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Suitability of two seaweeds, *Gracilaria lemaneiformis* and *Sargassum pallidum*, as feed for the abalone *Haliotis discus hannai Ino*

Zhanhui Qi [a,b], Hongmei Liu [c], Bin Li [b], Yuze Mao [b], Zengjie Jiang [b], Jihong Zhang [b], Jianguang Fang [b,*]

[a] South China Sea Fisheries Research Institute, Chinese Academy of Fisheries Sciences, Guangzhou 510300, China
[b] Yellow Sea Fisheries Research Institute, Chinese Academy of Fisheries Sciences, Qingdao 266071, China
[c] Yantai Institute of Coastal Zone Research Chinese Academy of Sciences, Yantai 264003, China

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ABSTRACT

The suitability of two algae species, *Gracilaria lemaneiformis* and *Sargassum pallidum*, for use as food sources for the abalone *Haliotis discus hannai Ino* was evaluated. Abalones were fed one of five experimental diets: 1) kelp *Laminari japonica*; 2) *G. lemaneiformis*; 3) *S. pallidum*; 4) a mixed diet of *L. japonica* and *G. lemaneiformis* (1:1); and 5) a mixed diet of *L. japonica* and *S. pallidum* (1:1) for a period of 4 months. The survival, growth (shell length and body weight), condition index (body weight/shell length), and feed utilization were measured. Survival was excellent (100%) in all groups. Growth rate (body weight) was highest in the abalone fed kelp exclusively, followed by the mixed diet of kelp and *G. lemaneiformis*. The abalone fed *S. pallidum* had the lowest increase in body weight and the lowest feed intake. The mixed diet of kelp and *G. lemaneiformis* yielded the biggest increase in shell length. There were no significant differences in the condition index among all the treatment groups. The mean daily feed intake of *L. japonica* was highest, followed by the mixed diets of kelp and *G. lemaneiformis*, and was lowest for *S. pallidum*. Abalone exhibited a preference for *L. japonica*. The feed conversion efficiency was highest for *G. lemaneiformis*. Results suggest that *G. lemaneiformis* can be used as a partial substitute for kelp in the diet of cultured abalone.

1. Introduction

Abalone is one of the most highly valued seafoods throughout the world. Until recently, they were harvested exclusively from the wild. Because of high demand, the wild populations in many regions have been over-exploited, resulting in serious declines in natural production (Guzmán del Proó, 1992; Parker et al., 1992; Shpigel et al., 1999; Tegner et al., 1992). Decreased wild catches combined with increasing global demand for abalone have resulted in market opportunities for cultured abalone (Oakes and Ponte, 1996). This has accelerated the development of intensive abalone aquaculture (Coote et al., 1996; Gordon and Cook, 2001; Shpigel et al., 1999).

Abalones are cultured extensively in China. In north China, particularly the Shandong and Liaoning provinces, *Haliotis discus hannai* is the most commonly cultured species. The successful culture of this species depends on proper nutrition and rapid growth. Developing a sustainable, cost-effective food supply for the long grow-out period is one of the key problems facing the abalone culture industry (Shpigel et al., 1999).

Artificial diets are generally expensive and their use is often not feasible (e.g., rapid leaching) for the ocean based long-line culture of abalone. Because of this, formulated diets are only used during the nursery stage, when the juvenile abalones are reared in land-based hatchery facilities. During the ocean-rearing period, abalones are primarily fed seaweed. A variety of seaweeds, including *Ecklonia maxima*, *Laminaria japonica*, *Ulva rigida*, *Carpoblepharis flaccida*, *Gracilaria gracilis*, *Ulva lactuca* are used for abalone culture (Alcantara and Noro, 2006; Demetropoulos and Langdon, 2004; Mai et al., 1996; Naidoo et al., 2006; Nie and Yan, 1985; Taylor and Tsvetnenko, 2004; Troell et al., 2006).

In north China, the majority of *H. discus hannai* is cultured on suspended long lines, and are often co-cultured with kelp (*L. japonica*). In this region, *L. japonica* is the most common, and often the only, food source for abalone. Sanggou Bay, located on the eastern side of the Shandong Peninsula, is one of the largest mariculture sites along the northern China coast (Guo et al., 1999). The entire bay is used for the culture of invertebrates and microalgae (Fang et al., 1996). In recent years, the culture of *H. discus hannai* has expanded rapidly in the bay, mostly co-cultured with kelp on long lines that are suspended in the water column.

