in the Canary Islands.

1. Introduction

Abalone species (*Haliotis spp.*) are found worldwide and are becoming important for aquaculture diversification due to their high market price and the over exploitation of wild stocks. In Europe, abalone industry is currently focussed on the production of the ormer *Haliotis tuberculata* Linnaeus (1758). Ireland, the Channel Islands (Huchette and Clavier, 2004) and France are currently the only established producing countries. A subspecies of ormer, the abalone *Haliotis tuberculata coccinea* Reeve (1846), is also considered a good candidate for European aquaculture, as it is highly appreciated for its delicate taste, reaches a large enough size to be commercialized and its culture techniques have been successfully developed (Bilbao et al., 2004, 2010a, b; Courtois de Viçose et al., 2007, 2009, 2010; Toledo et al., 2000; Viera et al., 2003, 2005, 2007, 2009a, b). A limiting factor for further expansion of abalone aquaculture is the restricted availability of an economically and environmentally sustainable feed, as this culture frequently requires large quantities of wild harvested macroalgae. Such feed would be particularly important in areas where wild algae are not commercially available (Viera et al., 2005).

Among different nutrients, protein constitutes the most costly component and is a major determinant of the nutritional value in diets of the abalone (Bautista-Teruel et al., 2003; Britz, 1996a; Britz, 1996b; Britz and Hecht, 1997; Gómez-Montes et al., 2003; Mai et al., 1995b; Reyes and Fermin, 2003; Sales et al., 2003; Shipton and Britz, 2001; Uki et al., 1985b; Uki and Watanabe, 1986). In an aquaculture integrated system, nitrogen enriched waste water of intensively cultured organisms, may be transformed into a valuable algal biomass, seaweeds production being an added income as feed for shellfish (Evans and Langdon, 2000; Neori et al., 2004; Schuenhoff et al., 2003). Besides,

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several studies have shown that the culture of macroalgae in nutrient-rich waters increases their protein content (Boarder and Shpigel, 2001; Naidoo et al., 2006; Robertson-Andersson et al., 2006; Shpigel et al., 1999; Viera et al., 2005). Thus, biofilter produced seaweed have been shown to support fast growth rates for *Haliotis tuberculata* (Neori et al., 1998; Shpigel et al., 1999), *Haliotis discus hannai* (Corazani and Illanes, 1998; Shpigel et al., 1999), *H. roei* (Boarder and Shpigel, 2001) and *H. tuberculata coccinea* (Viera et al., 2005).

Among the different macroalgae produced, *Ulva spp.* and *Gracilaria spp.* are good candidates as feed for abalone since their mass production technologies are well developed and their nutrient uptake capacities are among the highest known (Martínez-Aragón et al., 2002). The valuable rhodophyte *Hypnea sp.* has also been successfully cultured in mariculture biofilters (Harlin et al., 1978; Neori et al., 2004; Viera et al., 2005).

In the wild, abalone consumes different macroalgae species, obtaining their required nutrients from a combination of algal species. Although *H. tuberculata coccinea* feeds on a diverse assemblage of macroalgae (Espino and Herrera, 2002), its nutritional needs and the relative importance of these algae are unknown.

It is well recognized that consumer knowledge and attitudes to an aquaculture product have a significant role to play in commercial success. As abalone in Europe currently has low levels of production, it gives the sector an excellent opportunity to set standards that will meet consumer expectations for sustainable, eco-friendly production methods which fit into the strongly growing EU eco-sector for shellfish products. Abalone producers may be enticed to adopt effluent treatment procedures more readily if shown that enriched macroalgae can be suitable as a feed for local abalone species promoting higher growth performance than the one achieved with seaweeds harvested or reared in fresh seawater.

In this study, the comparative performance of juvenile abalone *Haliotis tuberculata coccinea* fed on various enriched *vs* non enriched macroalgae was examined. We determined: (1) Algal nutritional and fatty acid composition; (2) Survival; (3) Growth (shell growth rate; specific growth rate and weight gain); (4) Consumption (daily feed intake); (5) Feed efficacy of feed utilization (food conversion ratio (FCR) and protein efficiency ratio (PER); (6) Biochemical composition of the animals and (7) Soft-body to shell ratio (SB/S) of the abalone after being fed these diets for 12 weeks. Performance promoted by the various diets was related to a range of nutritional parameters including crude protein (CP), total lipid (TL), gross energy (GE), protein-energy ratio (PE) and fatty acids (FA) of the diets fed.

2. Materials and methods

2.1. Algal culture

Ulva rigida J. Agardh, *Hypnea spinella* (C. Agardh) Kützing and *Gracilaria cornea* J. Agardh were grown at the Centro de Biotecnología Marina (CBM-ULPGC), Gran Canaria, Spain. Eight feeding regimes were evaluated: three monospecific ones with macroalgae produced in fresh seawater *Ulva rigida* (U), *Hypnea spinella* (H) and *Gracilaria cornea* (G), and a mixture of equal parts from the three algae (M); and the same four feeding regimes using algae produced out of fishpond waste water effluents (UN; HN; GN and MN). Effluents were channeled from the fishponds to a 11 m^3 sedimentation pond for the removal of suspended particles and then, pumped at a flow rate of 10 $m^3\ h^{-1}$ to the seaweed tanks located in a greenhouse, where maximum irradiance was approximately of 1600 μmol photons $m^{-2}\ s^{-1}$. Semi-circular fiberglass tanks with a surface of 1.8 m^2 and a volume of 0.75 m^3 were provided aeration through a bottom-central linear pipeline and were employed for the cultivation of macroalgae. Algal stocking densities were adjusted to the optimal values obtained from previous experiments (1, 3 and 4 g l^{-1} for *U. rigida*, *H. spinella* and *G. cornea*, respectively). Water exchange rate in the seaweed culture tanks was 4 vol day^{-1} and TAN (total ammonia nitrogen) inflow into the biofilter ranged between 10 and 400 μM.

2.2. Abalone and feeding trial conditions

Abalone (*Haliotis tubercalata coccinea*) were produced within the experimental hatchery production unit of the Instituto Canario de Ciencias Marinas (Canary Islands, Spain).

Animals were initially fed a mixed diet of *Navicula sp.* and *Nitzschia sp.* for 4 to 5 months. Feeding of all abalone was then gradually switched to the green macroalgae *Ulva rigida* cultured in the laboratory biofiltration system for a period of 1 month prior to the beginning of the experiment. A total of 600 juvenile abalones (25/tank) with an average shell length and weight of 12.5 ± 1.6 mm and 0.27 ± 0.18 g, respectively, were selected for the trial.

Individuals were blot dried, weighed to the nearest 0.1 mg (total wet body weight: TWBW), measured with manual caliper with 0.1 mm accuracy (total shell length: SL) and assigned to an experimental unit. Abalones were homogeneously distributed among tanks to avoid significant differences in SL or TWBW. Each experimental algal regime (8 feeding diets tested in triplicates) was fed for 12 weeks to the abalones and tested in triplicates in a flow-through system. Eight control units containing the same feeding regimes without abalone, were used as controls to estimate percentage of modification in algal weight (computed as: $((A-B)/A)\times 100$; where B is the final weight of algae and A is the initial weight of algae in the control unit determined at 1 week interval).

