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Effects of Protein Skimming on Water Quality, Bacterial Abundance and Abalone Growth in Land Based Recirculating Aquaculture Systems

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ABSTRACT

Recirculating aquaculture systems can reduce many challenges associated with open culture systems but maintaining suitable water quality in recirculating system is difficult. Protein skimmer removes organic matter from the water before it breaks down into nitrogenous waste. Therefore, the aim of this study was to evaluate the effects of protein skimming on water quality, bacterial abundance and abalone growth in recirculating aquaculture system. Two recirculating systems utilizing artificial seawater were housed in an air-conditioned, insulated recycle frozen container (4.3×1.9×1.9 m) to maintain optimum water temperature (19.2±0.8°C) for abalone growth. Each system consisted of two biofilters (100 and 200 L) and two abalone culture tanks (each 200 L) containing three plastic baskets (each 50×34×6 cm, with 12 mm mesh). One culture system incorporated a protein skimmer. Over an experimental period of 87 days, protein skimming resulted in significantly better water quality, heterotrophic bacterial abundance and abalone growth. Results indicate recirculating abalone culture systems with protein skimmer housed in an air-conditioned, insulated recycle frozen container may provide a viable alternative to current land-based, flow-through systems. More research is needed to further increase the efficiency of this system.

Key words: Abalone, recirculating aquaculture systems, protein skimmer, water quality, bacteria

INTRODUCTION

Abalone aquaculture was first developed in Japan over 60 years ago (Fleming and Hone, 1998) and has grown dramatically in recent decades due to increased demand and dwindling catches of wild abalone (Coote *et al.*, 1996; Taylor and Tsvetnenko, 2004; Ali *et al.*, 2009). In Japan, abalone are generally cultured in coastal floating net cages and land-based flow-through systems (Alcantara and Noro, 2006; Sugawara and Kadowaki, 2006; Pereira and Rasse, 2007). Ocean-based cage systems are vulnerable to predation, poaching, unfavorable environmental conditions and offshore mariculture regulation and discharge their waste directly into the marine environment (Ghiraldelli *et al.*, 2006; Anyanwu *et al.*, 20011). Land-based flow-through systems reduce problems

associated with predation, poaching and weather (Fallu, 1991; Leonard, 1993) but still face challenges maintaining optimum water temperature (Nie *et al.*, 1996). This system also discharges nutrient-rich waste directly or indirectly into coastal waters. Moreover, land-based flow-through systems require large areas of land, which is often very costly and not easy to find in a country like Japan. However, these problems reduce economic success of land-based flow-through systems.

Major challenges associated with open culture systems could be reduced or eliminated by using closed systems. Such systems could enable the treatment of waste water within a closed loop, offer improved control of effluent discharge and allow complete environmental control (El-Marakby *et al.*, 2006; Shanmugam *et al.*, 2008; Akinyemi and Buoro, 2011). Closed systems could also confer ecological and economic advantages by reducing the amounts of water, energy and land required, making them accessible to farmers with limited resources. Use of artificial seawater in these systems would enable their establishment inland, away from expensive coastal areas.

However, it is considerably more difficult to maintain suitable water quality in closed than open, flow-through systems (Palpandi *et al.*, 2007). Toxic by-products (e.g., NH₃ and NO₂) from decomposition of abalone faeces and excess feed, tend to accumulate in closed systems. These toxic by-products lead to detrimental changes in tissue structure, cell function, blood chemistry, osmoregulation, disease resistance and growth of abalone (Harris *et al.*, 1998; Cheng *et al.*, 2004; John *et al.*, 2011). Build-up of toxins in recirculating seawater systems can be alleviated through the addition of Protein Skimmers (PS) which remove organic matter from the water before it breaks down into nitrogenous waste. The addition of PS to closed culture systems will increase costs but these may be outweighed by their beneficial effects on water quality and abalone growth, although this has never been empirically tested. Therefore, the main objective of this study was to evaluate the effects of PS on water quality, bacterial abundance and growth of abalone in a small-scale, recirculating aquaculture system.