Abalone can consume up to 35% of their body weight per day of seaweed (Tahil and Juinio-Menez, 1999). Typically, it takes about 3 years for *H. discus hannai* to reach commercial size in north China. Because of the long culturing period and high feeding rate, feed consumption and costs are considerable. In the last decade, the price of kelp has more than doubled, from 0.5 to 1.2 RMB/kg (based on fresh

* Corresponding author. Tel.: +86 532 85822957.
E-mail addresses: qizhanhui006@163.com (Z. Qi), Fangjg@ysfri.ac.cn (J. Fang).

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weight). Concurrently, the price of abalone has decreased from 300 RMB/kg in the late 1990s to 160 RMB/kg (based on fresh weight). The decrease in price is due to improvements in culture technology and increased production. The increase in food costs has led to a decline in net profit for abalone farmers. Given this, there is considerable demand for developing alternative, cheaper food sources.

A number of studies have evaluated the feasibility of using other red macroalgae, including *G. gracilis*, *Gracilaria cornea*, and *Gracilaria tenuistipitata* (Li et al., 2007; Naidoo et al., 2006; Viera et al., 2005). Different diets result in differential survival (Daume et al., 2003) and growth rate (Bautista-Teruel et al., 2003; Boarder and Shpigel, 2001; Britz, 1996; Guzmán and Viana, 1998; Leighton, 1974; Shpigel et al., 1999). Furthermore, the rate of growth is highly variable among species, even when fed identical diets. This is likely due to differences in nutrition requirements (Taylor and Tsvetnenko, 2004). Negative growth may even occur if the diet does not provide adequate nutrition.

Wild *Gracilaria lemaneiformis* and *Sargassum pallidum* often grow abundantly on structures that are used for kelp cultivation. *Gracilaria lemaneiformis* is also commercially cultured during the summer and autumn seasons in north China (Yang et al., 2005). The cost of abalone production may be lowered by using these seaweeds as a substitute (full or partial) for kelp. However, there is no information on the suitability of *G. lemaneiformis* and *S. pallidum* as feed for abalone.

The objective of this study was to evaluate growth and feed utilization of *H. discus hannai* fed *G. lemaneiformis* and *S. pallidum*, aimed to decrease the production costs.

2. Materials and methods

2.1. Experimental area

The study was conducted between May 20 and September 20, 2008, in Sanggou Bay, a 140-km^2 coastal embayment in north China (37°01′–37°09′N, 122°24′–122°35′E). Water exchange between the bay and the Yellow Sea is driven by semi-diurnal tidal exchange (tidal range 2 m). The average depth of the bay is 8.0 m. The experimental farm is located in a polyculture area of kelp and abalone. During the experiment, the water temperature ranged from 10.4 to 22.8 °C.

2.2. Experimental diets

The effects of three seaweeds *L. japonica*, *G. lemaneiformis*, *S. pallidum* on abalone were evaluated. The abalone were fed one of five experimental diets consisting of: 1) *L. japonica*, 2) *G. lemaneiformis*, 3) *S. pallidum*, 4) a mixture of *L. japonica* and *G. lemaneiformis*, and 5) a mixture of *L. japonica* and *S. pallidum* (D_L, D_G, D_S, D_{L+G}, and D_{L+S}, respectively). The two algae were combined at a ratio of 1:1 in the mixed diets. All the algae were collected fresh from suspended long lines.

2.3. Experimental animals and culturing condition

Hatchery-reared abalone (≈2 years old) that had an initial shell length (SL) of 75.78 ± 2.62 mm and an initial body weight (BW) of 62.31 ± 2.68 g were used in the experiment. The abalone were cultured in cylindrical plastic cages (diameter: 40 cm, height: 50 cm, N = 20 per cage). The abalone were distributed randomly among 15 cultivation cages. The cages were aligned in a single row and were suspended from long lines at a depth of 4.5 m.

2.4. Experimental procedures

The cages were identified using a numbered (1–15) tag. The cages were randomly assigned one of the five diet treatments to each cage (n = 3 cages per treatment). The abalone were acclimated for 1 month (April to May) to minimize the effects of switching diets. During the acclimation period, abalone from each treatment group were fed one of the five diets at a satiation level. BW and SL of each abalone were weighed and measured at the end of the acclimation period. There was no significant difference in SL and BW among the groups at the end of acclimation (P>0.05).