The experimental unit consisted in a 1 l lidded (plastic net of 2 mm mesh) PVC plastic container (20 × 14 cm), located in a 100 l cylindrical tank filled with 50 μm filtered seawater provided with constant aeration. Seawater temperature ranged between 22 and 24.5 °C and flow was set at 2.4 l/min. Abalone were subjected to a natural photoperiod of approximately 12 h L/12 h D. Algae were supplied in excess to guarantee *ad libitum* feeding and replaced once a week during the growth trial.

2.3. Growth and algal consumption

To determine feed intake, freshly collected algae were blotted dry and accurately weighed as well as the remaining algae at the end of the week. The weight of unconsumed food was deducted from the total weekly ration. Besides, weight of uneaten algae was corrected by calculating the natural weight variations of the algae in the control units during the same feeding period. Average daily intake by individual abalone was calculated by dividing the algal biomass eaten each week by the feeding days and the number of abalones in each experimental unit.

SL and TWBW of each animal were determined every four weeks. Besides, the following indices were calculated for all treatments and the end of the trial:

$$\text{Shell growth rate} = (L_2 - L_1) / \text{days of culture} \times 1000$$

$$\text{Specific growth rate, SGR} = (\text{Ln}W_2 - \text{Ln}W_1) / t \times 100$$

$$\text{Weight gain}(\%) = ((W_2 - W_1) / W1) \times 100$$

$$\text{Food conversion ratio} = \text{total feed intake (gwet)} / \text{total weight gain (gwet)}$$

$$\text{Protein efficiency ratio} = (\text{increase in body wet weight (g)}) / (\text{protein intake (g)})$$

where L_1 is the initial mean length of animals; L_2 is the final mean length of animals; W_2 is the weight at time t (days of culture), and W_1 is the initial weight.

At the end of the experiment, ten abalones were collected from each experimental unit, and the soft tissue was shucked from the shell. Shell and meat were then weighed separately in order to calculate the condition index (wet weight of soft flesh/wet weight of shell, SB/S in W/W) as an indicator of the abalone nutritional status.

2.4. Proximate and fatty acid analysis

Homogenized samples of visceral mass and foot muscle of the selected abalone and algae from each feeding regime were analyzed in triplicate for nutrient composition. Fatty acids of the algae were also analyzed. The algae, abalone visceral mass and foot muscle were cleaned, washed with freshwater and frozen at −80 °C. Dry matter was determined by drying samples at 110 °C until constant weight was attained. Ash content was determined by incinerating samples at 600 °C for 24 h. Protein content was analyzed according to AOAC (2005) standard methods. Total lipids were extracted by a chloroform–methanol (2:1) mixture as described by Folch et al. (1957). Fatty acids in the lipid extracts were transesterified to methyl esters (FAMEs) with 1% sulphuric acid:methanol complex (Christie, 1982). FAMEs samples were extracted into hexane and stored at −80 °C. Fatty acids were analyzed in a Thermo Finnigan-GC Focus gas chromatograph equipped with a flame ionization detector (260 °C) and a capillary column (Supelcowax 28 m × 0.32 mm × 0.25 i.d.), using helium as the carrier gas under the conditions described by Izquierdo et al. (1989).

2.5. Statistical analysis

All data were statistically treated by one-way ANOVA and Tukey's test was applied for multiple comparison of means at a 5% significance level ($P<0.05$). When data did not follow a normal distribution, a non-parametric one-way ANOVA on ranks of Kruskal–Wallis was applied (Zar, 1984).

3. Results

3.1. Algal nutritional composition

Nutritional composition and caloric content of the eight seaweed treatments are shown in Table 1. Gross energy values in diets ranged from 3.5 to 4.1(kcal g^{-1}). Protein:energy ratios increased by 82–159% (dry weight) with the enrichment among the different diets. Protein content was significantly higher ($P<0.05$) in the seaweeds reared using fishpond waste water effluents, increasing their protein content from 11.3–16.6% to 29.3–33.8%. Lipid content ranged from 1.4 to 7.2%, being also generally higher in enriched seaweeds, the highest being found in GN and the lowest in *H. spinella* (H). The carbohydrate contents varied inversely to protein contents, showing significantly higher values in non enriched treatments. No significant differences were observed in the ash content for all diets.

3.2. Survival, growth, consumption and condition index

Growth performance, feed utilization and survival of juvenile *H. tuberculata coccinea* fed the eight experimental diets are shown in Table 2. Survival was generally high, ranging from 85 to 100% for those fed with G and H, respectively. In general, abalone fed enriched algae performed better than those fed on non enriched macroalgae, displaying higher shell growth rate, specific growth rate and weight gain. Similarly, animals fed the mixed diets, both enriched and non enriched, performed significantly better than those that were fed with a single algal diet. Daily feed intake on different algal rations recorded for 12 weeks showed that, except with *Gracilaria* treatments (G and GN), all diets were very well accepted by the abalone. Nevertheless, a significantly ($P<0.05$) higher feed intake of the mixed diet, followed by *H. spinella* and *U. rigida* was registered. Hence, at the end of the trial, animal fed on the enriched mixed macroalgae diet (MN) presented a significantly ($P<0.05$) higher growth performance, length (151 ± 3.9 μm day^{-1}), weight gain ($561.3 \pm 20.3\%$) and specific growth rate (2.3%) than those on any other diets. For abalone fed non enriched macroalgae, final shell length and weight were significantly highest in animals fed the mixed diet (22.3 ± 3.5 mm and 1.4 ± 0.6 g), followed by *U. rigida* and *H. spinella*, and were the lowest in those fed *G. cornea* (14.3 ± 1.7 mm and 0.3 ± 0.1 g). Regarding feed utilization efficacy, except for *Hypnea* diets (H and HN), food conversion ratio (FCR) values were inversely related to protein level, being significantly lowest in animals fed UN and MN (7–10.1) and highest in those fed non-enriched *G. cornea* (188.1 ± 8.9). However, protein efficiency ratio (PER) values were generally higher in abalone fed non-enriched macroalgae, declining from 3.7–1 to 2.4–0.5, respectively. Soft-body to shell ratio were significantly influenced by the diets. The lowest SB/S ratios (2.4) resulted from feeding both *G. cornea* diets, which produced the poorest growth performance, whereas UN and both mixed diets, produced the best growth performance, yielded the highest SB/S ratios (3.3–3.1).

3.3. Fatty acid composition of macroalgae

Fatty acid profiles of the eight macroalgae diets are summarized in Table 3. The lipids of all algae tested consisted mainly of saturated fatty acids (SFA) (43–60%), with palmitic acid (16:0) as the most abundant fatty acid (FA) (40–47%) of total FAME. A higher amount (5–7.2%) of myristic acid (14:0) was detected in both Rhodophyta species as compared to that in the green algae (1%). The green algae *U. rigida* showed higher levels of C_{16} (58%) and C_{18} (32%) fatty acids and lower level of C_{20} fatty acids (1.8–1.1%) than that of red algae. 18:1n-7 was the predominant mono-unsaturated fatty acid of this Chlorophyta. All red algal treatments contained considerable levels of mono-unsaturated fatty acid predominantly 18:1n-9. Linoleic acid (18:2n-6) was present at similar levels in all selected algae, whereas linolenic acid (18:3n-3) was higher in *U. rigida*. The levels of $\sum$ n-6 PUFA (7–9%) were generally lower than those of $\sum$ n-3 PUFA (12–18%). Eicosapentanoic acid (20:5n-3) (EPA) highest percentage was found in red algae *H. spinella* (6.3–6.9%), being ten times higher than the one of *U. rigida* (0.7%). All macroalgae presented very low level of arachinodic acid (0.1–1.6%)

Table 1
Proximate composition and caloric content of the eight macroalgae treatments (g/100 g DW) (Mean ± S.D.) fed to abalone along the experimental trial.

