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GROWTH OF JUVENILE ABALONE, *HALIOTIS FULGENS* PHILIPPI, FED DIFFERENT DIETS

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ABSTRACT Growth rates of juvenile *Haliotis fulgens* (green abalone) were evaluated with four different diets over 106 days. Three diets were based on the algae palm kelp *Eisenia arborea*, giant kelp *Macrocystis pyrifera*, and *Gelidium robustum*, and one on seagrass *Phyllospadix torreyi*. One artificial diet was used as a control. The best growth rates and specific growth rates were found in abalone fed *M. pyrifera*, which were significantly different from growth achieved on the other natural diets. The pattern of growth in juveniles fed an artificial diet was similar to juveniles fed *M. pyrifera*. The highest mortality (11%) was in juveniles fed the red algae *G. robustum*.

KEY WORDS: *Haliotis fulgens*, green abalone, growth, algae, diets

INTRODUCTION

In recent years, dietary research in abalone has focused on the production of formulated feeds in countries with a history of abalone culture (Uki & Watanabe 1992, Wee et al. 1992, Viana et al. 1993, Viana et al. 1994, Britz 1996a, Britz 1996b, Fleming et al. 1996, Knauer et al. 1996, Britz et al. 1997, Clarke & Creese 1998, López et al. 1998, Monje & Viana 1998) because of potential use of these feeds in commercial production. Information on natural diets is important in understanding the biology of abalone species. Abalone food preferences have been studied from both analysis of gut contents and feeding experiments in species such as *Haliotis cracherodii* Leach, *H. discus hannai* Ino, *H. fulgens* Philippi, *H. tuberculata* (L.), and *H. rufescens* Swainson (Sakai 1962, Leighton & Boolootian 1963, Culley & Peck 1981, Uki 1981, Uki et al. 1986, Leighton 1966, Mercer et al. 1993, Corazani & Illanes 1998, Leighton & Peterson 1998, Simpson & Cook 1998). Shepherd and Steinberg (1992) reviewed the literature on the feeding biology of abalone. In Mexico, research into natural diets has been limited to the brown alga *Macrocystis pyrifera* (L.) C. Ag. as a control in feeding trials (Viana et al. 1993, Viana et al. 1996). It has been assumed that abalone feed on the kelp alga *M. pyrifera*. Regional hatcheries use this species as a main source of natural food. *M. pyrifera* does not occur at the southern limit of the distribution of abalone species along the Baja California Peninsula. However, there are many subtidal algae along the coast that may be used as potential food. In Baja California Sur, the most common food items in gut content of adults of green abalone *Haliotis fulgens* were the seagrass *Phyllospadix torreyi* S. Watson and the macroalgae *Sargasum* sp., *Eisenia arborea* Aresh., *Cryptopleura crispa* Kylin, and *Rhodymenia* sp. (Serviere-Zaragoza et al. 1998). It is important to develop feeding experiments oriented to test growth rates and feed conversion efficiencies on single species diets to evaluate local algal species as diets for abalone. Day and Fleming (1992) mentioned that although an alga may not support sustained growth when fed alone, it may be of great value when part of a mixed diet by providing essential nutrients to the diet. This study was designed to assess the growth of juvenile green abalone fed with common species in the benthic environments inhabited by abalone along the western coast of Baja California.

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MATERIALS AND METHODS

Diets

Macrocystis pyrifera (MP) was selected as the primary alga species (the control) because it is the dominant algal species of southern California (Dawson et al. 1960) and it is believed by fishermen that Mexican abalone species feed extensively on this alga. The brown alga *Eisenia arborea* (EA), red alga *Gelidium robustum* (GR), and the sea grass *Phyllospadix torreyi* (PT) are thought to be important species in abalone communities as potential food along Baja California (Guzmán del Próo et al. 1972, Guzmán del Próo et al. 1991, Serviere-Zaragoza et al. 1998). Natural diets were harvested, dried, and stored at the beginning of the experiment, because chemical composition varies during the year. The growth rate of abalone fed with algal diets is low and variable over time. Therefore, we decided to include an artificial diet as a control (AD). AD was manufactured by the nutrition group of Instituto de Investigaciones Oceanológicas, B.C. The dietary formulation is in Table 1. Proximate analyses (crude protein, ether extractables, crude fiber, and ash) were performed using the methods of the Association of Official Agricultural Chemists (1995).