The experiment was carried out between May 20 and September 20, 2008. The abalone were fed with the appropriate diet once every 5 days. The wet weight of algae prior to feeding wase recorded and any uneaten algae were collected and weighed. Prior to weighing the excess water was removed by blotting with tissue paper. For the mixed diets, the two algae species were weighed separately. Consumption for each species of seaweed was calculated.

At monthly intervals, all the abalone cages were briefly brought to the surface for cleaning. All fouling organisms on the cages and the shells of the abalone were removed. BW and SL of all the abalone at the end of the experiment were measured.

2.5. Calculations and statistical analysis

The following variables were calculated:

Daily increase in body weight (DIBW) = $(W_t - W_0)/t$
Daily increase in shell length (DISL) = $(L_t - L_0)/t$
Feeding rate (FR % body weight day^{-1}) = $100 \times D/[t \times N \times (W_t + W_0)/2]$
Mean total feed intake (MTFI) = D/N
Feed conversion efficiency (FCE: %) = $100 \times (W_t - W_0) \times N/D$
Condition index (CI) = W_t/L_t

Where n: number of abalone in each cultivation tank, W_0: mean initial body weight, W_t: mean final body weight, L_0: mean initial shell length, L_t: mean shell final length, t: experimental duration (d), D: diet intake (g).

Differences in DIBW, DISL, FR, CI, FCE and MTFI among the treatment groups were analyzed using one-way analysis of variance (ANOVA). When overall differences were significant at the 0.05 level, Duncan's multiple range test was used to compare the mean values of individual groups. Data are reported as the mean ± standard deviation (S.D.). All statistical analysis was performed using Statistica 6.0 (Statsoft, Tulsa, OK, USA).

3. Results

3.1. Survival, growth performance and condition index

The survival rate was 100% for all treatment groups (Table 1). The growth rate (DIBW) of the abalone fed *L. japonica* was significantly higher than the other treatment groups, except D_{L+G}. The body weight of abalone fed *S. pallidum* was significantly lower than the other groups (P<0.05) (Table 1).

Abalone that were fed a mixed diet of *L. japonica* and *G. lemaneiformis* had the highest DISL (26.25 μm day^{-1}). This was significantly

Table 1
Mean body weight, daily increase in body weight (DIBW), and survival of *H. discus hannai* fed one of five experimental diets over a 4-month period.

Diet treatment	Mean initial body weight (g)	Mean final body weight (g)	DIBW (g·day^{-1})	Survival (%)
D_L	64.19 ± 3.21	76.82 ± 8.86^a	0.105 ± 0.005^a	100
D_G	66.02 ± 4.40	76.00 ± 9.04^a	0.083 ± 0.002^b	100
D_S	65.60 ± 2.11	74.94 ± 8.49^{bc}	0.078 ± 0.009^{bc}	100
D_{L+G}	63.94 ± 5.09	75.62 ± 7.32^{ab}	0.097 ± 0.002^a	100
D_{L+S}	64.20 ± 4.67	73.93 ± 7.82^c	0.081 ± 0.006^b	100

Values represent the mean of triplicate groups. Values in the same row with the same letters are not significantly different (P>0.05, Duncan's test).

higher than the remaining treatment groups ($P<0.05$) which all had similar DISL values (Table 2). The abalone that were fed *S. pallidum* had the lowest DIBW and DISL values.

There was no significant difference in the CI among all the groups ($P>0.05$) (Table 2).

3.2. Feed utilization

The feeding rate (FR) was highest in abalone fed *L. japonica*, followed by the mixed diets D_{L+G} and D_{L+S}. These were significantly higher than in those fed D_G and D_S ($P<0.05$). The FR was lowest in those fed D_G. Among the mixed diets, the abalone consumed less *G. lemaneiformis* or *S. pallidum* than *L. japonica*.

The feed conversion efficiency (FCE) of abalone fed *G. lemaneiformis* (2.79 ± 0.33%) was significantly higher than that of the remaining groups ($P<0.05$). The FCE of abalone fed *L. japonica* (2.29 ± 0.21%) was low and was only higher than the combination of *L. japonica* and *S. pallidum*.