	Diets							
	U. rigida	*H. spinella*	*G. cornea*	*Mixed diet*	*Enriched U. rigida*	*Enriched H. spinella*	*Enriched G. cornea*	*Enriched Mixed diet*
Moisture	82.09 ± 0.8^{b}	84.2 ± 1^{ab}	83.9 ± 1.8^{ab}	83.3 ± 1.4^{ab}	82.04 ± 0.3^{b}	83 ± 2.9^{ab}	84.94 ± 0.6^{a}	83.25 ± 1.9^{ab}
Crude protein	16.6 ± 3.8^{b}	13.2 ± 1.7^{b}	11.27 ± 1.1^{b}	13.7 ± 3^{b}	33.76 ± 0.5^{a}	33.09 ± 6^{a}	29.35 ± 2^{a}	32.1 ± 3.9^{a}
Crude lipid	3.7 ± 1^{abc}	1.4 ± 0.4^{c}	5.4 ± 3.5^{abc}	3.4 ± 2.5^{bc}	4.4 ± 0.8^{abc}	6.6 ± 2.6^{ab}	7.21 ± 2.7^{a}	6.06 ± 2.1^{a}
Carbohydrate[1]	56.4 ± 8.5^{ab}	65.4 ± 9.5^{a}	58 ± 8.5^{a}	60.7 ± 7.6^{a}	40.5 ± 6.1^{bc}	39.2 ± 11.1^{bc}	31.8 ± 11.3^{c}	41.01 ± 3.6^{c}
Ash	25.2 ± 6	21.4 ± 7.9	24.1 ± 3.6	23.6 ± 5.9	21.5 ± 5.2	21.3 ± 2.7	32.3 ± 7	25.24 ± 7.3
GE[2] (kcal g^{-1})	3.5	3.5	3.6	3.6	3.9	4.1	3.6	4.04
Protein:energy ratio[3]	47.5	37.7	31.4	38.05	86.6	80.7	81.5	79.4

[1]Calculated by difference (AOAC, 2005).
[2]Gross energy.
[3]Metabolizable energy was calculated based on the physiological values at 5.6 kcal g^{-1} protein, 9.5 kcal g^{-1} lipid and 4.1 kcal g^{-1} carbohydrates(Cho et al., 1982).
Values in the same row with different letters are significantly different ($P<0.05$) $n=3$.

Table 2
Growth performance, feed utilization and survival of juvenile abalone (*H. tuberculata coccinea*) fed the selected 8 macroalgae diets for 12-weeks.

	Macroalgae treatments fed to the abalone							
	U. rigida	*H. spinella*	*G. cornea*	*Mixed diet*	*Enriched U. rigida*	*Enriched H. spinella*	*Enriched G. cornea*	*Enriched Mixed diet*
Initial length (mm)	12.6 ± 1.6	12.5 ± 1.6	12.6 ± 1.6	12.5 ± 1.6	12.5 ± 1.5	12.6 ± 1.6	12.5 ± 1.6	12.5 ± 1.5
Final length (mm)	19 ± 3^{c}	19.3 ± 2.1^{c}	14.3 ± 1.7^{d}	22.3 ± 3.5^{b}	21.6 ± 2.3^{b}	20.1 ± 1.9^{c}	14.4 ± 1.8^{d}	25.1 ± 2.6^{a}
Shell growth rate ($\mu m\ d^{-1}$)	77.7 ± 3.2^{c}	81.9 ± 7.8^{c}	20.8 ± 0.8^{d}	118.4 ± 7.9^{b}	110.1 ± 0.8^{b}	90.5 ± 8.4^{c}	23.0 ± 1.2^{d}	150.9 ± 3.9^{a}
SGR (% day^{-1})	1.5 ± 0.02^{c}	1.4 ± 0.2^{c}	0.2 ± 0.0^{d}	2 ± 0.1^{b}	1.9 ± 0.0^{b}	$1.4 \pm 0.\ 1^{c}$	0.3 ± 0.04^{d}	2.3 ± 0.04^{a}
Initial weight (g)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.9	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.9
Final weight (g)	0.9 ± 0.3^{c}	0.8 ± 0.4^{c}	0.3 ± 0.1^{d}	1.4 ± 0.6^{b}	1.2 ± 0.4^{b}	0.9 ± 0.3^{c}	0.3 ± 0.1^{d}	1.8 ± 0.6^{a}
Weight gain (%)	239.9 ± 6.5^{c}	255.9 ± 7^{c}	17 ± 3.3^{d}	410 ± 37.5^{b}	371.6 ± 12.6^{b}	229.9 ± 3^{c}	33 ± 4.3^{d}	561.3 ± 20.3^{a}
Feed intake (mg $abalone^{-1}\ day^{-1}$)	63.5 ± 2.5^{d}	147.4 ± 10.0^{b}	31.8 ± 2.5^{e}	168.7 ± 18.3^{ab}	81.0 ± 0.8^{c}	173.7 ± 17.1^{ab}	35.4 ± 0.1^{e}	189.9 ± 5.0^{a}
FCR	9.0 ± 0.5^{e}	21.0 ± 2.8^{c}	188.1 ± 8.9^{a}	12.9 ± 0.6^{cd}	6.8 ± 0.3^{e}	22.4 ± 0.4^{c}	57.1 ± 6.0^{b}	10.1 ± 0.2^{e}
PER	3.7 ± 0.2^{a}	2.3 ± 0.3^{b}	1 ± 0.0^{d}	3.4 ± 0.2^{a}	2.4 ± 0.1^{b}	0.8 ± 0.01^{d}	0.5 ± 0.2^{e}	1.8 ± 0.04^{c}
CI (%)	2.9 ± 0.4^{b}	2.9 ± 0.4^{b}	2.4 ± 0.5^{c}	3.1 ± 0.4^{ab}	3.3 ± 0.3^{a}	2.8 ± 0.4^{b}	2.4 ± 0.4^{c}	3.3 ± 0.3^{a}
Survival (%)	93.3^{ab}	100^{a}	85.3^{b}	97.3^{a}	93.7^{ab}	96^{ab}	92^{ab}	98.7^{a}

Values in the same row with different letters are significantly different ($P<0.05$) $n = 25 \times 3$.

(ARA) and docosahexaenoic acid (DHA) (22:6-n-3), with the highest percentage been found in enriched *G. cornea* with 3% of total FAME.

