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PROTEIN CONTENT DETERMINES THE NUTRITIONAL VALUE OF THE SEAWEED *ULVA LACTUCA* L FOR THE ABALONE *HALIOTIS TUBERCULATA* L. AND *H. DISCUS HANNAI* INO

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ABSTRACT The nutritional value to abalone of *Ulva lactuca* L. with different tissue nitrogen levels was studied. The seaweed was cultured at two levels of ammonia-N enrichment. Cultures receiving 0.5 g ammonia-N m^{-2} d^{-1} ("low-N") yielded 164 g m^{-2} d^{-1} of fresh thalli containing 12% crude protein in dry matter and 12 kJ g^{-1} energy; cultures receiving 10 g ammonia-N m^{-2} d^{-1} ("high-N") produced 105 g of fresh thalli m^{-2} d^{-1} containing 44% protein and 16 kJ g^{-1} energy. High-N and low-N algae and a "standard" mixed diet of 75% *U. lactuca* and 25% *Gracilaria conferta* (w/w) containing 33% protein and 15 kJ g^{-1} energy were fed to juvenile (0.7–2.1 g) and adult (6.9–19.6 g) *Haliotis tuberculata* and *H. discus hannai* in a 16-week feeding trial. Voluntary feed intake of the high-N and standard diets were significantly lower than the low-N diet in all the cases. Clear differences in performance between treatments were found in the juvenile and adult abalone of both species. Juveniles fed high-N and standard diets grew significantly faster (specific growth rate of *H. tuberculata* was 1.03% day^{-1} on high-N algae as compared to 0.72% on low-N algae; *H. discus hannai* grew 0.63 and 0.3% day^{-1} on high- and low-N algae, respectively) and showed much better food conversion ratios. The nutritional value of *Ulva lactuca* to abalone is greatly improved by a high protein content, attainable by culturing the seaweed with high supply rates of ammonia.

KEY WORDS: integrated mariculture, abalone, seaweed, protein content, nutrition, FCR

INTRODUCTION

Reduced fishery landings and increasing global demand for abalone have resulted in major market opportunities for cultured abalone (Oakes and Ponte 1996). The European abalone, *Haliotis tuberculata* L., and the Japanese *H. discus hannai* Ino, are valuable shellfish (Mgaya and Mercer 1994, Oakes and Ponte 1996), stimulating considerable effort into the development and optimization practices of their culture.

The supply of sufficient amounts of adequate food throughout the abalone's long growout phase continues to limit the development of intensive abalone culture; suitable artificial diets are generally too expensive (Fleming and Hone 1996) unless fed in conjunction with macroalgae (Uki and Watanabe 1992). Traditionally, cultured abalone have been fed wild-gathered macroalgae; however, the increasing intensity of abalone culture, the unreliable and seasonal nature (availability and quality) of the food harvest (Uki and Watanabe 1992, Mai et al. 1995), tougher environmental legislation, and the establishment of cultures in areas with no access to wild seaweed populations (Shpigel et al. 1996) have lead to increasing interest in the use of cultured macroalgae as abalone food (Tenore 1976, reviewed in Uki 1989, Marsden and Williams 1996, Shpigel and Neori 1996).

The robust chlorophyte *Ulva lactuca* L. has been successfully adapted to vegetative tank culture (DeBusk et al. 1986). It has been a highly effective biofilter species, removing dissolved nutrients from mariculture effluents and sustaining rapid production of seaweed biomass (Tenore 1976, Vandermeulen and Gordin 1990, Cohen and Neori 1991, Neori et al. 1991, 1996). Cultured and wild *Ulva lactuca* have been successfully used as diets for abalone (Tenore 1976, Shpigel et al. 1996); but to obtain commercially acceptable abalone growth rates, it has been necessary to feed other algal species in conjunction with *U. lactuca* (Mercer et al. 1993, Stuart and Brown, 1994). Given the worldwide distribution, ease of culture, biofiltration potential, and high productivity of *Ulva lactuca*, combined with its high palatability to abalone (Stuart and Brown 1994), learning to manipulate the dietary value of this seaweed to the nutritional requirements of abalone and understanding these requirements are of considerable value.