MATERIALS AND METHODS

Experimental system: The experiment was carried out in two small-scale recirculating systems (with and without protein skimmer) housed in an air-conditioned container (recycled frozen-transport container) at the Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan. Each recirculating system consisted of two biofilter (100 and 200 L) and two abalone culture tanks (each 200 L) (Fig. 1). One of these systems also incorporated a protein skimmer. Biofilter tanks were continuously aerated from the bottom via 25-mm PVC tubing with 1-mm perforations every 5 cm. Each abalone culture tank contained three plastic baskets (each 50×34×6 cm) with a mesh size of 12 mm. Thus each recirculating system had 6 plastic baskets. Both recirculating systems were housed in an insulated, frozen-transport container (4.3×1.9×1.9 m). An air-conditioner (AC) was used to maintain the desired water temperature. Artificial salt water was used instead of natural seawater. Artificial sea salt was collected from Marine-Tech Ltd., Japan and mixed with freshwater up to desired salinity. Water circulation was maintained at 43 L per minute. The experiment was continued for 87 days between March and August 2009 with zero water exchange.

Abalone culture and management: Hybrid abalone (*Haliotis discus hannai*×*H. sieboldii*) of 5.3±0.08 g individual weight (including shell) and 3.5±0.8 cm shell length were stocked in the plastic baskets (stocking density = 20 individuals per basket). Abalone and pelleted artificial feed

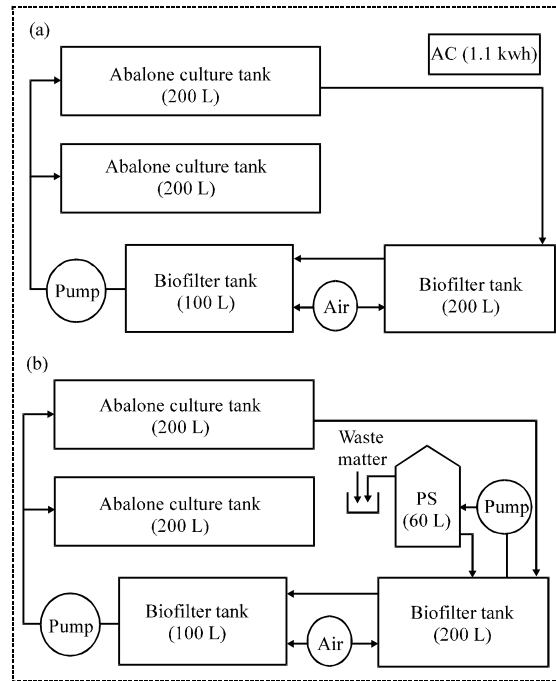


Fig. 1: Schematic diagram of recirculating system without PS (a) and with PS (b). Dotted line indicates container (4.3×1.9×1.9 m), PS indicates Protein skimmer

used in this experiment were collected from Kosumo Ocean Ranching Co. Ltd., Japan. Pelleted artificial feed was supplied six days per week at 2.3% of abalone body weight per day throughout the experimental period. The artificial feed contained 34% crude protein, 4% fat, 1.6% calcium, 1% phosphorous and 20% total mineral. Residual feed and faeces were collected next day after feeding.

Water quality analysis and total bacterial load assessment.

Physico-chemical parameters including Dissolved Oxygen (DO), pH, Total Ammonia Nitrogen (TAN), nitrite, nitrate, phosphate were monitored daily between 10:00 and 11:00, from the day before abalone stocking until the day of abalone harvesting. Digital meters were used to measure DO and pH. Total ammonia nitrogen (TAN), nitrite nitrogen (NO₂-N), nitrate nitrogen (NO₃-N) and phosphate phosphorous (PO₄-P) were quantified using a HACH DR/2000 spectrophotometer (HACH Co., Loveland, USA).

The heterotrophic bacterial loads of both recirculating systems were also estimated following the same time schedule of water quality analysis. Water samples were collected from each basket with sterile glass bottles for transport to the laboratory. Total count water testers (Millipore S.A.S. 67120 Molsheim, France) and an incubator were used to estimate total number of bacteria per ml of water in the abalone culture tanks.

Abalone harvesting: At the end of the experiment all abalone were individually weighed. Wet weight measurements were used to calculate specific growth rates (SGR, % body weight day⁻¹), which was calculated for each basket using the formula of Day and Fleming (1992):

$$SGR = [\ln W_t - \ln W_i] \times 100 / T$$

where, WT_f is the average final abalone weight (g), WT_i is the average initial abalone weight (g) and T is the duration of the experiment (days). Shell length of all abalones were also measured to calculate shell growth using formula:

$$\text{Shell growth } (\mu\text{m/day}) = (L_f - L_i) / T$$

where, T is time interval in days, L_f is the length of an abalone at the beginning of the experiment and L_i is the length of an abalone at the end of the experiment. Food Conversion Ratio (FCR) was calculated from feed intake and growth using formula:

$$\text{FCR} = \text{feed intake (g)} / \text{weight gained (g)}$$

Data analysis: All data were analyzed using SAS version 8 (SAS Institute, Inc., Cary, NC, USA). Data were analyzed using t-test and ANOVA. A t-test (at P = 0.05 level of significance) was performed to compare main fixed treatment effects (protein skimmer vs. without protein skimmer) on different growth parameters. A repeated measure ANOVA was used to examine temporal changes in all water quality parameters and heterotrophic bacteria abundance for each treatment. All percentage data were arcsine transformed before analysis and checked for normal distribution and homogeneity of variance, but non-transformed data are presented in all tables and figures.

