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Status of the digestive gland and feed index in juvenile green abalone *Haliotis fulgens* fed rehydrated macroalgae

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Abstract

One of the bottlenecks in cultivating juvenile green abalone *Haliotis fulgens* is the lack of well-adapted natural or formulated food for optimal growth. The goal of this study was to analyse the digestive gland structure of juvenile green abalone fed rehydrated natural feed, *Ulva* sp. (Chlorophyta), *Eisenia arborea*, *Macrocystis pyrifera*, *Egregia menziesii* (Phaeophyta) and *Porphyra perforata* (Rhodophyta), using histochemical techniques. Structure of the digestive gland was described, and proteoglycan granules were detected in the digestive cells. The abundance of granules was variable, depending on the feed provided to the abalone, and this was reflected in their growth. Granular content in digestive cells fed *Ulva* sp. was scarce, leading to low growth rate and high feed conversion ratio (FCR). Digestive cells of juveniles fed *E. menziesii* led to the best nutritional condition, including many proteoglycans cellular granules, best weight growth rate and a low FCR. Histochemical analysis of the digestive gland, differentiated by a modified Goldner trichrome method that included Alcian blue, was a useful tool for determining the nutritional status of farmed abalone, therefore recommended for assessing adjustments to the natural feed or formulation to meet the nutritional needs of abalone.

KEY WORDS: abalone, aquaculture, digestive gland, feeding, natural diets

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Introduction

Green abalone, *Haliotis fulgens* (Philippi, 1845), is a marine resource of ecological and economic importance along the coast of the Baja California Peninsula. It is an important source of income in several exporting countries, but it is an overexploited resource. Abalone farming is well developed in China, Taiwan and Japan and less developed in Australia, Chile, Iceland, Ireland, New Zealand, South Africa, Thailand, the USA and Mexico (Gordon & Cook 2003). One of the bottlenecks in intensive abalone farming is the natural or formulated food for optimal growth at a reasonable price (Viana *et al*. 1993; Fleming *et al*. 1996; Knauer *et al*. 1996; Kawamura *et al*. 1998). In Mexico, abalone farms use natural diets, principally *Macrocystis pyrifera* to rear abalone spat of 10 mm (MacBride 1998); however, abundance and biochemical content of *M. pyrifera* changes with environmental conditions.

On the Pacific coast of North America, the main species of macroalgae associated with abalone habitats are *M. pyrifera, Eisenia arborea, Laminaria farlowii, Pterygophora californica, Desmarestia lingulata, Cystoseira osmundacea, Sargassum muticum* and *Crytopleura crispa*. Along the southern coast of the Baja California Peninsula, algae species associated with abalone banks are brown algae (*C. osmundacea, E. arborea, Sargassum sinicola*, *Spatoglossum howelli*) and red algae (*Corallina officinalis* var. *chilensis, C. vancouveriensis, Cryptopleura crispa, Gelidium robustum, Prionitis cartilagineum* and *P. lanceolada*) (Serviere-Zaragoza *et al*. 2003).

Several studies describe the nutritional value of macroalgae consumed by abalone. Artificial diets have been extensively tested over the past three decades, chiefly to support refinements in abalone aquaculture (Uki & Watanabe 1992; Leighton 2000). These studies tried to find the best

alternatives of feed for abalone that lead to high growth rates under cultivation. For example, Uki *et al*. (1986) evaluated 57 macroalgae as diets for juvenile *Haliotis discus hannai* and obtained the best growth with *Eisenia bicylis*. Mercer *et al*. (1993) showed that *Palmaria* was the best macroalgae for juvenile *H. tuberculata* and *H. discus hannai*. Corazani & Illanes (1998) evaluated the growth in juvenile *H. discus hannai* and *Haliotis rufescens* fed three natural diets (*Ulva rigida*, *Macrocystis integrifolia* and *Lessonia trabeculata)* and an artificial diet. They found that the highest growth for both abalone species was with the artificial diet and the natural diet of *U. rigida* for *H. discus hannai* and *M. integrifolia* for *H. rufenses*. Serviere-Zaragoza *et al*. (2001) evaluated three macroalgae *Eisenia arborea, Gelidium robustum, M. pyrifera* and a seagrass (*Phyllospadix torreyi*) in juvenile *H. fulgens*, finding that *M. pyrifera* led to the highest growth rate (53 ± 2 μm d^{-1}; survival of 93%). Nelson *et al*. (2002) tested three macroalgae (*Egregia menziesii, Chondracanthus canaliculatus* and *Ulva lobata*) in juvenile *H. fulgens*, obtaining the best results with *E. menziesii*. Pérez-Estrada *et al*. (2011) found that *E. menziesii*, *M. pyrifera* and *P. perforata* are more efficient in promoting growth in juvenile green abalone than *Ulva* sp. and *E. arborea*.

Natural feed of abalone is a mixture of macroalgae, diatoms and biofilm; however, little is known of the important nutrient requirements of juvenile abalone in terms of energy level, proteins and micronutrients (Pérez-Estrada *et al*. 2011). One major concern of all aquaculture enterprises is to satisfy the nutritional requirements of the organisms during the first stages of life. This is a fundamental factor for developing abalone production, particularly in the Baja California Peninsula. Shawl & Davis (2006) point out that the major bottleneck in intensive gastropod farming is the lack of formulated food for optimal growth of larvae and juveniles in hatcheries at a reasonable price. The objective was to improve husbandry techniques for grow-out of juveniles, using diets that allow a growth rate equal to or higher than wild juveniles. Most studies of the digestive tract and digestive gland of microphagous prosobranch gastropods have been performed on species living in intertidal environments to determine the influence of tidal variations on the digestive gland (Nelson & Morton 1979). Cui *et al*. (2001) observed that digestive cells of *H. discuss hannai* contain a number of vesicles that participate in the heterophagic function of the cells. These cells have considerable variation in size and appearance and are involved in accumulating ingested material.