The availability of suitable quantity and quality of dietary protein are considered to be a prime factor governing the growth of abalone fed natural diets (Mai et al. 1995, Britz 1996). Previous studies have noted that the relative amounts of tissue nitrogen, predominantly in the form of protein and free amino acids (Duke et al. 1989b, Pedersén 1994), can vary in *Ulva* spp., depending upon the alga's growth conditions and the availability of inorganic nitrogen in the growth medium (DeBusk et al. 1986, Duke et al. 1989a, Vandermeulen and Gordin 1990, Cohen and Neori 1991, Neori et al. 1991). However, such studies have not considered possible subsequent effects upon the macroalgivores. Attempts to manipulate the chemical composition of a seaweed by adjusting the culture environment have so far been restricted to macroalgae of direct commercial value, such as the agarophyte genus *Gracilaria* spp. (e.g., Lapointe and Ryther 1979, Lignell and Pedersén 1987). Some important progress has, however, been made in enhancing the dietary value of certain phytoplankton species fed to bivalves, by adjusting the nutrient composition of the algal culture medium (Engirt et al. 1986, Herrero et al. 1991).

The present study was established with two main objectives: (1) to develop *Ulva lactuca* cultures of substantially different and stable tissue nitrogen levels; and (2) to determine the nutritional values of these *U. lactuca* cultures for the abalone *Haliotis tuberculata* and *H. discus hannai*.

MATERIALS AND METHODS

Abalone

Fifty juvenile European Abalone, *Haliotis tuberculata* (18.2–24.7 mm shell length, 0.8–2.1 g individual live weight; 76–89 g total biomass) or Japanese Ezo Awabi, *H. discus hannai* (17.9–

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24.4 mm, 0.7–1.8 g; 56–63 g biomass) were stocked to 28-L aquaria. Each aquarium contained two half-pipe shelters, and received 50 volume exchanges d^{-1} of 10 μm filtered seawater at 21.7–23.1°C. An airline suspended in the center of each aquarium supplied vigorous aeration to circulate food material, which was retained by a 1-mm mesh covering the outlet pipe. Aquaria were also established with adult animals: 10 *H. tuberculata* (37.0–50.2 mm, 6.9–19.6 g each; 123–125 g total biomass) or 10 *H. discus hannai* (34.0–50.8 mm, 5.0–16.1 g; 79–81 g biomass) individually identified by Dymo™ tags. All animals had previously received a mixed diet of *U. lactuca* and *G. conferta*.

Diets and Algal Cultures

Three distinct live seaweed diets were produced for the abalone. Two were monospecific *Ulva lactuca,* grown at two different levels of nitrogen enrichment. A "standard" diet consisted of a mixture of medially enriched *U. lactuca* together with *Gracilaria conferta.* This mixed diet had been found in our preliminary observations to support good growth in weaned abalone juveniles.

Macroalgal cultures were established in November 1995 in the land-based facilities of the National Center for Mariculture, Eilat, Israel. The cultures were supplied with 5–6 volume exchanges d^{-1} of 10-μm filtered water pumped from the Red Sea at 20-m depth (41 ppt, 19.5–25.3°C). Vegetative *Ulva lactuca* thalli, isolated from the Red Sea as in Vandermeulen and Gordin (1990), were grown in $1m^2$, 600-L tanks agitated with vigorous aeration. the technique has been described in Vandermeulen and Gordin (1990) and in Cohen and Neori (1991). Inorganic nutrients were added to the media in a concentrated solution, containing disodium phosphate (DSP, at a flux of 0.6 g P m^{-2} d^{-1}), and ammonium sulphate (at fluxes determined by the experimental treatment). The solution was dripped into the cultures over a 4-h period every morning, this being considered ample time for *Ulva* spp. to take up its daily ammonia-N requirement (Fujita et al. 1988). The ammonia-N was added at two levels, 0.5g N m^{-2} d^{-1} ("low-N" *U. lactuca* culture) and 10 g N m^{-2} d^{-1} ("high-N" culture). These levels were chosen based on Cohen and Neori (1991), who suggest that these N-fluxes would produce *U. lactuca* of considerably different tissue-nitrogen levels, while sustaining sufficient production to permit harvesting for feeding. Several cultures of high-N and low-N *U. lactuca* were established. Every week, the vessels were emptied and cleaned, the algae were centrifuged (500 r.p.m. for 3 min) to remove surface water, weighed to assess biomass production, and restocked at the original density. The mixed standard diet was obtained by harvesting *U. lactuca* and *Gracilaria conferta* grown in simulation biofilters (Cohen and Neori 1991) receiving 0.6 g DSP m^{-2} d^{-1} and 4 g ammonia-N m^{-2} d^{-1}.

Feeding and Growth

A preliminary test was conducted to determine whether the N content of *Ulva lactuca* was decreasing after days of immersion in the abalone tanks. High-N and low-N *U. lactuca* were stocked to separate aquaria without animals. Subsamples were removed after 0, 12, 18, 24, and 48 h, rinsed in deionised water, freeze dried, and the nitrogen content was determined.

