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Effect of Feed Type on Abalone (*Haliotis discus hannai*) Growth in Recirculating Aquaculture Systems

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ABSTRACT

Recirculating aquaculture systems (RAS) can improve the production of farmed abalone, but there are issues with using such systems. An important issue is that the health of shellfish reared in such systems is largely determined by feed type. Therefore, it is important to optimize diets to improve the production of abalone. In this study, *Haliotis discus hannai* in a recirculating aquaculture system were fed with three types of dry seaweed *Saccharina japonica*, *Undaria pinnatifida*, and *Pyropia tenera*) together with formulated diet (FD) in five different ratios. The different feed treatments were run for 90 days to examine the effects on abalone growth. The highest growth rate was 12.35%, which was observed for shell length in the group fed *Pyropia tenera*, with 7.01 and 7.14% growth rates in the groups fed *Undaria pinnatifida* and formulated diet, respectively. A 3.36 % growth rate was observed in the group fed *Saccharina japonica* alone. However, the addition of appropriate proportions of seaweed to the mixed diet led to better growth, indicating protection against the nutritional deficiencies of FD. The $CaCO_3$ abalone shell mainly comprises aragonite and calcite. Rearing in RAS and natural seawater resulted in 7:3 and 6:4 aragonite to calcite ratios, respectively, with no feed type-based difference. We, therefore, speculate that shell composition is influenced more by the environment than the feed in that the metastable aragonite crystal structure is converted to a stable calcite crystal structure in natural seawater, where minerals are abundant.

Keywords: Abalone, *Haliotis discus hannai*, Feed type, $CaCO_3$, Recirculating aquaculture system

INTRODUCTION

Abalone is classified into the phylum Mollusca, class Gastropoda, order Archaeogastropoda, family Haliotidae, and genus Haliotis. There are approximately 90 species of abalone globally (Sales and Janssens, 2004), and as of 2020, abalone has been mainly farmed in China, South Korea, South Africa, Chile, and Australia, with the main aquaculture species comprising *Haliotis rufescens* (red abalone), *H. iris* (Paua), *H. midae* (South African abalone), *H. discus hannai* (Pacific abalone), and *H. rubra* (blacklip abalone). The total global production of farmed abalone reached approximately 227,536 t in 2020, with the main producers being China (203,485 t, 89.43%) and South Korea (20,059 t, 8.82%) accounting for approximately 98% of the total production (FAO, 2022). Particularly in South Korea, abalone is popular as a high-end food ingredient and accounts for a large proportion of the shellfish aquaculture species consumed, with the approximately 23,000 ton produced in 2022 valued at nearly $530,000. Although abalone only accounts for approximately 5% of shellfish aquaculture species in South Korea, its production value is high enough to account for 60% of the total value (KOSIS, 2022).

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Most abalone in South Korea is farmed in 2.4 × 2.4 × 2.5 m^3 net cages that contain shelters constructed of polypropylene or polyvinyl chloride. Wave-type all-in-one shelters and apartment-type shelters divided into compartments with plates are used. These farming methods enable high-density breeding; however, environmental changes such as high temperatures, red tides, and low salinity often lead to mass mortality, which is common on the southern coast of South Korea. Aquaculture damage in South Korea, the main aquaculture production area for abalone, mainly occurs during winter (January to March) and summer (July to October) (Han and Lee, 2022), with the observed damage closely related to the geographical features of the country. Temperature variation is common around the Korean Peninsula, mainly owing to the Tsushima Warm Current that diverges from the hot and highly salty (20–30 ℃, 34-34.8 psu) Kuroshio Current; the peninsula is also affected by monsoon events (Ministry of Land, Infrastructure and Transport and National Geographic Information Institute, 2020; Seo *et al.*, 2011). Heavy precipitation can lead to a low salinity phenomenon on the coast, also leading to extreme conditions.