Gros *et al*. (2009) describe digestive cells with large Alcian blue-positive granules in *Strombus gigas* that contain large quantities of proteoglycans that are exported to the stomach through physiological destruction of digestive cells undergoing halocrine secretion. Aldana Aranda & Frenkiel (2012) analysed the structure of the digestive gland using histochemical techniques and measured the growth and survival of queen conch (*S. gigas*) with nine formulated feeds. Proteoglycan granules and acidophilic granules were detected in the digestive cells. The abundance of both types of granules was variable, mostly related to the nutritional state of the animals.

The extracellular matrices in various animal tissues consist of several common components: fibrous collagen proteins; hyaluronan (or hyaluronic acid), a large mucopolysaccharide; and covalently linked polysaccharides and proteins in the form of proteoglycans (mostly carbohydrate), and glycoproteins (mostly protein). However, the exact composition of the matrix in different tissues varies, reflecting the specialized function of the tissue (Lodish *et al*. 2000). The proteoglycan molecules in connective tissue form a highly hydrated, gel-like 'ground substance' in which fibrous proteins are embedded. The polysaccharide gel resists compressive forces on the matrix, while permitting the rapid diffusion of nutrients, metabolites and hormones between the blood and the tissue cells (Lodish *et al*. 2000).

The goals of this study were to compare the status of the digestive gland of juvenile green abalone fed one of five natural diets under controlled laboratory conditions and using histochemical techniques to differentiate proteoglycans to obtain a feed index.

Material and methods

Diets

Five macroalgae: *Ulva* sp. (Chlorophyta), *Eisenia arborea, Macrocystis pyrifera, Egregia menziesii* (Phaeophyta) and *Porphyra perforata* (Rhodophyta), were tested. *Ulva* sp. and *E. arborea* were collected from the Pacific coast of the central part of the Baja California Peninsula, and *M. pyrifera, E. menziesii* and *P. perforata* were collected from the northern coast of the peninsula. The proximate composition of the dry feed was determined using the methods of AOAC (1995), and its results were summarized in Table 1. Gross energy was determined using a bomb calorimeter (Model 1261; Parr Instrument, Moline, IL, USA), as described in Pérez-Estrada *et al*. (2011).

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*

Biochemical composition (%)/Diet	UL	EA	MP	EM	PP
Protein	47.8 ± 4.6^{c}	120.0 ± 0^{b}	164.0 ± 1^{a}	119.0 ± 0^{b}	169.0 ± 2^{a}
Lipids	35.0 ± 1.2^{a}	39.5 ± 0.1^{a}	36.3 ± 1.9^{a}	37.0 ± 1.8^{a}	14.6 ± 1.3^{b}
Ash	365 ± 2^{b}	288 ± 2^{d}	421 ± 1^{a}	314 ± 3^{c}	240 ± 0^{e}
Crude fibre	18.2 ± 0.4^{c}	30.6 ± 1.5^{b}	29.4 ± 0.8^{b}	59.1 ± 0.1^{a}	18.4 ± 0.2^{c}
NFE	534 ± 5^{b}	522 ± 3^{b}	349 ± 2^{d}	469 ± 3^{c}	558 ± 3^{a}
Gross energy (KJ g^{-1})	8.3 ± 0.2^{d}	12.4 ± 0.2^{c}	12.3 ± 0.0^{b}	11.4 ± 0.1^{b}	4.1 ± 0.1^{a}

NFE, Nitrogen Free Extract is mainly carbohydrates. UL, *Ulva* spp; EA, *Eisenia arborea*; MP, *Macrocystis pyrifera*; EM, *Egregia menziessi;* PP, *Porphyra perforata.*
Mean values with different letters in each column indicate significant differences ($P < 0.05$).

Feeding trial

The feeding trials lasted 136 days under controlled laboratory conditions with hatchery-produced juvenile green abalone *H. fulgens* (31.67 mm ± 0.11 SE in length) and 2.46 g ± 0.02 SE in weight. The juveniles were placed in 52-L fibreglass tanks at a concentration of 34 juveniles per tank, with three replicates for each diet. Abalones were cleaned twice a week to avoid growth of microalgae and biofilm that could be used as a food source. We used a randomized pattern of diets among tanks. Before beginning the experiment, an ANOVA was conducted to ensure homogeneity of initial size and weight among tanks. Sea water was filtered at 10 μm and 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; salinity of 37.4 g L^{-1} ± 0.5 SD; concentration of dissolved oxygen of 7.0 g L^{-1} ± 0.1 SD, and pH at 8 ± 0.1.

Rehydrated macroalgae were fed *ad libitum* every 2 days in the afternoon, and the uneaten food was removed. Feeds were rehydrated for 5 min in sea water before feeding. Once a month, maximum shell length of tagged individuals was measured with a vernier calliper, and wet body weight was measured with an electronic balance (Explorer, Ohaus, Parsippany, NJ, USA). Before weighing the specimens, excess water was removed by blotting with paper towels (Pérez-Estrada *et al*. 2011).