3.4. Effects of algal diets on the general biochemical composition of animals

Nutritional analysis of the abalone at the end of the experimental period revealed that diet did significantly affect the soft body tissues of the animals (Table 4). Protein levels in foot muscle (74–76% DW) did not differ significantly ($P<0.05$) among treatments. Generally the biofilter cultured algae produced better growth and resulted in significantly lower moisture levels in viscera than others. Lipid levels in viscera varied considerably within the feeding regimes, being higher in abalone fed non enriched macroalgae. Abalone fed both enriched and non enriched macroalgae, showed higher lipid levels, stored in the viscera (11–20%), rather than in the foot muscle (5–8%), the highest being found in those fed with M and U diets. Ash content, both in viscera and muscle, in animals fed non enriched algae, were generally higher than that of abalone fed with macroalgae produced out of fishpond waste water effluents.

4. Discussion

Gross energy is the total amount of energy supplied by food and is an important quantitative measurement of calorific value which may be an useful indicator of the seaweed nutritional value (Hernández-Carmona and Carrillo-Domínguez, 2009; Lamare and Wing, 2001). Despite the ability of abalone to utilize a wide variety of energy sources, being a mollusc, the metabolic rate of abalone is low and consequently energy requirements are low. The caloric content (gross energy) of the algae tested (3.5–4.1 kcal g^{-1}) was in the range of values reported for other diets used as feed for abalone generally reported around 4 kcal g^{-1} (García-Esquivel and Felbeck, 2006; Reyes and Fermin, 2003; Shipton and Britz, 2001). Protein content (11–17% DW) of the non enriched macroalgae was within the range of values reported for other species of red and green seaweeds used as feed for abalone (13–18% DW), (Bautista-Teruel and Millamena, 1999; Jackson et al., 2001; Mercer et al., 1993; Reyes and Fermin, 2003; Wong and Cheung, 2000). The protein content of seaweed species varies greatly and demonstrates a dependence on factors such as season and growing conditions. The high protein content of macroalgae produced using fishpond waste

Table 3
Fatty acid composition (% total fatty acids) of the eight macroalgae treatments.

FA	*U. rigida*	*H. spinella*	*G. cornea*	*Mixed diet*	*Enriched U. rigida*	*Enriched H. spinella*	*Enriched G. cornea*	*Enriched Mixed diet*
14:0	1.1	6	5.2	4.1	1	7.2	5	4.4
16:0	46.8	41.3	50.3	46.1	40.5	44.4	47.5	44.1
18:0	1.9	5.4	4.2	3.8	1.9	3.3	4.4	3.2
∑ SFA	49.8	52.7	59.7	54.1	43.3	54.9	56.9	51.7
14:(1n-5)	1.64	2.42	0.47	1.51	1.57	3.24	0.44	1.75
16:(1n-7)	6.1	2.5	2.3	3.6	9.6	2.9	3.5	5.3
16:(1n-5)	0.2	0.3	1.8	0.8	3	0.3	0.6	1.3
18:(1n-9)	5	10.6	8.8	8.1	3.5	9.7	10.5	7.9
18:(1n-7)	9	2.5	3	4.8	8.3	3.1	3.5	5
22:(1n-11)	1.9	0.3	0.1	0.8	2	0.3	0.1	0.8
∑ MUFA	23.9	18.6	16.5	19.7	27.9	19.5	18.6	22
16:(4n-3)	2.7	0.8	1.4	1.6	3.8	0.5	1.6	1.9
18:(2n-6)	7	7.7	5.3	6.7	6.1	5.5	6.1	5.9
18:(3n-3)	5.7	1.9	0.6	2.7	7	1.5	0.8	3.1
18:(4n-3)	3.3	1	0.7	1.6	4.2	0.9	0.6	1.9
20:(4n-6)	0.1	0.4	1.6	0.7	0.1	0.5	0.7	0.4
20:(4n-3)	0.4	2.9	7.1	3.4	0.3	3.8	4.5	2.9
20:(5n-3)	0.6	6.3	0.8	2.6	0.7	6.9	1.9	3.2
22:(5n-3)	1.6	0.5	0.3	0.8	1.5	0.4	0.5	0.8
22:(6n-3)	0.5	1.1	0.9	0.9	0.5	0.5	3	1.3
∑ PUFA	21.8	22.5	18.7	21.0	24.2	20.4	19.6	21.4
Other	4.5	6.2	5.1	5.2	4.6	5.2	4.9	4.9
∑ n-3-FA	14.7	14.5	11.8	13.6	18.0	14.5	12.9	15.1
∑ n-6-FA	7.1	8.1	6.9	7.4	6.2	6.0	6.7	6.3

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Other includes all components <1%: 14(1n-7), 15:00, 16:0ISO, 16:(2n-6), 16:(2n-4), 17:00, 16:(3n-3), 16:(3n-1),16:(4n-1), 18:(1n-5), 18:(2n-4), 18:(3n-6), 18:(3n-4), 20:00, 20:(1n-9 + n-7), 20:(1n-5), 20:(3n-6), 22:(1n-9), 22:(4n-6), 22:(5n-6).

Table 4
Proximate composition of foot tissues of *Haliotis tuberculata coccinea* reared on the experimental diets (g/100 g DW) (Mean ± S.D.).

Diet	Moisture		Crude protein		Crude lipid		Carbohydrate[1]		Ash	
	Viscera	Muscle	Viscera	Muscle	Viscera	Muscle	Viscera	Muscle	Viscera	Muscle
U. rigida	73.0 ± 0.3^{b}	75.7 ± 0.1^{a}	63.4 ± 0.0^{b}	74.0 ± 0.1	19.4 ± 1.8^{a}	7.1 ± 0.2^{ab}	9.4 ± 0.9^{d}	9.9 ± 0.4^{ab}	10.1 ± 0.7^{a}	9.1 ± 0.2^{c}
H. spinella	73.7 ± 0.9^{ab}	75.3 ± 0.3^{a}	55.4 ± 1.3^{d}	74.5 ± 1.5	16.8 ± 1^{b}	4.8 ± 1.3^{b}	18.8 ± 2.1^{b}	10.1 ± 0.5^{ab}	9.8 ± 0.1^{ab}	$10 \pm .1^{ab}$
G. cornea	75.3 ± 2.4^{a}	75.5 ± 0.0^{a}	63.1 ± 0.1^{b}	74.1 ± 0.2	15.3 ± 1.4^{bc}	5.8 ± 1.3^{ab}	11.6 ± 1.1^{c}	10.6 ± 1.7^{ab}	9.1 ± 0.2^{abc}	9.7 ± 0.2^{bc}
Mixed diet	73.8 ± 0.6^{ab}	74.5 ± 0.4^{b}	63.3 ± 1.4^{bc}	74.1 ± 0.2	20.5 ± 0.5^{a}	6.7 ± 1.2^{ab}	7.7 ± 1^{de}	8.2 ± 1.3^{c}	8.4 ± 0.2^{c}	11.0 ± 0.3^{a}
Enriched *U. rigida*	70.2 ± 0.2^{c}	73.9 ± 0.3^{b}	61.3 ± 0.3^{bc}	74.4 ± 0.5	16.2 ± 0.3^{b}	5 ± 0.2^{b}	14.1 ± 0.3^{c}	12.6 ± 0.7^{a}	8.5 ± 0.4^{c}	8.1 ± 0.3^{d}
Enriched *H. spinella*	73.2 ± 0.1^{ab}	75.4 ± 0.3^{a}	73.7 ± 0.9^{a}	76.3 ± 1.2	12.8 ± 0.4^{cd}	7.3 ± 0.3^{ab}	4.9 ± 0.1^{e}	9.2 ± 0.7^{ab}	8.9 ± 0.5^{bc}	6.9 ± 0.4^{de}
Enriched *G. cornea*	71.4 ± 0.1^{bc}	74 ± 0.3^{b}	55 ± 0.2^{d}	76 ± 1.6	11.5 ± 0.6^{d}	7.9 ± 1.1^{a}	24.4 ± 0.1^{a}	7.4 ± 1.6^{c}	9.5 ± 0.1^{abc}	9.3 ± 0.6^{bc}
Enriched mixed diet	70.7 ± 0.4^{c}	75.6 ± 0.1^{a}	61.5 ± 0.6^{c}	75.9 ± 2.6	12.2 ± 1.1^{d}	5.6 ± 0.3^{ab}	19.5 ± 1.6^{b}	10.6 ± 3.1^{ab}	7.1 ± 0.3^{d}	7.9 ± 0.1^{de}