Experimental Procedure

Animals used in this experiment were reared in a commercial hatchery in Eréndira, Baja California, and transferred to the CIBNOR laboratory in La Paz, B.C.S. at age 8 mo. Feeding experiments commenced six months later. During this period, animals were fed the brown alga *Eisenia arborea*. Six hundred animals (17.3 (±2.2) mm shell length and 0.44 (±0.2) g body weight) were used to test the growth response of abalone fed four diets for 106 days. Experimental animals were held in 16-L fiber glass containers (50 × 30 × 35 cm, experimental units) with a concave bottom. Three replicates per treatment were made with 40 abalone per experimental unit (EU). Animals were marked with plastic tags attached to the shell. The EUs were supplied with temperature controlled (20 ± 1°C), fresh, filtered (10 μm) water with a flow rate of 73 mL/min. The water was aerated vigorously. Salinity, oxygen, pH, nitrate, nitrite, ammonium content, and phosphate were monitored every week. Dead animals were removed and replaced to maintain densities. Shell length was measured with a vernier caliper, and body weight was measured with an electronic balance (to 0.001 g) at the beginning, and at 30, 60, and 106 days.

TABLE 1.

Percent composition of the artificial diets tested in this study, given as percentage of dry matter.

Ingredients	g/100 g
Fish meal	30.0
Silage (dry basis)	2.0
Soy bean meal	10.0
Corn meal (whole)	12.0
Vegetable meal	15.0
Corn starch	19.37
Gelatin	6.0
Vitamin mixture	1.7
Mineral mixture	3.3
Choline chloride	0.11
Methionine	0.23
BTH	0.086
Sodium Benzoate	0.23

Diets were given *ad libitum*. Natural diets were fed every 42 hours in the afternoon and the artificial diet each night. The remaining food was carefully collected for drying and weighing; every other morning for natural diets and each morning for the artificial diet. This was undertaken throughout the feeding experiment. Algae growing on the inside walls of the EU were removed twice a week with a soft brush. For diets, the amount of dry matter lost in seawater was estimated during the experiment by using EUs without abalone under the same conditions as those of the growth experiments.

Growth rates were calculated by the equation:

$$GR_{SL} = (SL_f - SL_i)/T \text{ and } GR_{BW} = (BW_f - BW_i)/T$$

where SL_f = mean final shell length, SL_i = mean initial shell length, BW_f = mean final weight, BW_i = mean initial weight, and T = time in days.

Specific growth rate (SGR % day^{-1}) was calculated for shell length and body weight by the equation (Britz 1996b):

$$SGR_{SL} = \{(\ln SL_f - \ln SL_i)/T\} \times 100 \text{ and}$$

$$SGR_{BW} = \{(\ln BW_f - \ln BW_i)/T\} \times 100$$

where SGR_{SL} is percent shell length gain per day, SL_f = mean final shell length, SL_i = mean initial shell length, and T is time in days between measurements. SGR_{BW} is percent body weight gained per day, BW_f = mean final weight, and BW_i = mean initial weight.

Consumption was calculated in terms of dry weight with the equation (Uki & Watanabe 1992):

$$FC = (GS/100) - R$$

where G is the weight of food offered per animal per day (in grams), S is the percentage of food recovered, obtaining a factor for each diet (from the controls without abalone), and R is the remaining food (in grams) after the abalone had fed.