The use of the recirculating aquaculture system (RAS), a land-based aquaculture method in which the breeding environment (e.g., water temperature, dissolved oxygen, and salinity) can be controlled, is used to control such risk factors. Using this system allows the optimal water temperature for abalone growth to be maintained, leading to rapid and continuous growth. Furthermore, in the event of a disaster, the artificial control enables an appropriate breeding environment to be maintained, preventing mortality and economic damage. However, the RAS is associated with other types of problems, in which recirculating seawater leads to increased pH, decreased minerals (such as calcium), and increased nitrogen compounds resulting from the decay of residual food, fish carcasses, and feces (Koizumi and Tsuji, 2017).

Abalone growth is influenced by diverse factors in the feeding environment, such as water quality (Harris *et al.*, 1999; Hoshikawa *et al.*, 1998; Leitman, 1992), feed type (Bautista-Teruel and Millamena, 1999; Capinpin Jr. and Corre, 1996; Capinpin *et al.*, 1999; Haaker *et al.*, 1998; Lopez *et al.*, 1998; Viana *et al.*, 1994; Viana *et al.*, 1996), and rearing density (Clarke and Creese, 1998; Day and Fleming, 1992; Mai *et al.*, 1995; Mgaya and Mercer, 1995; Mgaya *et al.*, 1995). Of these factors, feed exerts a wide range of effects on health and growth (Guillaume, 2001), and the health of animals bred in RAS systems is highly associated with feed type; thus, it is important to choose an appropriate diet that contributes to growth while also considering the economic feasibility and changes in the water environment until the abalone becomes commercially viable. In 2022, the seaweed production in South Korea amounted to 1,729,871 t, with 550,000 t of *Pyropia* spp., 560,000 t of *Saccharina japonica*, and 580,000 t of *Undaria* spp. being produced. Owing to its high production volume, seaweed is easily accessible as a feed source and has the advantage of providing a continuous supply during the cultivation period, depending on the type (dry or live). The seaweed we used in this study is actively utilized in abalone farming and as an additive in compound feeds. Given that supplying a combination of seaweed and properly formulated compound feeds may be more beneficial than providing seaweed alone, especially in the RAS environment, we hypothesized that this approach could support abalone growth. Therefore, this preliminary study was conducted as part of research aimed at minimizing environmental stresses related to climate change and establishing a stable foundation for production. We utilized an RAS to determine optimal growth conditions for abalone based on variations in feed types and feed mixture ratios.

MATERIAL AND METHODS

1. Acquisition and treatment of abalone

Abalone reared in an inland abalone aquaculture farm (Yeosu, Republic of Korea) with a average shell length (SL) of 50.34 ± 2.03 mm, average shell width of 33.13 ± 1.28 mm, and average total weight (TW)