[1]Calculated by difference (AOAC, 2005).
Values in the same column with different letters are significantly different (Tukey test, $P<0.05$ $n=3$).

water effluents (29–34%) compared with those reared in fresh seawater, would be related to its production under the high nitrogen culture conditions of the biofilter system, as it has also been observed in previous research with *Ulva spp*., *Hypnea spp.* and *Gracilaria spp*., used as macroalgal biofilters (Boarder and Shpigel, 2001; Njobeni, 2006; Robertson-Andersson, 2003; Shpigel et al., 1996a, b, 1999; Viera et al., 2005). The crude lipid contents were low in the algae studied, ranging between 1.4 and 7.2% DW, and being generally higher in enriched seaweeds, which is comparable to the range reported for other macroalgae (0.6–6.15% DW) (Dawczynski et al., 2007; Hernández-Carmona and Carrillo-Domínguez, 2009; Wong and Cheung, 2000). Nevertheless, abalone species show a low lipid requirement, typical of herbivores molluscs and fish (Mai et al., 1995a). This low lipid requirement has been associated by some authors with a reduced use of dietary lipids as energy source by abalone based upon its low metabolic rate (Durazo-Beltrán et al., 2004). Studies on the digestive enzymes of some species of *Haliotis* reveal that abalone have low activities of lipases, chymotrypsim or aminopeptidase (García-Esquivel and Felbeck, 2006). Indeed, high levels of dietary lipid seem to affect negatively abalone growth (Thongrod et al., 2003). However, high levels of carbohydrate enhance growth of abalone presenting high amylases activities and other carbohydrate digestive enzymes, such as cellulase, agarase and alginate lyase, (Britzs, 1994; García-Esquivel and Felbeck, 2006; Mai et al., 1996 Thongrod et al., 2003), as well as a good capacity to synthesize non essential lipids from carbohydrates. Carbohydrates are the largest component in many algae. In the present study, carbohydrate contents were high and inversely related to protein contents with comparable values to those obtained for other algal species (Foster and Hodgson, 1998; Hernández-Carmona and Carrillo-Domínguez, 2009; Kaehler and Kennish, 1996). Ash was the second highest fraction in all the diets, after carbohydrates, with similar values to those reported previously for other species of the same genus (Hernández-Carmona and Carrillo-Domínguez, 2009; Wong and Cheung, 2000).

Abalone fed enriched macroalgae diets showed better performance in terms of growth rate per day, weight gain, increase in shell length and FCR values compared to those fed non enriched diets. This could be related to the high protein content of enriched macroalgae diets, suggesting that nitrogen may be a limiting factor for growth in *Haliotis spp*. (Boarder and Shpigel, 2001; Fleming, 1995). Previous studies support this view by stating that maximum growth can only be achieved when sufficient protein, in the correct proportions of amino acid, is supplied in the feed. Shpigel et al. (1996a; 1999), Boarder and Shpigel (2001), Viera et al. (2005) and Naidoo et al. (2006) respectively, stated that satisfying growth of. *H. tuberculata*, *H. roei*, *H. t. coccinea* and *H. midae* fed enriched macroalgae was attributable to a consistent supply of high protein diet.

Besides, abalone fed mixed algal regimes, both enriched and non enriched, performed significantly better than those that were fed with a single algal diet, in agreement with studies showing that "mixed" diets produce better growth rates than single-species diets (Naidoo et al., 2006). This suggest that abalone obtain a complete range of required nutrients by eating a mixed algal regime and that essential nutrients may become limiting in trials where animal are fed single-species diets. The growth rate of abalone increased significantly with an increasing PE ratio up to a level of 32% protein/6% fat. Similar observations have been reported for *H. midae* fed with several dietary protein and energy levels (Britz and Hecht, 1997). With the exception of animals fed both *G. cornea* treatments, growth rates values in this study (78–151 μm day^{-1}) were generally higher than both, those obtain by other authors with other species (50–100 μm day^{-1}; Viana et al., 1996, 2000; Guzmán and Viana, 1998; Jackson et al., 2001; Gómez-Montes et al., 2003) under similar experimental conditions, and those obtain under commercial conditions (80 μm day^{-1}; Gómez-Montes et al., 2003). SGR values in the present study (1.4 to 2.3%) were higher than those reported by Mercer et al. (1993) and Mai et al. (1995b) in abalone fed several species of macroalgae who reported SGR values of 0.8% for *H. tuberculata* and 0.7–1% for *H. discus hannai*, and similar to the results reported by Capinpin and Corre, 1996 (2.5%) for *H. asinina* fed *Gracilariopsis heteroclada*. In an aquaculture context, the rate of weight gain is vitally important, particularly in the case of haliotids, as they are relatively slow growing. Therefore, from an aquaculture perspective, an optimal dietary protein level should be defined in terms of growth rate as well as dietary ingredient cost. In the present study, except with *G. cornea* treatments, all feeding regimes produced a similar or better weight gain (230–561%) than *Gracilariopsis bailinae* (134%, Bautista-Teruel and Millamena, 1999) or compound diets (454%, Bautista-Teruel et al., 2003) in *H. asinina* under similar conditions. Regarding *G. cornea*, the lowest growth rate obtained with abalone fed this macroalgae, might be due to the lowest feed intake registered. Indeed, consumption rate in abalone may be influenced by various factors that may decrease algal palatability, such as, the structure, growth form, and thallus toughness (Steneck and Watling, 1982). Hence, the harder texture of *G. cornea* may have affected its consumption by *H. t. coccinea*, since in the wild, abalone prefer soft textured macroalgae (Shepherd and Steinberg, 1992). This preference seem to be related to the little capacity of the rhipidoglossan radula to penetrate the algal surface (Steneck and Watling, 1982) as its teeth have limited ability to exert force against the substrate. However when the animals were fed *G. cornea* as a proportion of a mixed diet, they exhibited excellent growth rates, suggesting that this red seaweed provided essential nutrients not found in the other algae fed.