Nutritional status of abalones was analysed using shell length, wet body weight, feed conversion ratio (FCR), histological features of the digestive gland and the feed index. 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 FCR was determined as dry feed consumed per wet weight gain per animal per day (Britz 1996). Dead specimens were removed and replaced with similar sized, tagged abalone to maintain the standard density. Replacements were excluded from sampling.

A nonparametric Tukey test (Sokal & Rohlf 1995) of the feed index and growth rates was used to test for significant differences ($P < 0.05$) among diets.

Histological analysis

Five juveniles from each diet treatment were sampled at the end of the feeding trial ($N = 15$). Stomachs of each individual were dissected in the midsection that contains the digestive gland and the sections used in histological examinations. Sections were fixed in Davidson's fluid for 24 h and processed with standard histological techniques (Gabe 1968; Luna 1969). After dehydration in an ethanol series, and clearing with Clarene, the sections were embedded in Paraplast wax (SPI Supplies, West Chester, PA, USA). Sections (5 μm thick) were stained with a modified Goldner trichrome method that included Alcian blue (8GX Sigma-Aldrich) at pH 2.5 to differentiate blue granules of proteoglycans from the digestive cells (Gabe 1968).

The slides were examined and recorded with a digital camera (Nikon DXm 1200F) mounted on a Nikon microscope. For every abalone, two slides were prepared, with five sections per slide. The number of blue granules in the digestive cells was counted. Counting was made in three fields of the five sections at 100× magnification, and the mean and SD for each diet was calculated. To determine the feed index, we used the technique described by Aldana Aranda & Frenkiel (2012). The feed index is the sum of blue granules in the large adenomer cells. Data were transformed to granule area (μm^2), using the circle area formula for the granules divided by the image area (μm^2) at 100×, which was always a constant 13 771 μm^2). This was multiplied by 100 to obtain per cent.

Results

Digestive gland structure

In juvenile abalone, the digestive gland has an array of adenomers (Fig. 1A), measuring 180–200 μm to 220–280 μm. Adenomers are secreting cells connected by small secondary ducts, which join larger ducts leading to the stomach. The secondary ducts are lined with simple epithelium composed of a single cell type. Adenomers of the digestive gland are composed of large functional groups of digestive cells containing granules stained blue and smaller areas stained red, containing vacuolated cells (Fig 1A–D). Digestive cells measured 86.33 μm by 6.67 μm, which contained large blue-stained granules, 2.0–4.5 μm (average 4.26 ± 1.63 μm) in diameter. The larger primary ducts were lined by epithelia devoid of cilia, but with blue-stained mucocytes, giving a positive histochemical reaction in the digestive gland. The connective tissue surrounding the digestive gland has small, round, red-stained amoebocytes (Fig. 1D).

The digestive gland and blue granules from specimens fed one of five natural diets were not similar in structure (Fig. 2A–E). Digestive cells of abalone fed *Ulva* sp. had cell membranes and intracellular granules giving the cell a degenerated appearance (Fig. 2A). The digestive gland of abalone fed this diet lacked blue granules or unstained granules, therefore, lacking the proteoglycan component. Abalone fed *E. arborea* contained a large number of blue granules, but these were small (4.0 ± 1.21 μm) (Table 2).

Abalone fed *M. pyrifera* or *E. menziesii* had a well-structured cellular appearance in the digestive gland, and the large granules of the digestive cells were stained blue (Figs. 2C,E). *Egregia menziesii, M. pyrifera* and *P. perforata* appear to be the most useful of the five natural diets, as indicated by higher growth rates and FCR (Table 2).

The highest density of blue granules occurred in abalone fed *P. perforata* (mean of 342 granules) and had a feed index of 32.97 ± 10.07. Abalone fed *E. arborea* had a feed index of 6.64 ± 5.21. Abalone fed *M. pyrifera* or *E. menziesii* had a feed index of 32.49 ± 7.16 and 32.97 ± 6.72, respectively. One-way ANOVA test showed significant differences among diets in the value of the feed index ($P < 0.0001$), and the Tukey test ($P \leq 0.05$) showed significant differences between the feed index of abalone fed *Ulva* sp. or *E. arborea* than abalone fed *E. menziesii, M. pyrifera* or *P. perforata* (Fig. 3).