RESULTS

Effects on water quality parameters: PS significantly influenced DO, TAN, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations but had no impact on temperature, pH and $\text{PO}_4\text{-P}$ (Table 1). DO concentrations were significantly higher in the system with PS than without (Fig. 2). An opposite trend was observed for TAN, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations in the water (Fig. 3). PS increased DO concentration by 7% and decreased TAN concentration by 31%, $\text{NO}_2\text{-N}$ concentration by 35% and $\text{NO}_3\text{-N}$ concentration by 13%. Water quality also varied significantly over time (Fig. 2, 3), with decreasing trends in salinity, pH and DO and increasing trends in TAN, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ over the 87 day culture period. However, there was no interaction effect of experimental period and PS on any water quality parameters except $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$. The effect of PS was very pronounced by the final month of the experiment.

Table 1: Effects of protein skimming on different water quality parameters in abalone tanks based on one-way repeated measure ANOVA. Ranges are presented in parentheses

Variable	Significance (p-value)		
	PS	Time	PS×time
Salinity (ppt)	NS	*	NS
Temperature (°C)	NS	**	NS
pH	NS	*	NS
DO (mg L ⁻¹)	**	**	NS
TAN (mg L ⁻¹)	*	**	NS
$\text{NO}_2\text{-N}$ (mg L ⁻¹)	**	**	**
$\text{NO}_3\text{-N}$ (mg L ⁻¹)	*	**	**
$\text{PO}_4\text{-P}$ (mg L ⁻¹)	NS	*	NS

*p = 0.05; ** p< 0.01; NS: Not significant. PS: Protein skimmer; Time, effects of sampling period; DO: Dissolved oxygen; TAN: Total ammonia nitrogen; $\text{NO}_2\text{-N}$: Nitrite nitrogen; $\text{NO}_3\text{-N}$: Nitrate nitrogen; $\text{PO}_4\text{-P}$: Phosphate phosphorous

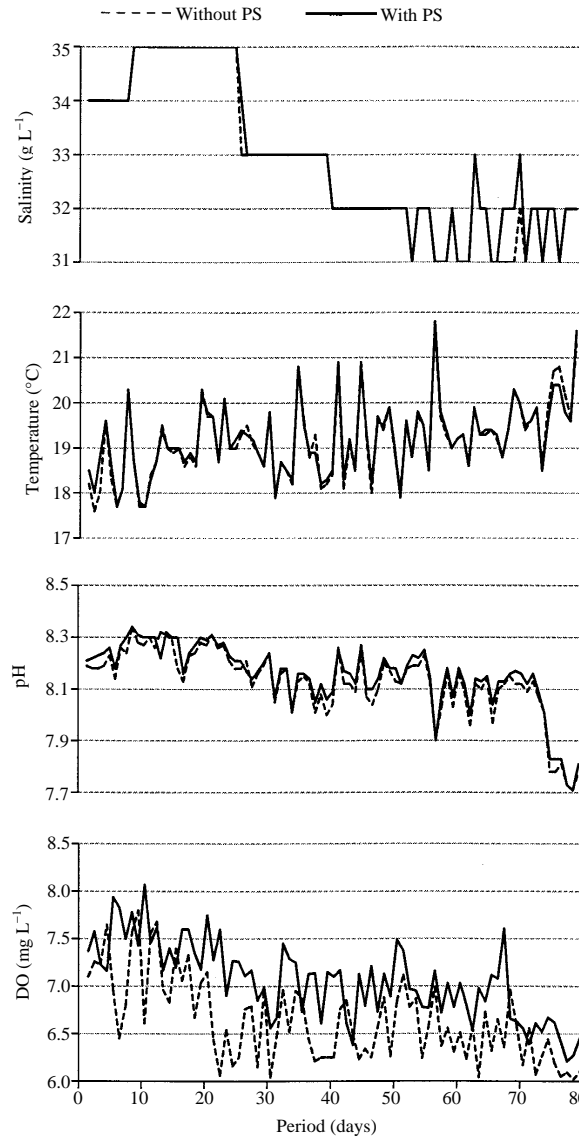


Fig. 2: Changes of salinity, temperature, pH and DO over time in system with and without PS