High-N and low-N *ulva lactuca* dietary treatments were supplied to triplicate aquaria with juveniles and duplicate aquaria with adult abalone of either species; in addition, a single aquarium for each abalone size class and species was fed the mixed diet. Animals were first stocked to the experimental aquaria on 8 February 1996 after individual live weights (following 2 min drying on absorbent paper ± 0.01 g) and shell lengths had been measured (±0.05 mm). An additional 25 juveniles and 10 adults were taken from the source populations of either species, weighed and held without food for 48 h; the soft body and shell were then separated and freeze dried.

Food algae were added to the aquaria in excess (equivalent to approximately 20% of the resident abalone biomass) at dusk and removed 16 h later, because abalone are assumed to show minimal daytime feeding activity (Barkai and Griffiths 1987, Uki and Watanabe 1992, Mgaya and Mercer 1994). This period was considered representative of total daily feeding. Feed intake rate assessments began following a 2-week acclimation to the diets; harvested algae were centrifuged (as described above), and a known weight (± 0.01 g) was supplied to each aquarium. The mixed diet consisted of *U. lactuca* and *G. conferta* offered in a wet weight ratio of 3:1. It was assumed that the abalone showed no preferential feeding behavior. Uneaten algae were collected by siphoning aquarium contents through a 1-mm mesh, allowing feces and detritus to be washed out; collected algae were centrifuged and weighed.

A control aquarium, identical to the experimental vessels, but without animals, was supplied with the effluent water of a randomly selected abalone aquarium from each treatment and was stocked with algae corresponding to the dietary treatment being offered. Change in algal wet weight was assessed, as described above, and the mean percentage weight change was calculated for each treatment (three dietary treatments, juveniles and adults, total $n = 6$) and used as a correction factor (C) of the initial weight of algae fed (see in Definitions, below).

On the occasion of each feed intake rate trial, algal samples were taken from each food-stock culture to produce samples of approximately 20 g wet weight per fortnight of each diet. The samples were weighed, freeze-dried, and reweighed (± 0.01 g) to determine water content. The three dried samples of each treatment collected during each 2-week period were combined and stored at −20°C for subsequent analysis of chemical composition.

At the end of each trial, abalone were removed from the experimental vessels, and individual weights and lengths were remeasured; all of the 10 adult abalone and 25 randomly selected juveniles from each vessel were then shucked and freeze dried for subsequent assessment of condition and soft body composition. The juveniles were not tagged. Thus, growth (in weight and length) in each aquarium was estimated by the difference between the average values of the population at the beginning of the experiment and the values of 10 randomly selected juveniles at the end of the experiment.

Analytical Procedures

The freeze-dried samples of algae and bodies of the abalone were homogenized in a mill before being subjected to analyses. Water content was calculated by weight loss after 24-h drying at 105°C. Crude protein was measured using the Kjeldahl technique and multiplying N by 6.25. Crude lipid was measured after chloroform–methanol extraction (Folch et al. 1957). Samples were homogenized with a high-speed homogenizer for 5 min, and lipid was determined gravimetrically after separation and vacuum drying. Crude carbohydrate was determined using the phenol–sulphuric acid method (Dubois et al. 1956) after boiling the sample in 1N H_2SO_4 for 1 h. The resulting color was measured by spectrophotometer against a glucose standard at 490 nm. Ash-free dry weight was calculated from the weight loss after incubation of

samples for 24 h at 550°C in a muffle furnace. Heat of combustion was measured in a Parr bomb calorimeter using benzoic acid as a standard.

Definitions

Net feed intake (I) was determined for each aquarium according to the equation

$$I = (A_{IN} \times C) - A_{OUT}$$

where A_{IN} and A_{OUT} represent, respectively, the measured weights of algae placed in, and removed from an aquarium and C is the treatment-specific correction factor used to compensate for endogenous changes in fresh weight. Daily feed intake was assessed in this way at 3 to 4 day intervals.

$$\text{Specific growth rate (SGR\%, \%/d)} = 100 \cdot (\ln W_1 - \ln W_0)/t$$

(W_0 is the weight of an animal at the beginning of each monitoring interval, and W_t is the weight after t days of growth at the end of the interval);

$$\text{Shell growth } (\mu m/day) = (L_2 - L_1)/t$$

(L_1 is the wet length of an animal at the beginning of each monitoring interval, and L_2 is the length at the end of the interval);

$$\text{Condition index (CI)} = \text{soft flesh (g wet)/shell (g wet)}$$

$$\text{Feed intake rate (mg algae/g abalone/day)} = \text{I/abalone standing stock}$$

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

$$\text{Protein productive value (PPV)} = 100 \cdot \text{protein gain (g)/protein consumed (g)}$$

$$\text{Energy productive value (EPV)} = 100 \cdot \text{energy gain/gross energy consumed}$$

Statistical Analyses

The responses of each abalone species and varied sizes to the various diets were analyzed separately. Growth and condition parameters examining the response to the treatments of individual animals were compared by analysis of variance (ANOVA) (Sokal and Rohlf 1995) and Duncan multiple range test. Feed intake and FCR parameters were analyzed for entire aquarium populations using *t*-test. All analyses were carried out with SPSS software.