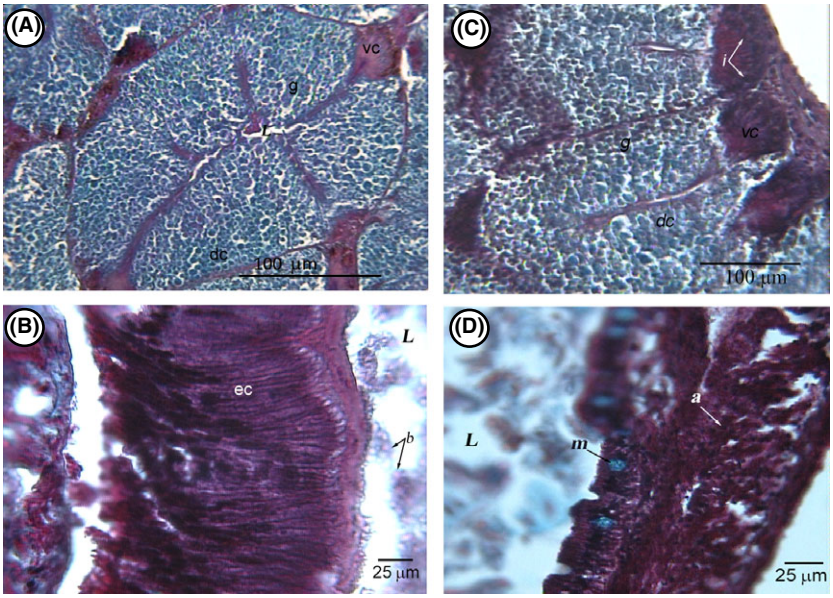

Figure 1 (A) Adenomers of digestive gland from a juvenile of *Haliotis fulgens*. Vacuolated cells (vc) with granules stained blue (g) and lumen (L) of adenomers. Microphotography at 40x. (B) Primary duct with one type of epithelium; plicate ciliated epithelium (ec) typical of primary ducts. This epithelium is characterized by abundant blebs (b) at the apical pole, and lumen (L). (C) Digestive gland showing two types of cells: digestive cells (dc) containing numerous large granules stained blue by Alcian blue (g) and of smaller crypt areas stained mostly red containing vacuolated cells (vc) with large round inclusions (i). (D) Epithelium of primary ducts with mucocytes (m) stained by Alcian blue that constitutes a positive control even if the digestive gland granules are not stained blue. The connective tissue possesses amoebocytes stained red (a), lumen (L).

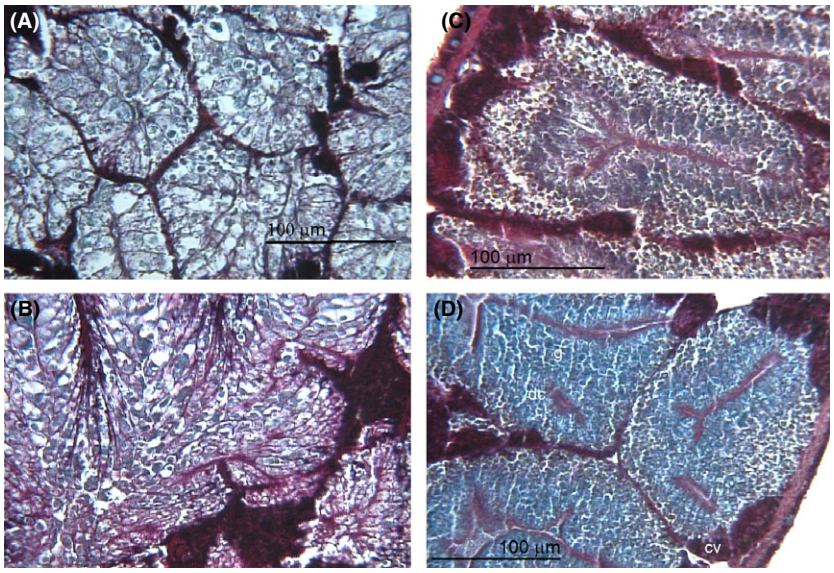

Figure 2 Comparison of digestive glands of *Haliotis fulgens* juveniles fed with various diets. All the pictures are of digestive glands at the same magnification with obj x40. (A) Digestive gland of an abalone fed with Ulva sp. showing large digestive or secreting cells without blue granules and crypt cells; (B) Juvenile fed on diet *Eisenia arborea* has twice number of those observed in Ulva sp. (C) Abalones feed with *Macrocystis pyrifera* showing smaller blue granules and a homogenous structure showing intracellular granules stained by Alcian blue; (D) Abalone fed on diet *P. perforate* showing that the digestive gland cells with granules stained and duct appears normal.

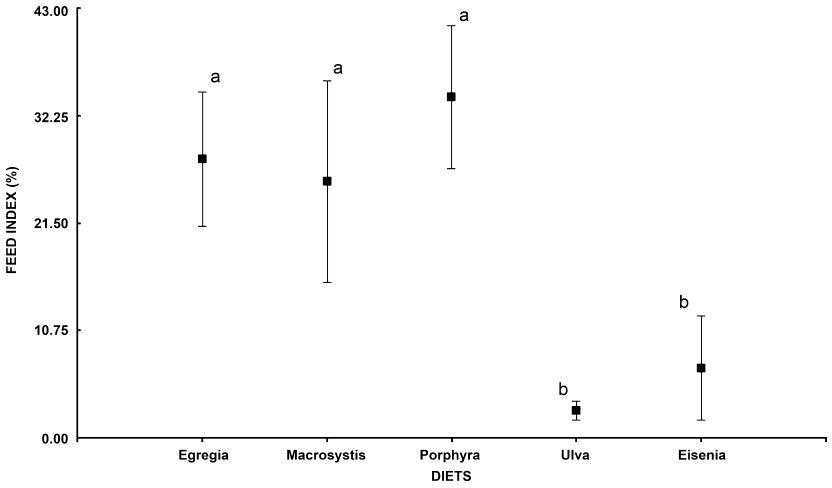

Figure 3 Feed index in juvenile H. fulgens fed with five different diets. Boxplots (n = 45 in each plot) showing feed index. The box contains 50% of the data (90% of data when whiskers are included), while dots indicate extreme values. Data are given as medians (horizontal line within each box) ± SD. Letters indicate significant differences between dietary treatments (Tukey test, $P \leq 0.05$).