Effects on bacterial abundance: PS also significantly reduced the mean abundance of bacteria in culture system waters (Fig. 4). The mean abundance of bacteria was 2.6 times lower in the system with PS than the system without PS. Bacterial abundance also varied significantly over time (Fig. 5), although a significant interaction effect of PS and time was also evident. Mean bacterial abundance generally increased over time in the system without PS, but remained fairly constant in the PS system with the exception of the last three weeks of the experiment.

Effects on survival, growth, FCR and faeces production of abalone: Survival rates were statistically similar in system with PS and without PS. Abalone consumed more feed and had significantly higher FCR and growth rates (higher shell growth and specific growth rate) in PS

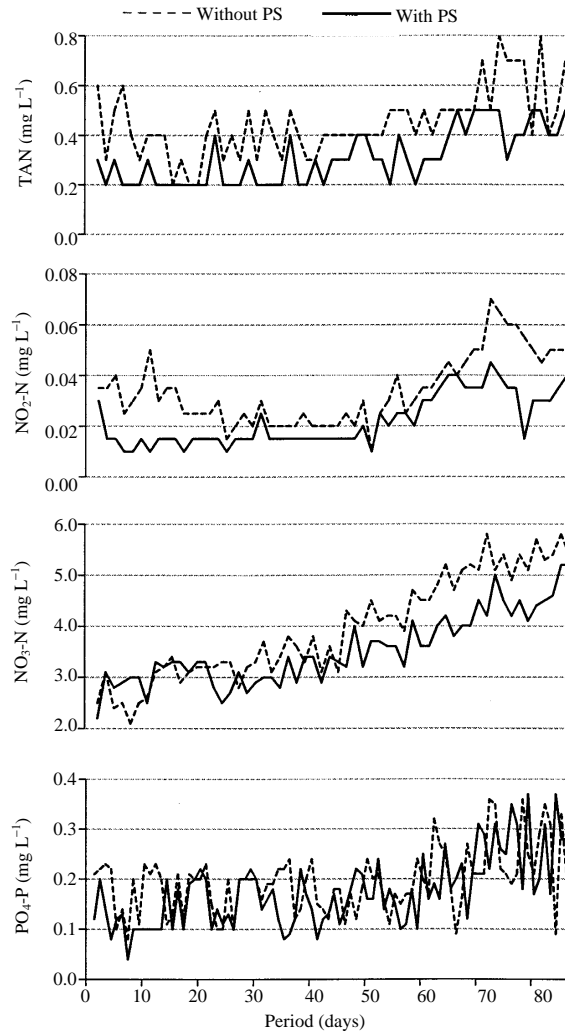


Fig. 3: Changes of TAN, NO₂-N, NO₃-N and PO₄-P over time in system with and without PS

Table 2: Effects of protein skimming on survival, growth, FCR and faeces excretion of abalone

Variable	Significance (p- value)	Treatment means ±95% confidence intervals	
		Without PS	With PS
Shell growth (µm day ⁻¹)	**	23.60±1.2	39.20±3.6
SGR (% day ⁻¹)	**	0.24±0.03	0.43±0.02
Feed consumption (% of feed supply)	**	56.00±2.7	68.70±2.6
FCR	**	3.40±0.2	2.10±0.2
Survival (%)	NS	86.70±6.8	88.30±11.3
Faeces (% of feed consumption)	*	10.00±0.4	8.30±0.4

* p = 0.05; ** p < 0.01; NS: Not significant; SGR: Specific growth rate; FCR: Feed conversion ratio; PS: Protein skimmer

system than non-PS system Table 2. Feed consumption by abalone in the PS system was 23% greater than the non-PS system. Shell growth and SGR in weight of abalone were 66 and 79%

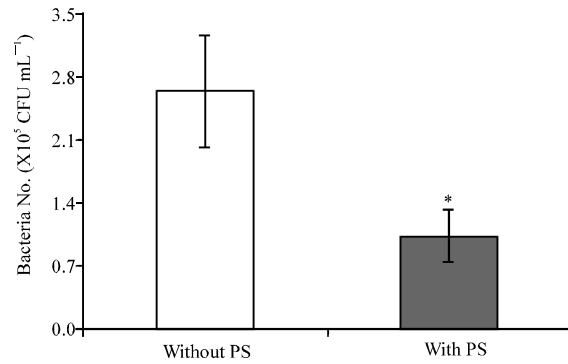


Fig. 4: Mean (bar:±95% confidence intervals) heterotrophic bacteria abundance in culture tanks without and with protein skimmer. *Represents significant treatment difference ($p>0.05$)