RESULTS

Algal Production and Composition

By the second week of culture, *Ulva lactuca* production had stabilized in all low-N and high-N cultures. From November 1995 to the end of feeding trials in May 1996, low-N cultures yielded a mean of 164 ± 6 g (SE) fresh *U. lactuca* m^{-2} d^{-1} (n = 57). High-N cultures yielded 105 ± 7 g m^{-2} d^{-1} (n = 38) and showed evidence of "perforation disease," described by Colorni (1989). High-N thalli were also considerably darker than low-N, but morphologically similar, flat, and sheet-like.

Nitrogen (expressed as crude protein), energy, water, and ash content all remained stable in the algal samples collected during the feeding trials. Water content was similar in all three diets, but the ash content of high-N *U. lactuca* was 30–35% lower than that of the low-N or mixed diet (Table 1). The mixed and high-N diets had similar calorific values, but the mixed diet had 25% less crude protein. Both the energy and the nitrogen content of low-N *U. lactuca* were low as compared to the other two diets. Samples of high-N *U. lactuca* placed in nonenriched seawater (data not shown) lost approximately 18% of their tissue N in 24 h, falling from 5.8 ± 0.08% N in dry tissue to a stable 4.8 ± 0.04% N (n = 3); whereas, low-N tissue nitrogen remained constant over 48 h.

Abalone Feed Intake and Performance

Juvenile Abalone

After 15.5 weeks, mean growth rate of juvenile *Haliotis tuberculata,* expressed in terms of SGR% or shell length increment, was significantly higher in aquaria receiving high-N diet as compared to those fed low-N diet (Table 2). Growth in the single aquarium that continued to receive a standard mixed diet was significantly faster than in the monospecific *Ulva lactuca* treatments. It was also noted that new shell growth during the feeding trials appeared light green in juvenile *H. tuberculata* fed low-N *U. lactuca,* in contrast to the characteristic red-brown shell increments of those animals fed high-N or mixed diets. Voluntary feed intake as apparent daily intake (mg alga per g of abalone biomass) also showed significant differences between the two *U. lactuca* treatments, with the feed intake rate of high-N *U. lactuca* being 68% that of low-N. The combined effects of relatively low feed intake rates and fast growth of juvenile *H. tubeculata* fed high-N *U. lactuca* resulted in a significantly lower (i.e., more efficient) FCR in the high-N treatment.

Although the growth and feed intake rates in juvenile *Haliotis discus hannai* after 16 weeks (Table 3) were considerably lower than in juvenile *H. tuberculata* (Table 2), similar patterns were apparent for the two species: overall feed intake rates of high-N algae were significantly lower ($p < .001$) than for low-N, and use of high-N or mixed diets resulted in significantly higher ($p < .01$) SGR% than with low-N *U. lactuca.* However, only the mixed diet treatment sustained significantly greater shell length growth, when compared to low-N, in *H. discus hannai.* Feeding high-N diet to juvenile *H. discus hannai* also resulted in a significantly more efficient ($p < .001$) FCR. However, only the mixed diet produced a significant effect on the condition (wet flesh: wet shell weight, $p < .001$) of juveniles of both species (Tables 2, 3), as compared to the high-N and low-N treatments. The loss of condition in juvenile *H. discus hannai* fed low-N seaweed was clearly apparent during the experiment as a progressive atrophying ("withering") of the foot muscle; two of the most reduced individuals in one replicate eventually died, these representing the only mortalities during the course of the experiment.

TABLE 1.

Composition of the three experimental diets, low N-*Ulva,* high-N *Ulva,* and the control diet Intermediate N-*Ulva* and *Gracilaria* in relation of 3:1 (w/w).

	Low N-*Ulva*	High N-*Ulva*	Control
Dry matter	13.41 ± 2.37	16.86 ± 1.79	16.69 ± 2.72
Crude protein	1.66 ± 0.29	7.38 ± 1.02	5.54 ± 1.09
Carbohydrate	3.62 ± 0.67	4.05 ± 0.51	3.81 ± 0.87
Lipid	0.14 ± 0.03	0.13 ± 0.03	0.14 ± 0.04
Ash	4.49 ± 0.86	2.91 ± 0.31	4.23 ± 0.91
Gross energy (kJ/g)	1.60 ± 0.29	2.63 ± 0.40	2.43 ± 0.43

Average values during the whole experimental period are given and components are expressed as % of fresh weight (±SD).