Discussion

Most histological studies of the digestive gland structure of molluscs have been achieved with bivalves (Purchon 1977; Morse & Zardus 1997). Gastropod digestive glands have been described by comparison with bivalve digestive glands (Fretter & Graham 1962). However, feeding methods and nutritional requirements are not similar in these two groups. Bivalves are suspension feeders and ingest small unicellular algae delivered to intracellular digestion in the digestive gland cells (digestive cells), whereas prosobranch gastropods, such as *Haliotis* spp., are grazers, usually feeding on macroalgae, when available, and on biofilm in the nearby surrounding area.

In the northern hemisphere, brown algae are the most common component in the abalone diet, and in the southern hemisphere, abalone feed mostly on abundant red algae (Shepherd & Steinberg 1992). Brown algae produce better growth than red algae (Leighton & Boolootian 1963; Hirose 1974; Uki *et al*. 1986; Mai *et al*. 1995); however, in

Table 2 Mean and SD throughout the digestive cells; counting of blue granules and their diameter (n = 45). Mean values (±SD, n = 3 for growth rate) of juvenile green abalone *Haliotis fulgens* fed different macroalgae diets

Diet	Digestive cells		Number of blue granules	Diameter of blue granules (μm)	Weight growth rate (mg day^{-1})*	FCR*
	Length (μm)	Width (μm)				
Ulva sp	87.55 ± 11.28	6.34 ± 1.05	23 ± 9.88^{b}	4.30 ± 2.0	1.4 ± 0.0^{c}	42.4 ± 7.5^{a}
Egregia menziessi	83.47 ± 4.66	5.27 ± 1.15	283 ± 67.96^{a}	3.98 ± 1.27	9.2 ± 1.9^{a}	16.0 ± 3.6^{b}
Macrocystis pyrifera	94.20 ± 18.50	5.17 ± 0.75	273 ± 97.08^{a}	3.82 ± 1.50	8.2 ± 0.4^{ab}	14.7 ± 1.5^{b}
Porphyra perforata	78.95 ± 7.94	10.10 ± 0.90	342 ± 70.40^{a}	4.00 ± 1.21	8.1 ± 0.1^{ab}	6.5 ± 1.0^{b}
Eisenia arborea	88.99 ± 15.70	6.50 ± 1.26	48 ± 14.96^{b}	5.22 ± 2.17	4.5 ± 0.7^{bc}	38.8 ± 6.4^{a}

*Information from Pérez-Estrada *et al.* 2011.
FCR: apparent feed conversion ratio.

our treatments, the red algae *P. perforata* produced growth rates that were not significantly different than brown algae (Pérez-Estrada *et al.* 2011). Moreover, the feed efficiency of *P. perforata* was superior to brown algae. Good results with red macroalgae for *Haliotis iris* and *Haliotis australis* were obtained by Poore (1972). Leighton & Peterson (1998) report that the red alga *Gracilaria* sp. supported a growth rate comparable to *E. menziesii* at an abalone farm in San Diego County, California. In the wild, the choice of macroalgae appears to be related to the nutritional content of the macroalga and is also influenced by the morphology and the presence of noxious metabolites in macroalgae (Shepherd & Steinberg1992; Leighton 2000).

Abalone show a moderate preference for the genus *Ulva* (Mercer *et al.* 1993). Good growth was reported in *H. tuberculata* that were fed *U. lactuca* (Uki & Watanabe 1992) and *H. discus hannai* that were 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 the feeding trial (47.8 ± 4.6 g kg^{-1}) of Pérez-Estrada *et al.* (2011). Higher FCR of this alga by juveniles did not compensate for its low protein content, which resulted in the lowest protein intake among the diets. Husbandry techniques for the grow-out of juveniles need diets that allow a growth rate equal to or higher than wild juveniles, which typically show growth rates of 174 μm d^{-1} (Sakai 1962; Uki 1981; Uki *et al.* 1986). López *et al.* (1998) and Preece & Mladenov (1999) reported growth rates of 10–135 μm d^{-1} for juveniles fed artificial diets. The highest growth rates in juvenile *H. discuss* were obtained by Uki *et al.* (1986) and in *H. fulgens* by Leighton & Peterson (1998), using a natural diet of *M. pyrifera* (Table 3). Viana *et al.* (1996), Leighton & Peterson (1998), and Serviere-Zaragoza *et al.* (2001) fed *M. pyrifera* to abalone and obtained

Table 3 Growth rates of various species of *Haliotis* spp. fed with different diets

Haliotis	Feed	Growth rates		Reference
		Length (μm d^{-1})	Weight (mg d^{-1})	
H. fulgens	*Ulva* sp. rehydrated	1.6	1.4	Pérez-Estrada *et al.* (2011)
H. fulgens	*Macrocystis pyrifera* rehydrated	18.1	8.2	Pérez-Estrada *et al.* (2011)
H. fulgens	*Porphyra perforata* rehydrated	15.1	8.1	Pérez-Estrada *et al.* (2011)
H. fulgens	*E. menziesii* rehydrated	17.5	9.2	Pérez-Estrada *et al.* (2011)
H. fulgens	*E. arborea* rehydrated	12	4.5	Pérez-Estrada *et al.* (2011)
H. discus hannai	Natural	80–174	–	Sakai (1962); Uki (1981); Uki *et al.* (1986)
H. midae	Natural	15–53	–	Simpson & Cook (1998)
H. asinia	Natural	2–70	–	Simpson & Cook (1998)
H. iris	Artificial	10–107	–	Preece & Mladenov (1999)
H. tuberculata	Artificial	134.8	3.20	López *et al.* (1998)
H. fulgens	*Macrocystis pyrifera* fresh	120–140	–	Leighton & Peterson (1998)
H. fulgens	*M. pyrifera* rehydrated	46	5.49	Serviere-Zaragoza *et al.* (2001)
H. fulgens	*Eiseniaarborea* rehydrated	19	1.52	Serviere-Zaragoza *et al.* (2001)
H. fulgens	*M. pyrifera* rehydrated	39	16.00	Ponce *et al.* (2004)
H. fulgens	*M. pyrifera* fresh	12–16	–	Viana *et al.* (1993, 1996)
H. fulgens	*Egregia* spp. fresh	43	56	Nelson *et al.* (2002)
H. corrugata	*E. menziesii* rehydrated	14	1.19	Robles (2003)