Food conversion efficiency ratio was calculated as (Uki & Watanabe 1992):

$$FCE = \text{wet weight gain (g)/dry weight of food consumed (g)} \times 100$$

This measure of food utilization is related to conversion efficiency, but is in fact the ratio of animal live or wet weight gain to the amount of dry diet consumed (Monje & Viana 1998).

Statistical Analyses

Data for experimental replicates were pooled because no significant differences were found between them by one-way analysis of variance (ANOVA) at a significance level of P = 0.05. Data from each different treatment were analyzed by ANOVA test and Tukey test to determine differences of means (Sokal & Rohlf 1995). Statistical analysis was done with software STATISTICA 6.0 for PC.

RESULTS

The highest protein content was found in *Gelidium robustum* (GR) followed by *Phyllospadix torreyi* (PT), *Macrocystis pyrifera* (MP), and *Eisenia arborea* (EA). The lipids were between 1.06% for *G. robustum* and 1.30% for *M. pyrifera* (Table 2). The daily average temperature was 20 ± 1°C. Water quality analyses showed the following averages (±*s*) for pH 8.03 (±0.06): oxygen 6.52 (±0.74) mg/L, salinity 40 (±1.29) ppm, nitrites 0.0052 (±0.0004) μmol/L, nitrates 0.1253 (±0.0029) μmol/L, ammonium 0.0226 (±0.00001) mg/L, and phosphate 0.00552 (±0.000017) μmol/L throughout the experiment.

Growth of Abalone

A significant difference in means was shown between shell length and body weight of juvenile green abalone fed with the natural diets ($P < 0.05$). At 30 days of the experiment, the mean length, 19.15 mm ± 0.23 (SE), and weight, 0.59 g ± 0.02 (SE), of juveniles fed MP were statistically different from the means of juveniles fed EA, 18.28 mm ± 0.21 (SE) and 0.02 g ± 0.18 (SE), and GR, 18.24 mm ± 0.19 (SE) and 0.50 g ± 0.02 (SE). After 60 days, the differences increased and were significant. Both mean length and weight of juveniles fed MP were statistically different from the other diets; EA, GR, and PT. Differences between juveniles fed these latter three diets were not detected.

Mean shell length and body weight increased over time on all diets (Fig. 1). The best growth in length and weight for green abalone was obtained with MP, 22.29 mm ± 0.33 (SE) and 1.0 g ± 0.06 (SE). The percent of survival was between 89% and 95% in natural diets. For AD, it was 97% (Table 3). The pattern of growth in juveniles fed the artificial diet, 22.01 mm ± 0.22 (SE) and 0.91 g ± 0.03 (SE), was similar to juveniles fed MP (Fig. 1).

TABLE 2.

Proximate analysis of the species and artificial diet used in experimental diets. EA, *Eisenia arborea;* MP, *Macrocystis pyrifera;* GR, *Gelidium robustum;* PT, *Phyllospadix torreyi;* and AD, artificial diet.

	Component				
Diet	Crude Protein	Ash	Crude Fiber	Ether Extract	N-free Extract
EA	7.60	27.13	6.44	1.15	57.68
MP	12.0	41.33	7.0	1.30	38.37
GR	17.61	21.26	10.19	1.06	49.88
PT	15.94	31.88	13.45	1.28	37.44
AD	35.85	10.70	5.85	7.09	40.51

Values are given as percent of dry matter.