4. Discussion

The experiment was carried out between May and September. During this period the water temperature in Sanggou Bay is ideal for the growth of *H. discus hannai*. Both feed intake and growth rate peak during this time.

The food preferences of abalone are species specific. The primary determinants appear to be habitat and food availability (Barkai and Griffiths, 1986; Dunstan et al., 1996). We evaluated the effect of feeding three species of seaweed. The feed intake of kelp was higher than *G. lemaneiformis* and *S. pallidum* in both single-species diets and in the mixed diets. This indicates a dietary preference for kelp in *H. discus hannai*. Our results are generally consistent with the findings of previous studies that show large juveniles and adults of *H. discus hannai* prefer to feed on brown Laminariales macroalgae (Kikuchi et al., 1967; Nie and Yan, 1985; Sakai, 1962; Uki, 1981). There are several possible explanations for this preference: 1) cultured abalone have been fed a diet of kelp for many generations. Furthermore, the abalone used in this experiment were fed kelp for a period of 2 years prior to the experiment. Even though the abalone were fed their respective diets for 1 month prior to the experiment, it is possible that a longer acclimation period may alter the dietary preferences. 2) The nutritive value of the different species of seaweeds may play a role in the preference. *Laminari japonica* contains more water (12.37%) than *G. lemaneiformis* (7.54%) (Qi et al., data unpublished). *Laminari japonica* also contains less protein (10.19% based on dry weight) than *G. lemaneiformis* (26.06%) (Liu et al., 2005; Zhou et al., 2002). Abalone may compensate for the lower nutritional value by increasing their feeding rate (Hahn, 1989). In our experiment, the abalone were able to compensate for a low FCE by increasing their feed intake. Thus, the abalone fed *L. japonica* had the highest DIBW (105.21 ± 0.54 mg day^{-1}).

Abalone feed using a rasp-like radula. The effectiveness of the radula is strongly influenced by the form and toughness of the food. The morphological structure of the radula allows *H. discus hannai* to graze the blades of *L. japonica* more effectively than the filamentous seaweeds *G. lemaneiformis* and *S. pallidum* (Kawamura et al., 2001; Nie and Yan, 1985). The low feed intake of *S. pallidum* may be caused by the toughness of the stipe. During the experiment, only the tender leaves were eaten, while the stipes were discarded. The poor growth of abalone fed *S. pallidum* was caused primarily by their lower feed intake (Table 3).

Like other herbivores, abalone are prone to nitrogen limitation (Mattson, 1980; White, 1978). This occurs because the protein content of plants is relatively low. The intake of digestible protein directly affects the growth rate of abalone (Boarder and Shpigel, 2001; Fleming, 1995; Shpigel et al., 1999). Maximum growth is achieved when the diet contains 28–35% protein (Mai et al., 1995; Uki and Watanabe, 1992). The protein content of the three seaweeds used in this trial was below that level. The nutritive value of the algae may explain the feed utilization profile. The FCE of abalone fed *G. lemaneiformis* was significantly higher than the other diets, most likely because of its nutrition profile (i.e., lower moisture and higher protein content). This explains why the increase in shell length of abalone fed *G. lemaneiformis* was similar to that of abalone fed kelp, even though feed intake was lower.

For commercial culture, it is important that the abalone reach a marketable size within an economically viable time. The DIBW of abalone fed the mixture of *L. japonica* and *G. lemaneiformis* was significantly higher than the other groups, except those that were only fed kelp. Moreover, the DIBL was significantly higher than the other diet treatments. This was consistent with previous studies showing that growth is improved when abalone are fed a combination of different algae compared with those fed a single species (Day and Fleming, 1992; Fleming, 1995; Gordon et al., 2006; Owen et al., 1984; Simpson and Cook, 1998; Stuart and Brown, 1994). Duncan and Klekowski (Duncan and Klekowski, 1975) also noted that essential nutrients may become limiting if the animals are fed single-species diets. Single species of seaweed are unlikely to meet the nutritional requirements of abalone. However, when given as part of a mixed diet, they may supply essential nutrients that are lacking in other algae (Day and Fleming, 1992; Fleming, 1995; Simpson and Cook, 1998). Interestingly, wild abalone typically feed on a variety of algal (Barkai and Griffiths, 1986; Barkai and Griffiths, 1987; Newman, 1968). Together, these data suggest that abalone are likely to perform better when fed more than one species of algae.