growth rates ranging from 18 to 120 μm d^{-1}. In the juvenile specimens in our study, growth rates ranged from 1.6 μm d^{-1} and 47.8 g kg^{-1} proteins and 35 g kg^{-1} lipids with *Ulva* sp. to 18.1 μm d^{-1} with 164 g kg^{-1} proteins and 36 g kg^{-1} lipids with a *M. pyrifera* diet. Here, abalone fed with *Ulva* sp. had suboptimal growth (Pérez-Estrada *et al.* 2011). Britz (1996) found that diets containing the highest level of dietary fat (100 g kg^{-1}) produced significantly lower growth rates and efficiencies of protein deposition in *H. midae* in comparison with abalone fed diets containing 60 or 20 g kg^{-1} fat. However, shell length and wet weight are not a sufficient measure to accurately determine the nutritional status of molluscs (Lucas & Beninger 1985).

In the digestive gland of juvenile *H. rubra*, Johnston *et al.* (2005) found structural and physiological changes: greater tubule number and density, higher enzyme production and increased digestive efficiency with age. In juvenile *H. rufescens*, García-Esquivel & Felbeck (2006) observed significant dietary effects on enzyme activity, especially in the digestive gland. Abalone fed 250 or 380 g kg^{-1} crude protein diets exhibited higher cellulase and lysozyme activities than abalone fed fresh kelp. In contrast, higher protease activity occurs in kelp-fed abalone than abalone fed crude protein diets, indicating that abalone can adjust enzyme levels to maximize ingestion of dietary protein and carbohydrates. The digestive gland appears to be the most important site for storage and secretion of enzymes in juvenile red abalone. Similar findings were reported for *H. fulgens* (Picos-García *et al.* 2000), indicating two main digestion regions: the stomach–digestive gland region, where high quantities of complex carbohydrases (cellulase and lysozyme) and the mouth–intestine region, where high activity of lipase and amino peptidase occurs. García-Carreño *et al.* (2003) found acid, serine proteinases and amino peptidases in the digestive organs of juvenile *H. fulgens*. Picos-García *et al.* (2000) found hydrolysed trypsin, chymotrypsin and acid phosphatase-specific substrates in extracts from the hepatopancreas of *H. fulgens*, showing that digestive enzymes in juveniles have activity peaks at acid pH.

In *H. discuss hannai*, Cui *et al.* (2001) observed that the digestive cells are capable of ingesting exogenous material in the lumen of the tubules and these contain a number of vesicles with heterophagic functions of cells, where the vesicles exhibit considerable variation in size and in different regions of the cells, which involve accumulation of ingested material. They show that the digestive cells secrete proteinase and can store food material. Aldana Aranda & Frenkiel (2012) determined that the juvenile herbivorous gastropod *Stombus gigas* grew and survived with formulated diets or natural food when they used histochemical techniques to examine digestive gland tissue. They observed proteoglycan granules in the digestive cells and determined that abundance of granules was variable, according to the nutritional status of the juveniles.

Cui *et al.* (2001) described two types of cells in the digestive gland of *H. discus hannai*: digestive and basophilic cells. The digestive cells are capable of ingesting fine exogenous material from the lumen of the tubule and contain vesicles concerned with the heterophagic function of the cells. The apical and subapical vesicles of these cells were called heterophagosomes, involved in accumulation of ingested material. Those in the mid-regions, called heterolysosomes, contain acid phosphatase and non-specific esterase that enhance intracellular digestion of ingested material and the vesicles in the basal regions that accumulate undigested and indigestible residues. Aldana Aranda & Frenkiel (2012) observed that digestive cells lost its blue granules when the queen conch was starved. When the conchs were later fed natural food or formulated diet, the digestive gland was restored to an almost normal status, with its digestive cells containing numerous blue granules. The authors observed that fine powder in the stomach is transported to the digestive gland tubules, and they found large quantities of secretion granules coming from the digestive cells in the stomach. In our study, we observed blue granules of different sizes in the stomach, mostly related to the specific diet. Further, using the digestive gland structure as a feed index, abalone fed *Ulva* sp. had digestive cells with no blue granules and abalone fed *M. pyrifera* or *P. perforata* had many blue granules in their digestive cells, indicating accumulation of proteoglycan granules.

The feed index was different for each of the five diets; therefore, a useful tool to test for micronutrients from algae to adapt natural feed to the real needs of juveniles. In the trials, the optimal nutritional status resulted in the best digestive gland structure, as well as the best shell length and weight. Similarly, the lowest FCR occurred with the *E. menziessi, M. pyrifera* or *P. perforate* diets. The histological study of the cell structure of the digestive gland demonstrated that the *M. pyrifera* diet provided the best appearance in abalone, as well as the best overall performance of the five diets.