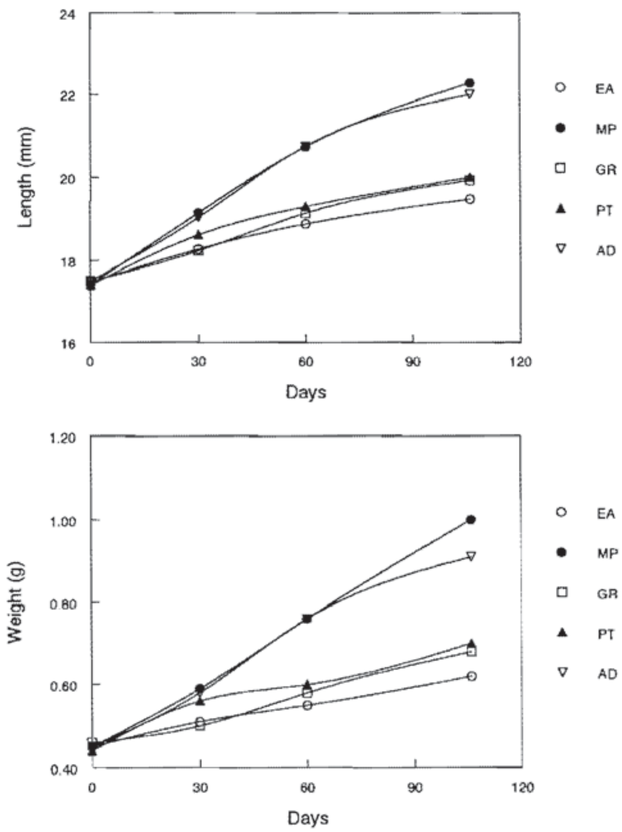

Figure 1. Mean growth of abalone fed with different diets. EA, *Eisenia arborea;* MP, *Macrocystis pyrifera;* GR, *Gelidium robustum;* PT, *Phyllospadix torreyi;* and AD, artificial diet.

Growth Rates

Significant differences occurred, between natural diets, in the daily growth rates of the shell length (SL) and body weight (BW) ($P < 0.05$). During the experimental period, 106 days, both mean SL and BW growth rates of animals fed MP were higher than mean growth rates of the juveniles fed the other natural diets ($P < 0.05$) (Table 3). The growth rates of the juveniles analyzed varied within the same diet during the experiment. In EA and PT a gradual decrease was observed during the experiment. For MP and GR, in the second month a gradual increase was measured, and at the end of the experiment the values had decreased. Juveniles fed EA showed the lowest shell length growth rates, 15 μm ± 1 (SE), during the third experimental month and the highest was in juveniles fed MP, 53 μm ± 2 (SE), during the second month. Body weight growth rates were between 0.92 mg ± 0.13 (SE) for GR during the first month and 6.70 mg ± 1.29 (SE) for MP during the third month.

The daily growth rates of juveniles fed artificial diet were similar to growth rates of juveniles fed MP (Table 3). The values ranged between 31 μm ± 2 (SE) in the third month to 57 μm ± 2 (SE) in the second month. Daily body weight rates were between 3.63 mg ± 0.18 (SE) in the first month and 6.13 mg ± 0.23 (SE) in the second month.

SGR

The ANOVA showed that there was no significant difference in mean SGR for any of the replicate diets. Using the Tukey test on the SGR (Tukey test $P < 0.05$) for green abalone, the existence of a significant difference in mean was shown between the SGR shell length and body weight of the abalone fed with MP and the other diets (EA, GR, PT). The same analysis revealed there was no significant difference in the mean SGR for EA, GR, and PT, except for SGR body weight of juveniles fed PT, which was higher than EA and GR (Fig. 2). SGRs obtained from juveniles fed the artificial diet were similar to juveniles fed MP.

The feed consumption rate of abalone ranged from 0.0033 g/day for EA to 0.0108 g/day for MP. Consumption did not differ significantly between EA and GR, and PT and MP ($P > 0.05$). Abalone consumed significantly greater amounts of PT and MP (Table 4). FCE ratios for the natural diets vary between 30% for PT and 63% for MP (Table 4). Differences between FCE ratios were not detected ($P < 0.05$).