Except in animals fed *G. cornea*, food conversion rate (FCR) values were within the range of those reported for *H. asinina*, *H. discus hannai*, *H. tuberculata* or *H. t. coccinea* fed seaweeds diets (Kunavongdate et al., 1995, Shpigel et al., 1999; Viera et al., 2005). The lower FCR attained for abalone fed enriched seaweeds in the present study seems to be explained, not only by the general composition of the algae, but also by a significantly higher protein:energy ratio than the rest of dietary treatments. PER values observed on the non enriched treatments agreed well with those reported for *H. discus hannai* (Uki et al., 1985a) and *H. midae* (Britz, 1996b) fed seaweeds or *H. midae* and *H. fulgens* fed compound feeds (Britz and Hecht, 1997; Gómez-Montes et al., 2003). Protein efficiency ratios were generally lower in abalone fed enriched

macroalgae. Similar observations have been reported by Uki et al. (1986), Britz (1996a) and Bautista-Teruel and Millamena (1999), who found that the growth rate of *H. discus hannai*, *H. midae* and *H. asinina* respectively, increased with an increase in protein content whereas the PER was negatively correlated with protein level. Despite the poorer efficiency of protein conversion by abalone fed the enriched macroalgae, higher weight gain and lower FCR were obtained with an increasing protein level in the algal diet. This increase in protein level, linked to the culture conditions of the integrated culture system, is of high economic significance for the production.

Soft-body to shell ratios of the experimental animals (2.4–3.3) were similar and even higher than those recorded by Mai et al. (1995a) for *H. tuberculata* (1.8–2.1) and *H. discus hannai* (2–2.4) fed with *P. palmata* and various levels of dietary lipids or by Sales et al. (2003) for *H midae* (2.9–3.2) fed different dietary crude protein level. Furthermore, the high survival of abalone noted for all treatments may well indicate a general balance of nutrients in the diets, although the low feed intake of both *G. cornea* diets may not have been enough to sustain comparable growth of abalone with those fed the rest of the experimental diets.

The algae studied presented typical fatty acid patterns of green and red algae in agreement with previous macroalgal studies (Li et al., 2002, Mai et al., 1996). Palmitic acid was the most abundant SFA, at similar contents of those reported for other species of seaweeds (Jackson et al., 2001; Li et al., 2002; Nelson et al., 2002). The fatty acid composition of the Chlorophyta *Ulva rigida* with predominat levels of C_{16} and C_{18} PUFAs and minimal levels of C_{20} fatty acids followed a pattern similar to the ones reported for other species of Ulvales such as *U. lactuca* (Mai et al., 1996) and *U. pertusa* (Li et al., 2002). Accordingly, the higher level of 18:3-n-3 and 18:1-n-7 relative to green algae, has been regarded as a characteristic of this phylum with a particular taxonomic value in Chlorophyta species (Johns et al., 1979). All macroalgae presented very low levels of 22:6-n3, with similar results found in other studies (Dawczynski et al., 2007). Hence DHA do not appear to be an essential FA in *H. t. coccinea* as they were detected in a very low level in all macroalgae tested despite they supported optimal growth of abalone. The growth promotion of EFA for abalone is generally dependent upon a collective effect of certain combination of different PUFA rather than on a single fatty acid.

Analyses of the abalone fed on the various diets showed that the biochemical composition of various organs were markedly affected by the diets. Lipid content in abalone tissues were generally higher than in their respective macroalgal diets (Nelson et al., 2002). Foot muscle contained significantly lower lipid levels than viscera indicating that selective storage of lipids occurs in the hepatopancreas/gonad assemblage. Similar observations have been reported by Webber (1970), Mercer et al. (1993) and Nelson et al. (2002), for *H. cracheroidii*, *H. tuberculata* and *H. discus hannai*, and *H. fulgens* respectively.

In conclusion, the dietary value of the macroalgal regimes tested can be divided into three categories based on the growth performances observed: best obtained with the mixed algal feeding regime, intermediate by using single *Ulva rigida* or *Hypnea spinella* feeding regimes and the lowest by *Gracilaria cornea.* Nevertheless, based on growth performance and nutritional indices, this study clearly demonstrates that the macroalgae produced in a biofiltering system are enriched in dietary protein and lipids and that their nutritional composition is matching the protein, lipid and carbohydrate requirements of abalone resulting in satisfying growth and survival of *H. tuberculata coccinea.* Results clearly indicate that *H. tuberculata coccinea* can be efficiently grown-out in an integrated-culture system suggesting that on-farm seaweed-abalone production could be a part of future development of abalone industry in the Canary Islands.