TABLE 2.

Growth of juvenile *Haliotis tuberculata* fed three algal diets (108 days).

	Low-protein *Ulva*	High-protein *Ulva*	Control
Initial wt (g)	1.74 ± 0.258	1.57 ± 0.176	1.71 ± 0.236
Final wt (g)	3.81[a] ± 0.880	4.79[b] ± 1.678	6.50[c] ± 1.805
SGR%[1]	0.725[a] ± 0.216	1.028[b] ± 0.288	1.234[c] ± 0.164
Shell growth (μm/day)	80.72[a] ± 20.38	121.47[b] ± 33.9	160.30[c] ± 35.8
Feed intake (mg algae/g abalone/day)	127.53[a] ± 11.07	86.19[b] ± 0.77	98.60
FCR[2]	18.17[a] ± 1.30	7.81[b] ± 0.794	7.70
Condition index[3]	0.586[a] ± 0.073	0.570[a] ± 0.077	0.682[b] ± 0.088
PPV (p)[4]	24.81[a] ± 2.69	15.06[b] ± 1.31	22.71
EPV (%)[5]	11.17[a] ± 1.34	13.38[a] ± 1.11	15.58

Average value ± SD.

[1] Specific growth rate = $(\ln W_2 - \ln W_1)$/days*100.

[2] Food conversion ratio = feed intake (g wet)/weight gain (g wet).

[3] Soft flesh (g wet)/shell (g wet).

[4] Protein productive value = protein gain (g)/crude protein consumed (g) *100.

[5] Energy productive value = energy gain (kJ)/gross energy consumed (kJ) *100.

[a,b,c] Values with the same superscript are not significantly different ($p < .05$) using ANOVA and Duncan multiple range test (SPSS).

TABLE 3.

Growth of juvenile *Haliotis discus hannai* fed three algal diets (112 days).

	Low-protein *Ulva*	High-protein *Ulva*	Control
Initial wt (g)	1.205 ± 0.148	1.172 ± 0.013	1.132 ± 0.082
Final wt (g)	1.680[a] ± 0.601	2.367[b] ± 0.650	2.571[b] ± 0.785
SGR%[1]	0.296[a] ± 0.201	0.627[b] ± 0.267	0.732[b] ± 0.262
Shell growth (μm/day)	31.70[a] ± 22.75	44.47[a,b] ± 25.61	54.93[b] ± 21.30
Feed intake (mg algae/g abalone/day)	85.0[a] ± 18.22	32.97[b] ± 4.6	44.23
FCR[2]	31.50[a] ± 5.83	5.54[b] ± 1.12	5.93
Condition index[3]	0.42[a] ± 0.069	0.45[a,b] ± 0.070	0.48[b] ± 0.08
PPV (%)[4]	9.15[a] ± 7.08	20.4[a] ± 4.93	22.32
EPV (%)[5]	5.99[a] ± 1.21	19.59[b] ± 1.28	17.70

Average value ± SD.

[1] Specific growth rate = $(\ln W_2 - \ln W_1)$/days*100.

[2] Food conversion ratio = feed intake (g wet)/weight gain (g wet).

[3] Soft flesh (g dry)/shell (g dry).

[4] Protein productive value = protein gain (g)/crude protein consumed (g) *100.

[5] Energy productive value = energy gain (kJ)/gross energy consumed (kJ) *100.

[a,b] Values with the same superscript are not significantly different ($p < .05$) using ANOVA and Duncan multiple range test (SPSS).

Juvenile *Haliotis tuberculata* fed low-N seaweed made more efficient use of the ingested protein (PPV) compared to individuals receiving high-N treatment (Table 2). The reverse was observed in juvenile *H. discus hannai,* where significantly less of the protein ingested as low-N diet was incoporated into abalone tissue, as compared to high-N diet (Table 3). On the other hand, the juveniles of both species utilized energy (EPV) better in the high-N diet, but only in *H. discus hannai* was this trend statistically significant.

Adult Abalone

Adults of both *Haliotis tuberculata* and *H. discus hannai* grew significantly better when fed with the mixed and high-N diets than with the low-N diet (Tables 4, 5, respectively). Adults of both species also voluntarily ate significantly more low-N *Ulva lactuca,* as compared with the high-N seaweed. As a mathematical consequence of these two observations, the FCRs for both species fed high-N *U. lactuca* were significantly and strikingly better (lower) than in the animals fed the low-N *U. lactuca.* In *H. tuberculata* the reduction in FCR by feeding high-N seaweed was by 64% and in *H. discus hannai* by 77% (Tables 4, 5).