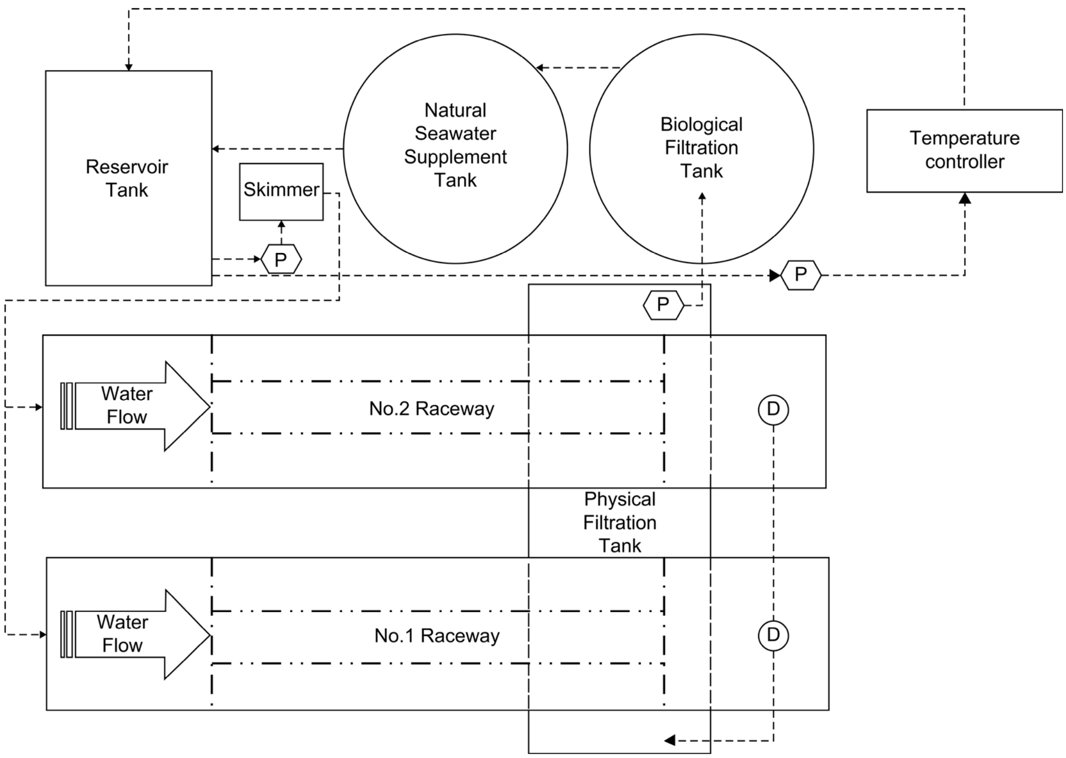

Fig. 1. Diagram of the used recirculating aquaculture system; (**P**): circulation pump, (**D**): drain, one point line (—·—): stainless steel net, two points line (—··—): polycarbonate plate in raceway. Water flowed in the direction of the arrows.

of 14.72 ± 2.15 g were used in this experiment. Two thousand and two hundred abalone were transferred from the inland abalone aquaculture farm to the biological aquaculture room and reared in RAS tanks set at the same natural water temperature. A total of 165 abalone were placed in each compartment. Acclimation was performed over 7 days, and the abalones were fed a diet consisting of three seaweeds (*Pyropia tenera*, *Undaria pinnatifida*, and *Saccharina japonica*) with commercially available formulated abalone food over the experimental period of 90 days.

2. Experimental conditions

Three 5.46-t RAS tanks, two raceway water tanks (300 × 100 × 40 cm^3), physical and biological filter tanks, skimmers, a natural seawater supplement tank, and a reservoir tank were used in the experiment. The inner raceway water tank was divided into 200 × 30 cm compartments using a 200 × 30 × 0.5 cm polycarbonate plate and a 100 × 30 cm^2 stainless steel net, providing a total of three feeding spaces. Water was filled to a height of 20 cm (Fig. 1) and maintained at a temperature of 20.1 ± 0.4 ℃, a pH of 7.9 ± 0.1, and a salinity of 31.5 ± 1 psu throughout the feeding period. Temperature and pH were measured using a pH logger MX2501 (Onset, USA), and salinity using the multi-item water quality meter, Yellow Springs Instrument (YSI) Pro2030 (YSI Inc., USA). Measurements were made twice per day. Sodium bicarbonate (500 g/2 days) was added to the reservoir tank to prevent the pH from falling below 7.9. The supplementary tank was supplied daily with natural seawater to a level comprising approximately 20% of the total water, which was filtered using a 200 μm sand filter and mixed with the biologically filtered breeding water before transfer to the reservoir tank. The breeding water was supplied through a feeding pump (5,000 L/h), and circulation of the water in the breeding tank was performed 200 times/day.

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3. Feeding conditions

Three types of dry seaweed (*S. japonica*, *U. pinnatifida*, and *P. tenera*) were fed together with formulated diet (FD) during the experiment, in five different ratios: [A] 100% seaweed, [B] 75% seaweed:25 % FD, [C] 50% seaweed:50% FD, [D] 25% seaweed:75% FD, and [E] 100% FD. Dry seaweed was soaked in water before being used as feed to soften it prior to supply. Daily feeding was performed based on 1% of the TW of the experimental animals. The main ingredients of the supplied feed are listed in Table 1.