In the digestive gland of *H. fulgens*, Pérez-Estrada *et al.* (2011) found that its biochemical composition closely reflected recently consumed food, compared with slower turnover of constituents in muscle tissue, which reflects retained nutrients. They observed simultaneous decreases in the concentration of nutrients in the digestive gland and

muscle and lower growth rates for abalone fed *Ulva* sp. and *E. arborea* and increases in concentration of carbohydrates and lipids of abalone fed *P. perforata*.

We observed that digestive gland structure, particularly the digestive cells appear to be a more sensitive tool to evaluate the efficiency of diets for juvenile green abalone. Aldana Aranda & Frenkiel (2012)showed that a period of 7–20 days of testing a diet is enough to see the effect of diet in the microstructure of the digestive gland, in contrast with measuring growth rate, which takes at least 3 months to observe the effect of nutrients. This histochemical technique represents a considerable advantage for those engaged in cultivating abalone.

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References

Aldana Aranda, D. & Frenkiel, L. (2012) Digestive gland structure as a feed index for juveniles of the queen conch, *Strombus gigas*, reared with formulated food. *Aquacult. Nutr.*, **18**, 581–588.

AOAC (1995) Official Methods of Analysis, 16th edn. Association of Official Analytical Agricultural Chemists, Gaithersburg, MD.

Britz, P.J. (1996) Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. *Aquaculture*, **140**, 55–61.

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**, 663–666.

Cui, L.B., Liu, C.L., Liu, X. & Lu, Y.H. (2001) The cell types and secretion of the digestive gland in *Haliotis discuss hannai* Ino. *Acta Zool. Sin.*, **47**, 32–37.

Fleming, A.E., Van Barnevel, R.J. & Hone, P.W. (1996) The development of artificial diets for abalone: a review and future directions. *Aquaculture*, **140**, 5–53.

Fretter, V. & Graham, A. (1962) British Prosobranch Mollusks, (Royal Society ed.). Bernard Quaritch, London.

Gabe, M. (1968) Techniques histologiques. Masson, Paris.

García-Carreño, F.L., Navarrete del Toro, M.A. & Serviere-Zaragoza, E. (2003) Digestive enzymes in juvenile green abalone, *Haliotis fulgens*, fed natural food. *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.*, **134**, 143–150.

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.

Gordon, H.R. & Cook, P.A. (2003) World abalone supply, markets and pricing: historical, current and future prospectives. 5th International Abalone Symposium, Quingdao, China.

Gros, O., Frenkiel, L. & Aldana Aranda, D. (2009) Structural analysis of the digestive gland of the queen conch, *Strombus gigas*, Linnaeus, 1758 and its intracellular parasites. *J. Molluscan Stud.*, **75**, 59–68.

Hirose, T. (1974) On the growth of the abalone, *Haliotis discus hannai* Ino, specially concerned with the food consumption in nature. *Bull. Tohoku Reg. Fish. Res. Lab.*, **33**, 87–94.

Johnston, D.T., Moltschaniwskyj, N. & Wells, J. (2005) Development of the radula and digestive system of juvenile blacklip abalone (*Haliotis rubra):* potential factors responsible for variable weaning success on artificial diets. *Aquaculture*, **250**, 341–355.

Kawamura, T., Roberts, R.D. & Takami, H. (1998) A review of the feeding and growth of postlarval abalone. *J. Shellfish Res.*, **17**, 615–625.

Knauer, J., Britz, P.J. & Hecht, T. (1996) Comparative growth performance and digestive enzyme activity of juvenile South African abalone, *Haliotis midae*, fed on diatoms and a practical diet. *Aquaculture*, **140**, 75–85.

Leighton, D.L. (2000) The Biology and Culture of the California Abalones. Dorrance Publishing, Pittsburgh.

Leighton, D.L. & Booloottian, R.A. (1963) Diet and growth in the black abalone, *Haliotis cracerodii. Ecology*, **44**, 227–238.

Leighton, D.L. & Peterson, D.L. (1998) The superiority of *Egregia* as a food in cultivation of the green abalone (*Haliotis fulgens*). In World Aquaculture Society and the National Shell Fisheries Association, Abstract, p. 331.

Lodish, H., Berk, A., Zipursky, S.L. *et al.* (2000) Molecular cell biology, 4th edn. W.H. Freeman, New York.

López, L.M., Tyler, P.A. & Viana, M.T. (1998) The effect of temperature and artificial diets on growth rates of juvenile *Haliotis tuberculata* (Linnaeus, 1758). *J. Shellfish Res.*, **17**, 657–662.

Lucas, A. & Beninger, P.G. (1985) The use of physiological condition indices in marine bivalve aquaculture. *Aquaculture*, **44**, 187–200.

Luna, G.L. (1969) Manual of Histologic staining methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York.

MacBride, S.C. (1998) Current status of abalone aquaculture in the Californias. *J. Shellfish Res.*, **17**, 593–600.

Mai, K., Mercer, J.P. & Donlon, J. (1995) Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. II. Aminoacid composition of abalone and six species of macroalgae with an assessment of their nutritional value. *Aquaculture*, **128**, 115–130.