DISCUSSION

Performance in Response to Dietary Treatment

Culturing *Ulva lactuca* at high- and low-ammonia fluxes yielded thalli that were of considerably different nutritional value to both abalone species. The animals fed N-enriched seaweed subsequently grew significantly faster, while consuming significantly less seaweed than the animals fed N-deprived seaweed. The above observations agree with the broad principles of herbivorous grazing. Feed intake rate is the main compensatory mechanism for diet quality in herbivores (Bowen et al. 1995), including abalone (Koike et al. 1979, Mgaya and Mercer 1994). Nevertheless, when fed low-quality food, a herbivore feeding to capacity may still be undernourished (White 1978). Bowen et al. (1995) and Britz (1996) suggested that dietary energy content also regulates abalone feed intake rate. In the present study, differences in feed intake rates of the abalone juveniles correlate numerically more closely with differences in energy content of *Ulva lactuca* than with its N content. Low-N and high-N feed intake rates are separated by a factor of 1.5 in juvenile *H. tuberculata* and 2.6 in *H. discus hannai*; high-N *U. lactuca* has 1.6 × energy and 4.4 × N of the low-N *U. lactuca.* Therefore, at least the *H. tuberculata* juveniles eating both diets had about the same energy intakes but very different N intakes. These results suggest that in addition to crude protein content, different energy contents in the *U. lactuca* diets may contribute to the observed differences in feed intake. To corroborate this explanation, it would be necessary to assess the gut capacity of the abalone to determine whether the animals were simply feeding to capacity.

Abalone somatic growth is considered to depend upon the amount of crude dietary protein (Uki and Watanabe 1992, Fleming 1995b, Mai et al. 1995, Britz 1996). Maximum abalone growth

TABLE 4.

Growth of adult *Haliotis tuberculata* fed three algal diets (106 days).

	Low-protein *Ulva*	High-protein *Ulva*	Control
Initial wt (g)	12.56 ± 3.17	12.26 ± 4.49	12.34 ± 3.73
Final wt (g)	15.47[a] ± 3.52	16.05[a] ± 5.15	18.27[a] ± 5.64
SGR%[1]	0.202[a] ± 0.107	0.271[a,b] ± 0.193	0.371[b] ± 0.154
Shell growth (μm/day)	43.95[a] ± 16.33	53.77[a] ± 23.09	59.88[a] ± 23.06
Feed intake (mg algae/g abalone/day)	77.0[a] ± 2.61	36.16[b] ± 0.94	36.15
FCR[2]	39.18[a] ± 1.7	14.20[b] ± 3.96	9.71
Condition index[3]	0.54[a] ± 0.106	0.51[a] ± 0.084	0.52[a] ± 0.102
PPV (%)[4]	−2.0[a] ± 9.87	2.32[a] ± 0.24	7.79
EPV (%)[5]	2.85[a] ± 1.91	1.91[a] ± 1.94	4.59

Average value ± SD.

[1] Specific growth rate = $(\ln W_2 - \ln W_1)$/days*100.

[2] Food conversion ratio = feed intake (g wet)/weight gain (g wet).

[3] Soft flesh (g dry)/shell (g dry).

[4] Protein productive value = protein gain (g)/crude protein consumed (g) *100.

[5] Energy productive value = energy gain (kJ)/gross energy consumed (kJ) *100.

[a,b] Values with the same superscript are not significantly different ($p < .05$) using ANOVA and Duncan multiple range test (SPSS).

TABLE 5.

Growth of adult *Haliotis discus hannai* fed three algal diets (106 days).

	Low-protein *Ulva*	High-protein *Ulva*	Control
Initial wt (g)	7.81 ± 2.22	8.06 ± 3.53	7.98 ± 3.94
Final wt (g)	9.16[a] ± 3.05	10.47[a] ± 3.98	11.24[a] ± 5.71
SGR%[1]	0.143[a] ± 0.109	0.264[b] ± 0.163	0.337[b] ± 0.227
Shell growth (μm/day)	17.68[a] ± 12.61	29.13[b] ± 18.14	43.18[c] ± 24.3
Feed intake (mg algae/g abalone/day)	54.15[a] ± 4.17	20.82[b] ± 2.32	19.01
FCR[2]	36.2[a] ± 6.76	8.4[b] ± 1.24	5.8
Condition index[3]	0.43[a] ± 0.086	0.46[a] ± 0.15	0.50[a] ± 0.104
PPV (%)[4]	−3.53[a] ± 7.21	9.58[a] ± 6.66	24.84
EPV (%)[5]	−0.77[a] ± 3.34	4.19[a] ± 2.85	14.27

Average value ± SD.