4. Biological indicators

Survival rate: the death of the experimental organisms was determined by the absence/presence of retraction of the mouths and tentacles of individuals, and the survival rate was calculated using the cumulative mortality during the feeding period.

Growth rate: fifty *H. discus hannai* individuals were randomly selected from experimental groups for measurement. The SL was measured to the nearest 100 μm (0.01 mm) using a digital vernier caliper; TW including shell and body weight excluding shell were measured to the nearest 100 μg (0.01 g) using an electronic scale. The meat of 30 *H. discus hannai* individuals randomly selected at the beginning of the experiment was separated from the shell, dehydrated, and measured, with the results used as baseline data. The daily SL and TW growth values for *H. discus hannai* were calculated using Equation (1):

$$\text{Daily growth} = (\text{Final value} - \text{Initial value})/\text{Days of culture} \times 1{,}000 \quad (1)$$

Condition index (CI) and meat weight rate (MWR): to calculate the initial ratio of the CI and MWR, the remaining 55 abalone that were not used in the experiment were measured. The average body weight of the measured individuals was then used as the baseline data for the CI and MWR. The CI and MWR were calculated using Equations (2) and (3):

$$CI = \frac{\text{Body weight (g)}}{\text{Shell length (mm)}^3} \times 1{,}000 \quad (2)$$

$$MWR = \frac{\text{Body weight (g)}}{\text{Total weight (g)}} \times 100 \quad (3)$$

5. Shell component ratio analysis

Sample preparation for X-ray diffraction (XRD) analysis: meat was completely removed and dried in an oven at 60℃ for 24 h. Shells were placed in a polyethylene bag and crushed with a rubber hammer before further crushing with a blender (7010S, Warning, USA). The resulting powder was filtered using a 200 μm sieve for use. Samples were divided into two groups: before feeding (Fig. 2. i; original shell) and after feeding (Fig. 2. ii; growth shell).

XRD: The shell component ratio was analyzed using an X-ray diffractometer (Empyrean, Malvern Panalytical Corp., the Netherlands). Shell powder (100 mg) was placed in the holder and rotated at 300 rpm on the specimen table for analysis. Results were obtained using a rotation angle of 2(θ) = 5–90°, an XRD intensity step of 0.02°, and a scanning speed of

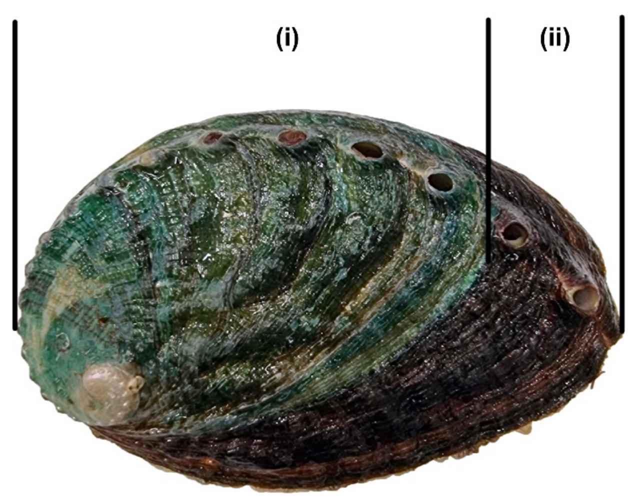

Fig. 2. Abalone shell imaged using X-ray diffraction analysis. The shell was obtained from abalone fed *P. tenera*, (i): pre-experimental existing grown abalone shell (green) and (ii): abalone shell particle grown during the experimental period (red brown)

1.41°/min. XRD patterns were imaged using Highscore Plus (Panalytical, the Netherlands) and compared with library specimens (Raman spectroscopy, XRD, and chemistry data for minerals, https://rruff.info). The composition ratios were analyzed using the Rietveld method.