Mercer, J.P., Mai, K.S. & Donlon, J. (1993) Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* Linnaeus and *Haliotis discus hannai* Ino. I. Effects of algal diet on growth and biochemical composition. *Invertebr. Reprod. Dev.*, **23**, 75–88.

Morse, P. & Zardus, J.D. (1997) Bivalvia. In: Microscopic Anatomy of Invertebrates (Harrison, F.W. & Kohn, A.J. eds.), vol **6A**: Mollusca II, pp. 7–118. Wiley-Liss, New York.

Nelson, L. & Morton, J.E. (1979) Cyclic activity and epithelium renewal in the digestive gland tubules of the marine prosobranch *Maoricrypta monoxyla* (Lesson). *J. Molluscan Stud.*, **45**, 262–283.

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, Biochem. Mol. Biol.*, **131**, 695–712.

Pérez-Estrada, C.J., Civera-Cerecedo, R., Hernández-Llamas, A. & Serviere-Zaragoza, E. (2011) Growth and biochemical composition of juvenile green abalone *Haliotis fulgens* fed rehydrated macroalgae. *Aquacult. Nutr.*, **17**, e62–e69.

Picos-García, C., García-Carreño, F. & Serviere-Zaragoza, E. (2000) Digestive proteases in juvenile Mexican green abalone, *Haliotis fulgens*. *Aquaculture*, **181**, 157–170.

Ponce, D.G., Serviere, Z.E., Racotta, S.I., Reynoso, G.T., Mazariegos, V.A., Monsalvo, S.P. & Lluch, B.D. (2004) Growth and tissue biochemical composition of *Haliotis fulgens* at elevated temperatures in Baja California under two dried brown algal diets. *J. Shellfish Res.*, **23**, 1051–1057.

Poore, G.B. (1972) Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda). *Mar. Freshw. Res.*, **6**, 11–22.

Preece, M.A. & Mladenov, P.V. (1999) Growth and mortality of the New Zealand abalone *Haliotis iris* Martín 1784 cultured offshore structures and fed artificial diets. *Aquac. Res.*, **30**, 865–877.

Purchon, R.D. (1977) The biology of the Mollusca. Pergamon Press, New York.

Robles, H.C.R. (2003) Crecimiento de juveniles de abulo´n amarillo Haliotis corrugata (Wood, 1828) alimentados con cinco especies de macrofi´tas. Licenciatura thesis. Universidad Auto´ noma de Baja California Sur, La Paz, Baja California Sur, Mexico.

Sakai, S. (1962) Ecological studies on the abalone, *Haliotis discus hannai* Ino. I. Experimental studies on the food habit. *Bull. Jpn. Soc. Sci. Fish.*, **28**, 766–779.

Serviere-Zaragoza, E., Mazariegos-Villarreal, A., Ponce-Díaz, G. & Montes, M.S. (2001) Growth of juvenile abalone, *Haliotis fulgens* Philippi, fed different diets. *J. Shellfish Res.*, **20**, 689–693.

Serviere-Zaragoza, E., García-Hernández, V. & Siqueiros-Beltrones, D. (2003) Diversity and distribution of macroalgae associated with abalone (*Haliotis* spp.) habitats in Baja California Sur, Mexico. *Bull. Mar. Sci.*, **72**, 725–739.

Shawl, A. & Davis, M. (2006) Effects of dietary calcium and substrate on growth rate and survival of juvenile queen conch cultured for stock enhancement. *Proc. Gulf Carib. Fish. Instit.*, **57**, 955–962.

Shepherd, S.A. & Steinberg, P.D. (1992) Food preferences of three Australian abalone species with a review of the algal food of abalone. In: Abalone of the World: Biology, Fisheries and Culture (Shepherd, S.A., Tegner, M.J. & Guzmán del Proo, S.A. eds), pp. 169–181. Fishing New Books, Oxford, UK.

Simpson, B.J.A. & Cook, P.A. (1998) Rotation diets: a method of improving growth of cultured abalone using natural algal diets. *J. Shellfish Res.*, **17**, 635–640.

Sokal, R.R & Rohlf, J.F. (1995) Analysis of Frequencies. In: Biometry the principles and practice of statistics in biological research. 3rd edn, 887pp. W. H. Freeman, New york.

Uki, N. (1981) Food value of marine algae of order Laminariales for growth of abalone, *Haliotis discus hannai*. *Bull. Tohoku Nat. Fish. Res. Inst.*, **42**, 19–27.

Uki, N. & Watanabe, T. (1992) Review of the nutritional requirements of abalone (*Haliotis* spp.) and development of more efficient diets. In: Abalone of the World: Biology, Fisheries and Culture (Shepherd, S.A., Tegner, M.J. & Guzman del Proo, S.A. eds), pp. 504–517. Fishing New Books, Oxford, UK.

Uki, N., Sugiera, M. & Watanabe, T. (1986) Dietary value of seaweeds occuring on the Pacific Coast of Tohoku for growth of abalone *Haliotis discus hannai*. *Bull. Jpn. Soc. Sci. Fish.*, **52**, 257–266.

Viana, M.T., López, L.M. & Salas, A. (1993) Diet development for juvenile abalone *Haliotis fulgens*. Evaluation of two artificial diets and macroalgae. *Aquaculture*, **117**, 149–156.

Viana, M.T., López, L.M., García-Ezquivel, Z. & Méndez, E. (1996) The use of silage made from fish and abalone viscera as an ingredient in abalone feed. *Aquaculture*, **140**, 87–98.