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References

AOAC, 2005. Official Methods of Analysis of the Association of Analytical Chemistry. Washington, DC. 1018 pp.
Bautista-Teruel, M.N., Millamena, O.M., 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein/energy levels. Aquaculture 178, 117–126.
Bautista-Teruel, M.N., Fermin, A.C., Koshio, S.S., 2003. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: animal and plant protein sources. Aquaculture 219, 645–653.
Bilbao, A., Viera, M.P., Courtois de Vicose, G., Socorro, J., Fernández-Palacios, H., 2004. Characterization of females broodstocks *Haliotis tuberculata coccinea R*. Eur. Aquac. Soc. Spec. publ. 34, 168.
Bilbao, A., Courtois de Viçose, G., Viera, M.P., Sosa, B., Fernández-Palacios, H., Hernández, C., 2010a. Efficiency of clove oil as anesthetic for abalone (Haliotis tuberculata coccinea, Reeve). J. Shellfish. Res. 29 (3), 679–682.
Bilbao, A., Tuset, V., Viera, M.P., Courtois de Viçose, G., Fernández-Palacios, H., Haroun, R., Izquierdo, M., 2010b. Reproduction, fecundity and growth of abalone (*Haliotis tuberculata coccinea*, Reeve 1846) in the Canary Islands. J. Shellfish. Res. 29 (4), 959–967.
Boarder, S.J., Shpigel, M., 2001. Comparative performances of juvenile *Haliotis roei* fed on enriched *Ulva rigida* and various artificial diets. J. Shellfish. Res. 20 (2), 653–657.
Britz, S.J., 1994. The development of an artificial feed for abalone farming. S. Afr. J. Sci. 90, 6–7.
Britz, P.J., 1996a. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. Aquaculture 140, 55–61.
Britz, P.J., 1996b. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone. Haliotis midae Aquac. 140, 63–73.
Britz, S.J., Hecht, T., 1997. Effect of dietary protein and energy levels on growth and body composition of South African abalone, *Haliotis midae*. Aquaculture 156, 195–210.
Capinpin, E.C., Corre, K.G., 1996. Growth rate of the Philippine abalone *Haliotis asinina* fed an artificial diet and macroalgae. Aquaculture 144, 81–89.
Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25–41.
Christie, W.W., 1982. Lipids Analysis. In: Christie, W.W. (Ed.), Pergamon Press, Oxford, pp. 17–23. 51–61.
Corazani, D., Illanes, J.E., 1998. Growth of juvenile abalone, *Haliotis discus hannai* Ino 1953 and *Haliotis rufescens* Swainson 1822, fed with different diets. J. Shellfish. Res. 17 (3), 663–666.
Courtois de Vicose, G., Viera, M.P., Bilbao, A., Izquierdo, M.S., 2007. Embriology and complete larval development of *Haliotis tuberculata coccinea* Reeve: an indexed micro-photografic sequence. J. Shellfish. Res. 26, 1–8.
Courtois de Vicose, G., Porta, A., Viera, M.P., Bilbao, A., Fernández-Palacios, H., Izquierdo, M.S., 2009. Potential value of *Navicula incerta, Proschkinia* sp., *Nitzschia* sp. and *Amphora* sp. as feed for *Haliotis tuberculata coccinea* post-larvae: effect of inoculums density on algal growth rates. Aquac. Soc. Spec. Publ. 38, 56–59.
Courtois de Vicose, G., Viera, M.P., Bilbao, A., Izquierdo, M.S., 2010. Larval settlement of *Haliotis tuberculata coccinea* in response to different inductives cues and the effect of larval density on settlement early growth and survival. J. Shellfish. Res. 29 (3).
Dawczynski, C., Shubert, R., Jahreis, G., 2007. Amino acids, fatty acids, and dietary fibre in edible seaweeds products. Food Chem. 103, 891–899.
Durazo-Beltrán, E., Viana, M.T., D'Abramo, L.R., Toro-Váquez, J.F., 2004. Effect of starvation and dietary lipid on the lipid and fatty acid composition of muscle tissue of juvenile green abalone (*Haliotis fulgens*). Aquaculture 238, 329–341.
Espino, F., Herrera, R., 2002. Seguimiento de poblaciones de especies amenazadas 2002 (*Haliotis tuberculata coccinea*, Nordsieck, 1975) Gran Canaria. Informe final presentado por Gesplan y la Consejería de Política Territorial y Medio Ambiente (Viceconsejería de Medio ambiente de Gran Canaria y Dirección General de Política Ambiental). Informe no publicado. 52 pp.
Evans, F., Langdon, C.J., 2000. Co-culture of dulse *Palmaria mollis* and red abalone *Haliotis rufescens* under limited flow conditions. Aquaculture 185, 137–158.
Fleming, A.E., 1995. Digestive efficiency of the Australian abalone *Haliotis rubra* in relation to growth and feed preference. Aquaculture 134, 279–293.
Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226, 479–509.
Foster, G.G., Hodgson, A.N., 1998. Consumption and apparent dry matter digestibility of six intertidal macroalgae by 11 (Mollusca: Vetgastropoda: Turbinidae). Aquaculture 167, 211–227.
García-Esquivel, Z., Felbeck, H., 2006. Activity of digestive enzymes along the gut of juvenile red abalone, *Haliotis rufescens*, fed natural and balanced diets. Aquaculture 261, 615–625.
Gómez-Montes, L., García-Esquivel, Z., D'Abramo, L.R., Shimada, A., Vásquez-Peláez, C., Viana, M.T., 2003. Effect of dietary protein:energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*. Aquaculture 220, 769–780.
Guzmán, J.M., Viana, M.T., 1998. Growth of abalone *Haliotis fulgens* fed diets with and without fishmeal compared to a commercial diet. Aquaculture 165, 321–331.
Harlin, M.M., Thorne-Miller, B., Thursby, G.B., 1978. Ammonium uptake by *Gracilaria sp* (Florideophyceae) and *Ulva lactuca* (Clorophyceae) in closed system fish culture. In: Jensen, A., Stein, J.R. (Eds.), Proc. IXth Int. Seaweed Symp. Sicience Press, Princeton, pp. 285–293.