6. Statistical analysis

One-way analysis of variance was performed using Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc., USA) to identify significant differences between the experimental groups. Post-hoc tests were conducted using Duncan's multiple range test at the $p < 0.05$ level.

RESULTS

1. Survival rate

The cumulative mortality rate per feed type and mixing ratio ranged from 0 to 5.5% at the end of the experiment, with a survival rate of 94.5–100% for *H. discus hannai* (Table 2).

Table 2. Survival rate of abalone based on feed type and mixing ratio

	Saccharina japonica				*Undaria pinnatifida*				*Pyropia tenera*				FD
Mixing Ratio*	[A]	[B]	[C]	[D]	[A]	[B]	[C]	[D]	[A]	[B]	[C]	[D]	[E]
Survival rate (%)	100	97.6	98.8	99.4	100	99.4	100	98.2	99.4	99.4	97.6	97.6	94.5

FD, formulated diet

Table 3. Growth evaluation of abalone based on feed type and mixing ratio

	Diet ary ratio (%)	Initial shell length (mm)	Final shell length (mm)	Shell length growth (μm/d)	Shell length growth rate (%)	Initial total weight (g)	Final total weight (g)	Total weight growth (mg/d)	Total weight growth rate (%)
Saccharina japonica	[A]	49.70 ± 0.99	51.37 ± 1.54	17.96	3.36[a]	13.67 ± 1.24	14.16 ± 1.82	0.52	3.54[a]
	[B]	50.33 ± 1.22	51.86 ± 1.18	16.50	3.05	14.45 ± 0.98	15.64 ± 1.89	12.81	8.25
	[C]	50.81 ± 1.16	53.35 ± 2.17	27.25	4.99	14.88 ± 1.14	15.95 ± 1.40	11.63	7.27
	[D]	50.42 ± 0.88	53.03 ± 1.80	28.09	5.18	14.39 ± 0.92	16.54 ± 2.65	23.06	14.90
Undaria pinnatifida	[A]	49.79 ± 0.75	53.28 ± 1.10	37.51	7.01[bd]	14.01 ± 1.01	15.59 ± 1.10	17.07	11.34[bd]
	[B]	51.41 ± 0.81	54.79 ± 0.85	36.28	6.56	14.59 ± 1.44	16.09 ± 1.01	16.13	10.28
	[C]	49.45 ± 0.73	52.49 ± 0.88	32.74	6.16	14.19 ± 0.75	15.37 ± 1.34	12.66	8.30
	[D]	49.45 ± 0.66	52.65 ± 0.88	34.46	6.48	14.19 ± 1.02	15.77 ± 0.97	16.96	11.12
Pyropia tenera	[A]	51.20 ± 0.89	57.52 ± 1.91	67.97	12.35[c]	15.16 ± 0.89	20.04 ± 2.34	52.48	32.20[c]
	[B]	50.28 ± 0.96	55.45 ± 0.99	55.56	10.28	14.50 ± 0.96	19.05 ± 1.50	48.92	31.37
	[C]	51.89 ± 1.21	56.85 ± 1.93	53.32	9.56	15.85 ± 1.21	19.92 ± 2.34	43.82	25.72
	[D]	49.66 ± 1.04	54.33 ± 1.21	50.19	9.40	13.49 ± 1.04	16.72 ± 0.89	34.69	23.91
Formulate d diets	[E]	49.90 ± 1.24	53.47 ± 2.36	38.31	7.14[bd]	14.64 ± 1.56	17.44 ± 2.41	30.45	19.13[bd]

No significant difference (mean ± SD, $p > 0.05$) for the mixed feed experimental group.
Significant difference (mean ± SD, $p < 0.05$) were observed among the experimental groups supplied with a single type of feed.