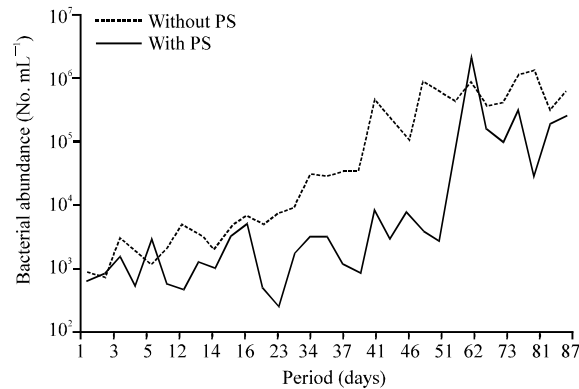


Fig. 5: Interaction effects of treatment (without and with protein skimmer) and sampling date on the abundance of heterotrophic bacteria in culture tanks

higher respectively in the PS system than the non-PS system. FCR decreased 38% in system with PS than the system without PS. Faeces production was also significantly lower (17%) in the PS system.

DISCUSSION

One of the critical problems for abalone aquaculture is maintaining optimum water temperature throughout the culture period. In recirculating systems, temperature is important both for maintaining optimum growth of the culture species and for increasing biofilter efficiency. Heating is a common method of maintaining water temperature in recirculating systems (Nie *et al.*, 1996; Losordo *et al.*, 1999; Martins *et al.*, 2009) but is typically very costly and reduces the economic viability of the culture system. In the present study, the systems used an insulated container and an Air Conditioner (AC), which successfully maintained optimum water temperature (18 to 20°C) for abalone in both recirculating systems (mean: 19.2±0.8°C) without significance difference between them (Fig. 2). Although several previous studies have examined the effects of temperature on abalone growth (Imai, 1977; Pereira and Rasse, 2007), none have focused on this particular hybrid (*Haliotis discus hannai*×*H. sieboldii*). However, *Haliotis discus hannai* growth is highest between 15 and 20°C (Sakai, 1962; Imai, 1977; Pereira and Rasse, 2007) and maximum growth

of *Haliotis midae* occurs at 20°C (Britz *et al.*, 1997). Collectively these previous studies suggest our experimental culture temperatures were close to optimal for abalone growth.

In the present study, PS reduced waste matter and subsequently aerobic bacterial decomposition, resulting in lower TAN, NO₂-N and NO₃-N concentrations in the system with PS. Decomposition of waste matter includes nitrification which is two-step oxidation of ammonia to nitrate. Two moles of oxygen are necessary for each mole of ammonia oxidized, hence decomposing waste matter left in closed culture systems consumes available oxygen within the water column.

Protein skimming reduced organic waste matter and bacterial abundance, resulting in less decomposition, lower TAN and NO₃-N concentrations and higher DO concentrations in the PS culture system. The lower decomposition, TAN and NO₃-N and higher DO in the water can also explain why the abundance of bacteria in the water was lower in system with PS than the system without PS.

Free Ammonia Nitrogen (FAN) is the most toxic type of nitrogenous waste to aquatic animal and chronic exposure may cause reduced growth and increased mortality (Hargreaves and Kucuk, 2001). Although there has been ample research on ammonia toxicity for finfish and prawns, few studies have examined ammonia toxicity in abalone. Harris *et al.* (1998) observed that free ammonia nitrogen (FAN) concentrations exceeding 0.031 mg L⁻¹ suppressed abalone (*Haliotis laevis*) growth rates. They also observed that FAN concentrations of 0.041 and 0.158 mg L⁻¹ reduced abalone growth (whole weight basis) 5 (EC₅) and 50% (EC₅₀), respectively.

In the present study the maximum TAN concentrations in the PS and non-PS systems were 0.50 and 0.80 mg L⁻¹, respectively. After conditioning, these concentrations were equivalent to 0.021 mg FAN L⁻¹ and 0.036 mg FAN L⁻¹ at pH 8.2, temperature 19.2°C and salinity 33 g L⁻¹. However, calculated maximum FAN concentrations in the PS system were much lower than the threshold concentration for abalone weight reduction reported by Harris *et al.* (1998) for *Haliotis laevis*. The maximum FAN concentration in the non-PS system was almost similar to EC₅ concentration reported by Harris *et al.* (1998).