[1] Specific growth rate = $(\ln W_2 - \ln W_1)$/days*100.

[2] Food conversion ratio = feed intake (g wet)/weight gain (g wet).

[3] Soft flesh (g dry)/shell (g dry).

[4] Protein productive value = protein gain (g)/crude protein consumed (g) *100.

[5] Energy productive value = energy gain (kJ)/gross energy consumed (kJ) *100.

[a,b] Values with the same superscript are not significantly different ($p < .05$) using ANOVA and Duncan multiple range test (SPSS).

was typically attained from diets of 35% protein (by dry weight; see in Uki 1989, Uki and Watanabe 1992, Mai et al. 1995, Britz 1996). These findings of previous researchers are corroborated in the present study by the rapid growth and efficient energy use (EPV) of abalone juveniles fed high-N (44% protein) *Ulva lactuca,* as compared to the apparent N-limitation in low-N (12% protein) fed abalone. The substantially more efficient food conversion ratios with high-N diet as compared with low-N diet in juveniles and adults of both species are typical of herbivorous nutrition (Mattson 1980). This has been shown to apply to juvenile *Haliotis discus hannai* with dietary crude protein up to 28–30% of dry matter (Uki 1989, Uki and Watanabe 1992) and to at least 47% for *H. midae* (Britz 1996). Britz (1996) and Uki and Watanabe (1992) found that the efficiency with which ingested protein was utilized for growth (PPV) in abalone increased with decreasing dietary protein level, as corroborated here by the significantly higher PPV of juvenile *H. tuberculata* fed low-N *U. lactuca.* The reverse is seen in juvenile *H. discus hannai.* It is suggested that the loss of condition of low-N fed *H. discus hannai*; that is, reduction in the soft tissue fraction of total dry weight, resulting in low (occasionally negative) levels of protein utilization, has caused this anomaly.

Growth rates of *Haliotis discus hannai* in the present study were lower than in *H. tuberculata* receiving the same diets. Faster *H. discus hannai* growth has been recorded elsewhere (e.g., Uki 1989 reports shell growth of up to 270 μm d^{-1} for young juvenile *H. discus hannai* grown at similar temperatures to those used here). However, this study concurs with the results of Mercer et al. (1993), who recorded *H. discus hannai* growth to be consistently inferior to that of *H. tuberculata* in comparative feeding experiments. They also found, as we have, that for optimal growth *H. discus hannai* required higher diet protein content than *H. tuberculata.*

Value of Ulva lactuca as a Dietary Alga for Abalone

The adults of both abalone species showed similar feeding behavior, with mean feed intake of high-N being less than half that of low-N *Ulva lactuca.* Food conversion ratios of *Haliotis discus hannai* adults resemble those found for juveniles, becoming more efficient in hign-N fed animals. Spawning activity was occasionally observed in adult *H. tuberculata* vessels, affecting individual feeding rates (as in Mgaya and Mercer 1994), as well as flesh weight and shell deposition (as in Mercer et al. 1993), and causing a negative protein utilization at the low-N treatment.

It seems that if high-N and low-N *Ulva lactuca* were offered as diets to abalone throughout the growout period, the high-N diet would sustain significantly faster growth in both species. Using high-N *Ulva,* both species can sustain high mean growth rates with less than half the food intake. However, previous studies have implied that the extrapolation of short-term abalone feeding trials may be unrepresentative. Day and Fleming (1992) found that abalone fed monospecific algal diets stopped growing after 50 to 200 days. They suggested it was unlikely that a single algal species could supply all essential nutrients.

The performance of juvenile abalone fed the mixed diet suggests that using only gross N and energy measurements to assess

the value of an algal diet has its limits. The values of gross N and energy in the mixed diet were intermediate between those of low-N and high-N *Ulva lactuca*, but abalone performance with the mixed diet was usually superior to that produced even by high-N diet. Problems also arise when using only energy and crude protein to assess relative nutritional values of a single algal species. In the current study, the N and energy content of low-N and high-N *U. lactuca* at the time of stocking to the abalone vessels is used as an indication of dietary value for the animals. These parameters may not be representative of the relative amounts that are ultimately available to the abalone. *Ulva* sp. in a rich medium where N does not limit growth will carry out luxury N uptake, storing excess N in intracellular pools of organic N, predominantly amino acids, and NH_4^+ (Fujita et al. 1988, Lundberg et al. 1989). Pedersén (1994) notes that if *Ulva* spp. is transferred from an N-rich medium to an N-starved medium, there is a fall in tissue N, as observed in the present study when *U. lactuca* is stocked to the abalone vessels. The NH_4^+ pool is highly soluble and considered physiologically impossible to maintain if external concentrations fall (Fujita et al. 1988). The release of intracellular NH_4^+ by *U. lactuca* has been recorded by Vandermeulen and Gordin (1990) and is likely to be the cause of decline in high-N nitrogen content found in the current study.