2. Growth indicators

The growth rate of individuals fed each experimental feed type was recorded based on their initial and final growth. The overall growth and daily growth rates are shown in Table 3. *H. discus hannai* that were placed in the experimental tank and fed the FD as a base showed SL and TW growth rates of 7.14 and 19.35% during the feeding period, respectively. No significant difference ($p > 0.05$) was observed in the SL or TW of the experimental group fed a mixture of seaweed and FD, regardless of the ratio. However, significant differences ($p < 0.05$) were observed for the experimental groups fed single-type feed, with SL in the order: *P. tenera* > *U. pinnatifida*, FD > *S. japonica*; TW in the order: *P. tenera* > FD > *U. pinnatifida* and *S. japonica*. *H. discus hannai* fed *S. japonica* showed SL and TW growth rates of 3.36 and 3.54%, respectively, with rates of 7.01 and 11.34% for *U. pinnatifida* and 12.35 and 32.2% for *P. tenera* feed, respectively. *P. tenera* was, therefore, associated with higher shell growth and weight gain than the other diets.

3. CI and MWR

The CI and MWR are indicators that confirm the ratios of shell growth and meat weight. The MWR, which indicates the proportion of meat weight relative to the TW, and fatness, which evaluates the ratio of meat weight to the SL, generally increased from the beginning of the experiment, but did not follow the same trend as the growth rate. No significant difference was observed for the CI and MWR according to feed. There were only differences in the rate of increase in the CI or MWR depending on the type of feed, and we, thus, concluded that there were no apparent growth imbalances based on the type of feed (Table 4).

4. Shell component analysis

The results of the shell component analysis for *H.*

Table 4. CI and MWR for abalone based on feed types and mixing ratios

	Dietary ratio (%)	Condition index		Meat weight rate (%)	
		Initial CI	Final CI	Initial MWR	Final MWR
Saccharina japonica	[A]	0.070	0.068	62.57 ± 5.82	64.97 ± 1.05
	[B]	0.067	0.071	59.18 ± 4.04	62.94 ± 0.34
	[C]	0.065	0.070	57.47 ± 4.30	66.27 ± 0.90
	[D]	0.067	0.072	59.41 ± 3.92	64.87 ± 1.16
				*8.67	
Undaria pinnatifida	[A]	0.069	0.080	61.05 ± 4.24	77.68 ± 6.15
	[B]	0.063	0.068	58.61 ± 2.77	69.93 ± 6.77
	[C]	0.071	0.082	60.26 ± 3.00	77.39 ± 9.47
	[D]	0.071	0.081	60.24 ± 4.52	74.71 ± 9.55
				*24.76	
Pyropia tenera	[A]	0.064	0.067	56.40 ± 3.99	63.49 ± 8.37
	[B]	0.067	0.087	58.96 ± 5.03	78.26 ± 9.18
	[C]	0.061	0.073	53.95 ± 3.28	67.33 ± 8.61
	[D]	0.070	0.077	63.38 ± 4.06	73.82 ± 7.64
				*21.64	
Formulated diets	[E]	0.069	0.075	58.41 ± 4.36	66.05 ± 1.02
				*13.07	

No significant difference (mean ± SD, $p > 0.05$) for the experimental group.
*Mean increase rate of the experimental group.

Table 5. Results of abalone shell component analysis obtained using XRD for feed type and mixing ratio

	Dietary ratio (%)	Growth shell area		Original shell area	
		Aragonite (%)	Calcite (%)	Aragonite (%)	Calcite (%)
Saccharina japonica	[A]	64.8	35.2	61.4	38.6
	[B]	72.2	27.8	57.1	42.9
	[C]	71.2	28.8	54.3	45.7
	[D]	74.9	25.1	57.0	43.0
Undaria pinnatifida	[A]	71.0	29.0	62.7	47.3
	[B]	75.6	24.4	53.7	46.3
	[C]	75.4	24.6	53.0	47.0
	[D]	68.7	31.3	56.6	43.4
Pyropia tenera	[A]	70.5	29.5	62.2	37.8
	[B]	71.2	28.8	59.2	40.8
	[C]	67.2	32.8	62.3	37.7
	[D]	74.8	25.2	56.0	44.0
Formulated diet	[E]	72.8	27.2	55.8	44.2
Ocean aquaculture abalone	*S. japonica* and *U. pinnatifida*	66.7	33.3	66.3	33.7