DISCUSSION

Mean shell length and body weight increased over time on all diets. The best growth in length and weight for green abalone was obtained with *Macrocystis pyrifera*. Feeding abalone on *Eisenia arborea*, *Gelidium robustum*, and *Phyllospadix torreyi* diets resulted in lower growth and FCE, 46% to 81% of the values obtained with *M. pyrifera*. These results indicate that the dietary value of the common species along the coast of Baja California Sur, *Eisenia arborea*, *Gelidium robustum*, and *Phyllospadix torreyi*, was inferior to that of the dominant algal species of southern California *M. pyrifera*. This result may be related to the trends described by Guzmán del Próo et al. (1976) about the size and weight means for *Haliotis* spp., which decrease from north to south along the Baja California Peninsula. In southern California, the

TABLE 3.

Survival, mean initial size, mean growth gain, and mean growth rate of green abalone fed with different diets. Diets as defined in Table 2.

Diet	Survival %	Mean Initial Size (mm)	Mean Growth Gain (mm)	Mean Growth Rate (μm day^{-1})	Mean Initial Size (g)	Mean Growth Gain (g)	Mean Growth Rate (mg day^{-1})
EA	93	17.47 (0.20)	1.91 (0.10)	19 (0.89)^a	0.45 (0.01)	0.15 (0.01)	1.52 (0.09)^a
MP	93	17.42 (0.20)	4.63 (0.23)	46 (1.96)^b	0.45 (0.01)	0.55 (0.06)	5.49 (0.54)^b
GR	89	17.49 (0.21)	2.22 (0.21)	23 (1.94)^a	0.46 (0.01)	0.20 (0.02)	2.07 (0.19)^a
PT	95	17.40 (0.20)	2.52 (0.11)	25 (0.95)^a	0.44 (0.01)	0.25 (0.01)	2.43 (0.10)^a
AD	97	17.50 (0.17)	4.47 (0.13)	42 (1.21)^b	0.45 (0.01)	0.46 (0.02)	4.39 (0.24)^b

Standard error in parentheses.

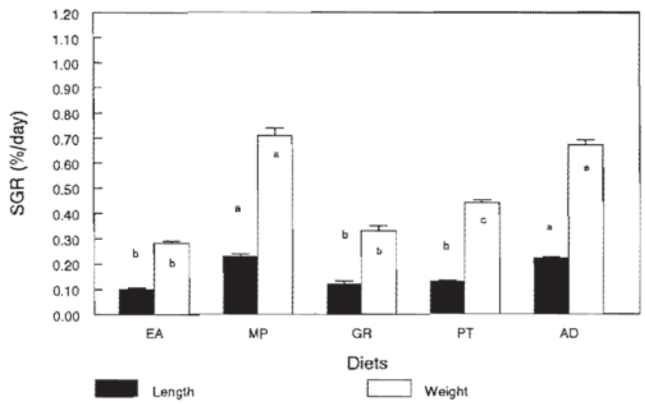

Figure 2. Mean specific growth of abalone compared between diets. Diets as defined in Figure 1.

TABLE 4.

Consumption (FC) and Food conversion efficiency (FCE) of green abalone fed with different diets. Diets as defined in Table 2.

Diet	FC (g)	FCE (%)
EA	0.0033 (0.0002)[a]	52 (5)
MP	0.0108 (0.0010)[c]	63 (16)
GR	0.0043 (0.0009)[ab]	33 (.01)
PT	0.0102 (0.0009)[c]	30 (7)
AD	0.0075 (0.0004)[b]	59 (2)

Standard error in parenthesis.
Items with different superscript letters are significantly different.

best growth rates for juveniles and young adults have been observed when the alariacean brown alga, *Egregia menziesii*, served as food. The giant kelp, *Macrocystis pyrifera*, is of relatively minor value as a food of green abalone (Leighton & Peterson 1998). *E. menziesii* does not occur in the natural habitats of the different abalone species in southern Baja California (Guzmán del Próo et al. 1991). Local studies oriented to evaluate common algae along southern Baja California as a diet of abalone are of interest in the knowledge of abalone biology and for abalone hatcheries developed along this area of the coast. Fishermen have been forced to develop abalone hatcheries to produce juveniles and to increase natural stock. Hatcheries are going to use microalgae and macroalgae as the diet for juveniles until an artificial diet is successfully developed.