Hernández-Carmona, G., Carrillo-Domínguez, S., 2009. Monthly variation in the chemical composition of *Eisenia erborea* J.E. Areschoug. J. Appl. Phycol. doi:10.1007/s10811-009-9454-5.
Huchette, S.M.H., Clavier, J., 2004. Status of the ormer (*Haliotis tuberculata* L.) industry in Europe. J. Shellfish. Res. 23, 951–955.
Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, Kitajima, C., 1989. Requirements of larval red seabream *Pagrus major* for essential fatty acids. Nippon Suisan Gakkaishi 55, 859–867.
Jackson, D., Williams, K.C., Degnan, B.M., 2001. Suitability of Australian formulated diets for aquaculture of the tropical abalone *Haliotis asinina* Linnaeus. J. Shellfish. Res. 20, 627–636.
Johns, R.B., Reid Nichols, P.D., Perry, G.J., 1979. Fatty acid composition of ten marine algae from Australian waters. Phytochemistry 18 (5), 799–802.
Kaehler, S., Kennish, R., 1996. Summer and winter comparisons in the nutritional value of marine macroalgae from Hong Kong. Bot. Mar. 39, 11–17.
Kunavongdate, P., Sakares, W., Muangsakorn, S., 1995. Experimental rearing on abalone, *Haliotis asinina* Linneus with three species of seaweed. Technical Paper, 39. Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand.
Lamare, M.D., Wing, S.R., 2001. Caloric content of New Zealand marine macrophytes. N. Z. J. Mar. Freshw. Res. 35, 341–355.
Li, X., Fan, F., Han, L., Lou, Q., 2002. Fatty acids of some algae from the Bohai Sea. Phytoquemistry 59, 157–161.
Linnaeus, C., 1758. Systema Naturae, Ninth edition. Theodor Haak, Leiden (Lugdunum Batavorum).
Mai, K., Mercer, J.P., Donlon, J., 1995a. Comparative studies on the nutrition of two species of abalone. Haliotis tuberculata L. and Haliotis discus hannai Ino. IV. Optimum dietary protein level for growth. Aquaculture 136, 165–180.
Mai, K., Mercer, J.P., Donlon, J., 1995b. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino: III. Response of abalone to various levels of dietary lipid. Aquaculture 134, 65–80.
Mai, K., Mercer, J.P., Donlon, J., 1996. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. V. The role of polyunsaturated fatty acids of macroalgae in abalone nutrition. Aquaculture 139, 77–89.
Martínez-Aragón, J.F., Hernández, I., Pérez Llorens, J.L., Vázquez, R., Vergara, J.J., 2002. Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with seabass (*Dicentrarchus labrax)* waste waters:1. Phosphate J. Appl. Phycol. 14, 365–374.
Mercer, J.P., Mai, K.S., Donlon, J., 1993. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata coccinea* Linneaeus and *Haliotis discus hannai* Ino. 1. Effects of algal diets on growth and biochemical composition. Invertebr. Reprod. Dev. 23, 75–88.
Naidoo, K., Maneveldt, G., Ruck, K., Bolton, J., 2006. A comparison of various seaweed-based diets and formulated feed growth rate of abalone in a land-based aquaculture system. J. Appl. Phycol. 18, 437–443.
Nelson, M.M., Leighton, D.L., Phleger, C.F., Nichols, P.D., 2002. Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgal diets. Comp. Biochem. Physiol. B 131, 695–712.
Neori, A., Ragg, N.L.C., Shpigel, M., 1998. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. Aquac. Eng. 17 (4), 215–239.
Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, 361–391.
Njobeni A., 2006. The cultivation of *Gracilaria gracilis* (Rhodophyta) in an integrated culture system for the production for abalone feed and the bioremediation of aquaculture effluent. MCs Dissertation, Cape Town University, Cape Town
Reeve, L.A., 1846. Descriptions of forty new species of Haliotis. Proceedings of the Zoological Society of London, 14, 53–59.
Reyes, O.S., Fermin, A.C., 2003. Terrestrial leaf meals or freshwater aquatic fern as potential feed ingredients for farmed abalone *Haliotis asinina* (Linnaeus 1758). Aquacult. Res. 34, 593–599.
Robertson-Andersson D.V., 2003. The cultivation of *Ulva lactuca* (Chlorophyta) in an integrated culture system for the production for abalone feed and the bioremediation of aquaculture effluent. MCs Dissertation, Cape Town University, Cape Town.
Robertson-Andersson, D.V., Leitao, D., Bolton, J.J., Anderson, R.J., Njobeni, A., Ruck, K., 2006. Can kelp extract (Kelpac) be useful in seaweed mariculture? J. Appl. Phycol. doi:10.1007/s10811-006-9030-1.
Sales, J., Truter, P.J., Britz, P.J., 2003. Optimum dietary crude protein level for growth in South African abalone (*Haliotis midae* L.). Aquac. Nutr. 9, 85–89.
Schuenhoff, A., Shpigel, M., Lupatsch, I., Ashkenazi, A., Msuya, F.E., Neori, A., 2003. A semi-recirculating, integrated system for the culture of fish and seaweed. Aquaculture 221, 167–181.
Shepherd, S.A., Steinberg, P.D., 1992. Food preference of three Australian abalone species with the review of the algal food of abalone. In: Shepherd, S.A., Tegner, M.J., Guzmán del Próo, S.A. (Eds.), Abalone of the World. Biology, Fisheries and Culture. Blackwell Scientific Publications, Oxford, pp. 169–181.
Shipton, T.A., Britz, P.J., 2001. The partial and total replacement of fishmeal with selected plant sources in diets for the South African abalone *Haliotis midae* L. J. Shellfish. Res. 20 (2), 637–645.
Shpigel, M., Marshall, I., Lupatsch, J.P., Mercer, J.P., Neori, A., 1996a. Acclimatation and propagation of the abalone *Haliotis tuberculata* in a land-based culture system in Israel. J. World Aquacult. Soc. 27, 435–442.
Shpigel, M., Neori, A., Marshall, A., 1996b. The suitability of several introduced species of abalone (Gastropoda: Haliotidae) for land-based culture with pond-grown seaweed in Israel. Israeli J. Aquacult.- Bamidgeh 48, 192–200.
Shpigel, M., Ragg, N.C., Lupatsch, I., Neori, A., 1999. Protein content determines the nutritional value of the seaweed *Ulva lactuca* for the abalone *Haliotis tuberculata* and *H. discus hannai*. J. Shellfish Res. 18, 227–233.
Steneck, R.S., Watling, L., 1982. Feeding cababilities and limitations of herbivorous mollusc: a functional group aproach. Mar. Biol. 68, 299–319.
Thongrod, S., Tamtin, M., Boonyaratpalin, M., 2003. Lipid to carbohydrate ratio in donkey's ear abalone (*Haliotis asinina,* Linne) diets. Aquaculture 225, 165–174.
Toledo, P., Haroun, R., Fernández-Palacios, H., Izquierdo, M., Peña, J., 2000. First culture experiences of *Haliotis coccinea canariensis* in a biofilter system. J. Shellfish. Res. 19 (1), 493–541.
Uki, N., Watanabe, T., 1986. Effect of heat-treatment of dietary protein sources on their protein quality for abalone. Bull. Jpn. Soc. Sci. Fish. 52 (7), 1199–1204.
Uki, N., Kemuyama, A., Watanabe, T., 1985a. Development of semipurified test diets for abalone. Bull. Jpn. Soc. Sci. Fish. 51 (11), 1825–1833.
Uki, N., Kemuyama, A., Watanabe, T., 1985b. Nutritional evaluation of several protein sources in diets for abalone *Haliotis discus hannai*. Bull. Jpn. Soc. Sci. Fish. 51 (11), 1835–1839.
Uki, N., Kemuyama, A., Watanabe, T., 1986. Optimum protein level in diets for abalone. Bull. Jpn. Soc. Sci. Fish. 52, 1005–1012 (in Japanese, with English abstract).
Viana, M.T., López, L.M., García-Esquivel, Z., Méndez, E., 1996. The use of silage from fish and abalone viscera as an ingredient for abalone feed. Aquaculture 140, 87–98.
Viana, M.T., Jarayabhand, P., Menasveta, P., 2000. Evaluation of an artificial diet for use in the culture of the tropical abalone *Haliotis ovina*. J. Aquac. Trop. 15, 71–79.
Viera, M.P., Courtois de Vicose, G., Fernández-Palacios, H., Roo, J., Valencia, A., 2003. Inducción al desove de la almeja canaria *Haliotis tuberculata coccinea* mediante el método del Peróxido de Hidrogeno. Actas del IX Congreso Nacional de Acuicultura. Consejería de Agricultura y Pesca. Junta de Andalucía, p. 289.
Viera, M.P., Gómez-Pinchetti, J.L., Courtois de Viçose, G., Bilbao, A., Suárez, S., Haroun, R.J., Izquierdo, M.S., 2005. Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve. Aquaculture 248, 75–82.
Viera, M.P., Gómez-Pinchetti, J.L., Courtois de Viçose, G., Bilbao, A., Izquierdo, M.S., 2007. Crecimiento comparative de juveniles de oreja de mar *Haliotis tuberculata coccinea* Reeve alimentados con macroalgas cultivadas en agua de mar y en efluentes marinos. Antonio Cerviño Eiroa, Alejandro Guerra Díaz y Carmen Pérez Acosta (EDS), 1, pp. 627–630. ISBN TOMO I: 978-84-611-9086-7.
Viera, M.P., Fidalgo, P., Haroun, R.J., Courtois de Viçose, G., Bilbao, A., Gómez-Pinchetti, J.L., Izquierdo, M.S., 2009a. Effect of different rearing conditios on the growth performance of the macroalgae *Ulva rigida*, *Hypnea spinella* and *Gracilaria cornea*. Proceedings of the 7th International Abalone Symposium, Pattaya-Thailand, p. 107.
Viera, M.P., Courtois de Viçose, G., Bilbao, A., Fernández-Palacios, H., Robaina, L., Izquierdo, M.S., 2009b. Requerimientos nutricionales y dietas artificiales en el cultivo de la oreja de mar *Haliotis spp.:* Revisión. In: Baez, D., Villaroel, M., Cardenas, S. (Eds.), Libro de Actas del XII Congreso Nacional de Acuicultura, 1. ISBN: 978-84-937611-0-3, pp. 46–47. Madrid.
Webber, H.H., 1970. Changes in metabolite composition during the reproductive cycle of the abalone *Haliotis cracheroidii* (Gastropoda: Prosobranchiata). Phisiol. 2, 213–231.
Wong, K.H., Cheung, P.C.K., 2000. Nutritional evaluation of some subtropical red and green seaweeds Part I — Proximate composition, amino acid profiles and some physico-chemical properties. Food Chem. 71, 475–482.
Zar, J.H., 1984. Biostatistical Analysis, 2nd ed. Prenting Englewood Cliffs, New Jersey.