discus hannai using XRD were compared with those obtained from the library, confirming that the shells both included aragonite and calcite. Rietveld analysis of all samples indicated an aragonite to calcite ratio of approximately 7:3 for shells including growth and approximately 6:4 for shells excluding growth. As a control for comparison with the growing shell component ratio of *H. discus hannai* reared in the RAS, the shell component ratio of cage-aquacultured *H. discus hannai* was analyzed, confirming 66% aragonite and 33% calcite. The shell component ratio obtained for RAS growth was, thus, not significantly different from that obtained from conventionally grown shells (Table 5).

DISCUSSION

In South Korea, brown algae, such as *S. japonica* and *U. pinnatifida*, are supplied to *H. discus hannai* depending on the season. Fresh *S. japonica* is supplied during the seaweed season from April to September, whereas salt-preserved *S. japonica* and *U. pinnatifida* are supplied from October to December, and fresh *U. pinnatifida* from January to March. In addition to seaweed, inland abalone farms also use FDs that include an appropriate combination of essential nutrients and amino acids. The effects of feed type and nutrient component ratios on abalone growth have been studied for some time (Cho *et al.*, 2006; Cho and Cho, 2009; Demetropoulos and Langdon, 2004; Jang *et al.*, 2018; Ju *et al.*, 2016; Kim *et al.*, 2015; Lee *et al.*, 1997; Ma *et al.*, 2022).

The survival rate of *H. discus hannai* according to feed conditions was lower in the experimental group supplied with only dried *U. pinnatifida* than in the group supplied with dried *U. pinnatifida* and FD (Lee *et al.*, 1997). The results of the present study differed from those obtained previously in that feeding *U. pinnatifida* did not significantly affect the survival rate. However, the results indicating no difference in survival rate when feeding FD with dried *S. japonica* are similar to those obtained previously (Cho *et al.*, 2006; Cho and Cho, 2009; Kim *et al.*, 2015). In the present study, a survival rate of 94.5–100% was observed for the studied *H. discus hannai*, and there was no difference in the survival rate for all experimental groups based on different feeding regimes.

The growth rate of *H. discus hannai* according to diet was in the order: *P. tenera* > *U. pinnatifida* = FD > *S. japonica*; this growth effect shows the same pattern as the content ratio of protein in Table 1. Ma *et al.* (2022) investigated the protein content of different feed types for a *H. discus hannai*/H. fulgens hybrid and confirmed that supplying 300 g/kg of protein had the best effect on shell growth and the weight gain rate. The protein efficiency in H. rufescens fed on kelp (Macrocystis pyrifera) with 380 g/kg of protein resulted in a daily shell growth of 70.5 ± 4.2 μm (Garcia‐Esquivel and Felbeck, 2009. The protein content of 386 g/kg for *P. tenera* was similar to the growth result of the experimental group fed only *P. tenera* (67.969 μm/d). In addition, the weight gain was also significantly higher in the experimental group fed *P. tenera*. However, for the other health indicators, CI and MWR, the increase in meat weight as compared to shell growth was stable in the experimental group fed *U. pinnatifida*. A high MWR ratio is assumed to suggest stable weight gain in line with shell growth.