Condition factor is an important determinant of the economic value of a diet. No differences in CI existed among the different diet treatments. The CI of abalone fed fresh kelp was 0.96 (±0.13) g mm^{-1}, which is much higher than the values reported by Naidoo et al. (Naidoo et al., 2006). According to Naidoo et al. (Naidoo et al., 2006), the BW/shell length of abalone (*Haliotis midae*) fed fresh kelp (*E. maxima*) was 0.463 g mm^{-1}. The difference among these studies is most likely due to

Table 2
Mean shell length, daily increase in shell length (DISL), and condition index in *H. discus hannai* fed one of five experimental diets over a 4-month period.

Diet treatment	Mean initial shell length (mm)	Mean final shell length (mm)	DISL (mm·day^{-1})	Condition index (g·mm^{-1})
D_L	77.80 ± 2.26	80.45 ± 3.41^a	0.022 ± 0.004^a	0.96 ± 0.13
D_G	77.82 ± 3.25	80.40 ± 2.76^a	0.021 ± 0.006^a	0.95 ± 0.10
D_S	77.62 ± 2.01	78.60 ± 1.92^b	0.018 ± 0.008^b	0.95 ± 0.11
D_{L+G}	76.19 ± 1.22	79.25 ± 1.97^a	0.026 ± 0.003^c	0.95 ± 0.09
D_{L+S}	76.38 ± 4.26	79.05 ± 2.58^a	0.020 ± 0.006^{ab}	0.94 ± 0.14

Values represent the mean of triplicate groups. Values in the same row with the same letters are not significantly different ($P>0.05$, Duncan's test).

Table 3
Feeding rate (FR), feed conversion efficiency (FCE), and mean total feed intake (MTFI) of *H. discus hannai* fed one of five experimental diets over a 4-month period.

Diet treatment	FR (% body weight day^{-1})	FCE (%)	MTFI (g·ind^{-1})
D_L	6.52 ± 0.08^a	2.29 ± 0.21^a	552.50 ± 3.13^a
D_G	4.20 ± 0.14^b	2.79 ± 0.33^b	358.13 ± 4.63^b
D_S	4.44 ± 0.16^b	2.49 ± 0.11^a	374.13 ± 7.50^b
D_{L+G}	6.03 ± 0.10^a	2.31 ± 0.30^a	505.13 ± 18.88^{ac} (*L*:295.63 ± 4.38; *G*: 209.50 ± 14.50)
D_{L+S}	5.85 ± 0.09^a	1.86 ± 0.25^c	484.50 ± 4.50^c (*L*: 295.00 ± 5.00; *S*: 189.50 ± 9.50)

Values represent the mean of triplicate groups. Values in the same row with the same letters are not significantly different ($P>0.05$, Duncan's test).

differences in the species of abalone and kelp, as well as differences in the experimental conditions.

At the end of the experiment, the shell color of *H. discus hannai* fed kelp was green. Conversely, the shell margin (new shell) of abalone fed diets containing *G. lemaneiformis* was purple. Previous studies have also noted that the coloration of abalone shells may be altered by the diet (Leighton, 1961; Olsen, 1968). It appears that the pigment in the red algae *G. lemaneiformis* is deposited into the abalone shell. This trait is desirable as purple shell abalone have a higher market value in north China because of their similarity to wild-caught abalone.

To ensure the results were applicable to commercial culture operations, our rearing conditions and feeding regimes were similar to those used in the majority of commercial enterprises. Based on our results, approximately 43.66 kg of kelp (cost: ≈52.45 RMB) is needed to produce one kilogram of abalone (at a FCE of 2.29%). The price of *G. lemaneiformis* was ≈0.6 RMB in 2008. Based on the feed consumption of *L. japonica* and *G. lemaneiformis* when fed as a mixed diet (Table 3), the total cost to produce 1 kg of abalone would be 41.14 RMB. This represents a decrease of 21.6% in the cost of feed.

In summary, a mixed diet consisting of *L. japonica* and *G. lemaneiformis* significantly increased the growth in shell length and improved feed conversion efficiency. Thus, *G. lemaneiformis* may be used as a partial substitute for kelp during the culture of *H. discus hannai*.

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