The nutritional value of food rations depends on many factors, including nutrient composition, bioavailability, palatability, and digestibility. Food palatability is an important factor in determining feeding rates (Leighton & Boolootian 1963, Leighton 1966, Poore 1972, Fleming 1995). In this study, the difference in growth between juveniles fed MP and EA or GR might be caused mainly by differences in palatability because of the lower amount consumed using both EA and GR. Differences between growth of juveniles fed MP and PT might be related to the digestibility of the protein sources because of the lower FCE of the seagrass. Poor abalone growth rates observed for seaweed diets could be attributed to a deficiency of essential nutrients or a low protein to energy ratio, because marine algae, in general, are rich in storage carbohydrates but low in protein. Thus the abalone fed macroalgal diets may satisfy their energetic requirements primarily from carbohydrate, but sufficient protein may not be available for tissue deposition (Britz 1996b). Beside variables associated with the food quality, digestion of the food is an important issue in nutrition. Our group hereby describes the enzymes responsible for digestion of this protein found in the organism's digestive system. In the adult green abalone trypsin and chymotrypsin activity was found in both intestine and rectum, but not in hepatopancreas and crop-stomach content. In juvenile green abalone digestive extracts revealed hepatopancreas and viscera hydrolyzed trypsin, chymotrypsin, and acid phosphatase specific substrates. (Serviere-Zaragoza et al. 1997, Picos-Garcia et al. 2000).

The growth rate of juvenile green abalone was slow and heterogeneous. The average growth rates of the juveniles analyzed varies from 19 μm day^{-1} and 1.52 mg day^{-1} for *Eisenia arborea* to 46 μm day^{-1} and 5.49 mg day^{-1} for *M. pyrifera*. Viana et al. (1993, 1996) reported averages of 12 μm day^{-1} and 16 μm day^{-1} for juveniles fed fresh kelp, *M. pyrifera*, and 18 μm day^{-1} for juveniles fed an artificial diet based on kelp meal for the same abalone species. *Gelidium robustum, Eisenia arborea*, and *Phyllospadix* spp. are of low acceptability for green abalone in Southern California and support minimal growth (Leighton pers. comm.). Simpson and Cook (1998) found shell length growth rates of *H. midae* ranged between 15 and 53 μm day^{-1} on single-species diets of *Ecklonia, Laminaria, Porphyra, Ulva, Aeodes*, and *Gracilaria*.

Using EA and PT, a gradual decrease was observed during the experiment. For MP and GR in the second month, a gradual increase was observed, and at the end of the experiment the values had decreased. This is similar to the trend reported by Viana et al. (1993); the daily growth rates decreased throughout time. Feeding trials on *H. rubra* using single species of dried algae revealed that abalone cease to grow after a period ranging from 50 to 200 days (Day & Fleming 1992). In the wild, abalone species consume more than one species. In *H. fulgens* the average number of plant species per gut was between two and four (Serviere-Zaragoza et al. 1998). This suggests they obtain the required nutrients for growth from combinations of species.

An artificial diet provides better growth rates than a natural one (macroalgae) in abalone cultures (Nie et al. 1986, Hahn 1989, Uki & Watanabe 1992, Viana et al. 1993, Viana et al. 1996). Nevertheless, in this study the growth of juveniles fed the artificial diet used as a control was similar to growth of juveniles fed MP. The lower growth of juveniles fed AD may be related to the leaching of some components during the transport or storage of the artificial diet. All environmental variables were constant during the experiment with abalone strongly attached and active throughout. Additionally, growth rates in juveniles fed MP were similar to that reported in the literature for the same species (Viana et al. 1993, Viana et al.1996).

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