Abalone shells are composed of $CaCO_3$, with different crystal structures occurring on the inner and outer sides of the shell; the inner part consists of aragonite, and the outer of calcite (Lin and Meyers, 2005). $CaCO_3$ constitutes both the skeleton and shape of marine calcified organisms such as mollusks, shellfish shells, and corals, and its crystal structure is divided into trigonal calcite, orthorhombic aragonite, and hexagonal vaterite depending on environmental conditions, such as temperature, pressure, pH, and saturation (Bots *et al.*, 2012; Loste *et al.*, 2003; Son *et al.*, 2017; Zhou *et al.*, 2010). Shell growth occurs via a biomineralization process that utilizes the protein Pif80, which is secreted in the mantle, with calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) in the seawater to produce aragonite (Bahn *et al.*, 2017; Suzuki *et al.*, 2009). Aragonite has a metastable crystal structure that is transformed into calcite comprising a stable crystal structure over time (Naka, 2007). The *H. discus hannai* in this experiment were transferred from a seed-producing field to an inland fish farm, where they were reared in natural seawater until reaching the initial size required for the experiment. The aragonite:calcite composition ratios in Fig. 2 (i) and (ii) occurred because the abalone initially inhabited a mineral-rich natural seawater environment that provided a high calcite content. However, interruption of the mineral supply, which resulted from transfer to the breeding water tank, led to a decrease in calcite conversion. Little difference was observed for the component ratio of the growth part to the existing shell for ocean-cultivated *H. discus hannai*; thus, the mineral-rich marine environment is assumed to have considerably influenced the conversion of unstable aragonite into stable calcite. The calcium concentration of natural seawater may vary in different regions (averaging approximately 400 mg/L), and decreases in a closed RAS; therefore, calcium chloride or chemicals containing calcium should be added to maintain levels that are comparable to those of seawater (Koizumi and Tsuji, 2017).

Ammonia is rarely found at levels that biologically affect marine organisms in the natural environment. However, it can be present at levels that affect biological functioning when living in closed aquaculture facilities with high density (Morash and Alter, 2016). In the present study, the water environment was measured using an automatic water quality analyzer (Quaatro, Bran+Luebbe, Germany) on days 0, 30, 60, and 90. Non-ionic ammonia (NH^3) was estimated from the measured ionic ammonia (NH^{4+}) results based on the inverse operation of the total ammonia nitrogen (TAN) using the Henderson–Hasselbalch equation. The 0.153–0.710 mg/L NH^{4+} obtained during the feeding period was, thus, back-calculated to give an estimate of 0.007–0.032 NH^3 mg/L, which is considerably higher than the acceptable levels suggested by Leighton and Robinson (2008) (i.e., TAN < 1.0 mg/L, NH^3 < 0.025 mg/L) obtained in a circulating abalone rearing system.

The type of feed, appropriate nutrient contents, minerals present, and stable operation of the filtration system are, thus, conditions that need to be considered for growth when rearing abalone in RAS. When using RAS for abalone rearing, considerations related to their growth should include not only the type of feed but also the appropriate nutrient content within the feed and sufficient supply of minerals for stable shell growth. Additionally, it is necessary to minimize environmental stress caused by water quality (nitrogen compounds, consume waste, and uneaten food) by maintaining adequate filtration devices and a stable system.

CONCLUSIONS

This study investigated the effect of feed type on abalone growth in an RAS. The SL and weight of *H. discus hannai* were high in the experimental group fed *P. tenera*, with the highest protein content, and no significant difference was observed between the groups fed *U. pinnatifida* and FD. The lowest growth was observed in the experimental group fed only *S. japonica*. No significant difference was observed according to the mixture ratio of seaweed to FD. Although there were no significant differences in growth rate observed based on the mixing ratios of each seaweed and FD, significant differences in growth were observed in the experimental groups where individual supplies (*P. tenera*, *U. pinnatifida*, and *S. japonica*) and FD were provided. The $CaCO_3$ composition of the shell mainly consists of aragonite and calcite, and no difference was observed in the composition ratio of aragonite to calcite according to feed type; however, minerals in the marine environment are assumed to influence the conversion of aragonite to calcite in the stable phase and differences observed in the ratio of abalone grown in RAS compared with those grown in the sea. Therefore, for abalone farmed in RAS systems, not only the feed type, but also the nutritional composition, mineral supply, and stable rearing environment, should be considered.

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