The quality of the digestible protein, expressed in terms of amino acid composition, may also vary between low-N and high-N *Ulva lactuca*. The amino acid profile of an alga may vary, depending upon the level of N available (Lignell and Pedersén 1987, Miyashita and Miyazaki 1993), hence affecting the dietary value of the alga for abalone (Mai et al. 1995). Other components of the algal composition might affect its nutritional value and vary according to the seaweed's nutrient status. For example, N-starved *Ulva* spp. has increased levels of high energy soluble carbohydrates (cf. DeBusk et al. 1986); whereas, Mercer et al. (1993) noted considerable variation in total lipid and carbohydrate levels in wild gathered *U. lactuca* used as an experimental abalone diet.

Morphologically simple, fast-growing opportunistic seaweeds that lack specific chemical defenses, such as *Ulva* spp., *Enteromorpha* spp., and *Porphyra* spp., are considered to be the most palatable taxa for abalone (Stuart and Brown 1994, Fleming 1995a). Such algae also tend to show the greatest range in tissue-N levels in field-gathered specimens and in culture (Kudoh 1987, Björnsäter and Wheeler 1990, Wheeler and Björnsäter 1992). Considerable intraspecific variations in tissue N have also been noted in other macroalgal species commonly fed to abalone, such as *Gracilaria* spp. (Friedlander et al. 1987, Lignell and Pedersén 1987, Jones et al. 1996).

The demonstration that abalone performance when fed a monospecific diet of *Ulva lactuca* can vary considerably, depending upon the nutrient status of the alga, explains conflicting conclusions reached in other reports considering the nutritional value of *Ulva* spp. Pickering (1990, reviewed in Stuart and Brown 1994) found differences in abalone growth when fed different ecotypes of *Gracilaria sordida*. However, other researchers have tended to consider only interspecific differences between macroalgae offered as diets for abalone (e.g., Day and Fleming 1992, Shepherd and Steinberg 1992, Mercer et al. 1993, Marsden and Williams 1996). The documented relative dietary value of *U. lactuca*, as compared to other seaweeds, provides a useful indication of the importance of considering the alga's nutritional status: *Ulva* spp. has been considered a good (Uki 1989) and preferred food spcies for *Haliotis discus hannai* (Shepherd and Steinberg 1992); whereas, Mercer et al. (1993) noted *H. discus hannai* growth rates when fed their *Ulva* sp. (13% crude protein) to be significantly lower than for any other algae tested; results using *H. tuberculata* tend to be in closer agreement with the present study, the seaweed being considered a preferred diet (Shepherd and Steinberg 1992) of intermediate nutritional value (Koike et al. 1979, Mercer et al. 1993). In other abalone species, Tenore (1976) found good growth performance for juvenile *H. discus* and *H. rufescens* fed biofilter grown *Ulva* sp. (30% protein); whereas, *Ulva* sp. (13.2% protein) was the only diet tested by Stuart and Brown (1994) that produced no significant growth in juvenile *H. iris*.

Implications for Commercial Abalone Production and Future Research

In nature, macrophyte development tends to be N-limited (Lignell and Pedersén 1987, Duke et al. 1989a); therefore, protein-N availability is suggested as being the main factor limiting field abalone growth (Fleming 1995b). The protein component of artificial diets represents the most costly ingredient of feeds (Mai et al. 1995) that are often prohibitively expensive (Fleming and Hone 1996). The culture of macrophytes in ammonia-enriched seawater, either by chemical supplement or by mariculture effluents, seems to be a logical procedure for removing N-limitation from the food chain. Our results show that enrichment of N content in *U. lactuca* in this way significantly improves all indices of growth and feed use in juvenile and adult abalone.

The present study suggests a considerable scope for modification of the nutritional value of the algal species commonly used as fresh diets for abalone and also a need for caution when considering interspecific differences between seaweeds without examining intraspecific variations in composition. Seasonal variability of wild seaweed populations with respect to protein content is proposed as critical for farmers harvesting natural stocks, in the selection of optimum sites for stock enhancement projects, and as an ecological tool to help elucidate the factors governing the food selection and population dynamics of abalone. There remains a need to investigate the long-term affects of algae cultured at a range of nutrient enrichment levels. It is also necessary to determine the nutritional requirements of developing abalone, particularly at the critical stages of weaning, rapid juvenile growth, and sexual development in adults.

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