Although comparatively low, FAN concentrations may have been the most significant environmental factor influencing abalone feed consumption, FCR and growth in the present study. All of these growth parameters were higher in the PS-system which had lower FAN concentrations. There are no previous studies comparing the effects of protein skimming on abalone food consumption and growth in recirculating systems. However, Sano and Maniwa (1962) reported decreasing food consumption and growth of *Haliotis discus hannai* with increasing FAN concentrations. Similar effects of FAN on feed consumption and growth were also observed in greenlip abalone (*H. laevis*) by Harris *et al.* (1998). Previous studies have suggested frequent water exchange as a method for managing FAN concentrations in traditional land-based abalone culture (Capinpin Jr. *et al.*, 1999; Evans and Langdon, 2000; Badillo *et al.*, 2007). However, frequent water exchanges increase operating costs and the present study suggests protein skimmers may present a viable alternative to water changes for maintaining water quality in recirculation systems for abalone culture.

Nitrite can be toxic as FAN for aquatic animals but its toxicity to abalone is not yet well understood. Nitrite is usually not a significant concern in brackish or seawater because chloride competes with nitrite for active transport across the gill (Lawson, 1995) but it can become a serious problem in recirculating systems where water is continually reuse Basuyaux and Mathieu (1999) observed a decrease in growth of *Haliotis tuberculata* at NO₂-N concentration of 0.5 mg L⁻¹. However, in the present study nitrite concentrations in both PS and non-PS systems (0.070 mg L⁻¹

Table 3: Reported SGR (specific growth rate) in weight and survival of *Haliotis discus hannai*

Abalone size (mm)	Culture period	Feed	SGR (% day ⁻¹)	Survival (%)	Reference
76.0	4 months	Various seaweed diet	0.11-0.12	100	Qi <i>et al.</i> (2010)
44.2	1 year	<i>Ulva</i> sp. and <i>Gracilaria</i> sp.	0.34	75.0	Neeri <i>et al.</i> (2000)
35.0	87 days	Artificial feed	0.43	88.3	Present study (system with PS)
23.4-34.9	106 days	<i>Gracilaria</i> sp. and <i>Laminaria</i> sp.	0.24-0.33	87.7-99.5	Wu <i>et al.</i> (2009)
20.0-30.0	-	<i>Ulva</i> sp.	0.71	-	Uki and Watanabe (1991)
21.0	-	<i>Ulva rigida</i>	0.69	-	Corazani and Illanes (1998)
20.0	6 months	<i>Ulva</i> sp. and <i>Gracilaria</i> sp.	0.31-1.02	93.3-97.2	Pereira and Rasse (2007)
7.1-7.3	163 days	<i>Laminaria</i> sp. and <i>Undaria</i> sp.	-	63.4-66.0	Nie <i>et al.</i> (1996)

and 0.045 mg L⁻¹, respectively) were too low to reduce abalone growth, presumably because the biofilters functioned properly and continually oxidized nitrite to nitrate.

In system with PS, although all water quality parameters were suitable for abalone culture, the survival rate of abalone was comparatively lower than those cited by other authors for the *Haliotis discus hannai* (Table 3). However, the SGR (0.43% day⁻¹) was mid-range compared to those observed in previous studies (Table 3). In the present study, comparatively low survival and growth of abalone were probably related to handling stress of abalone. Abalones were regularly stressed due to monitoring water quality and collection of excess feed and faeces. Uki (1989) and Hahn (1989) reported that abalone stopped feeding for one or more days after being handled. According to La Touche *et al.* (1993), handling can be an important source of mortality in culturing of abalone. Another factor explaining the lower survival and growth of abalone might be supplying commercial feed (Tung and Alfaro, 2011). Abalone generally prefers natural diet (seaweed) better than commercial diet (Naidoo *et al.*, 2006; Hernandez *et al.*, 2009). Therefore, in RAS, besides adding PS, higher growth and survival of abalone also can be attributed by supplying suitable seaweed as feed and minimizing handling stress. However, additional experiments with natural feed and minimal handling are needed.

CONCLUSION

Adding protein skimmer resulted in better water quality, heterotrophic bacterial abundance and abalone growth in recirculating abalone culture system. Recirculating abalone culture systems with protein skimmer housed in an air-conditioned, insulated recycle frozen container may provide a viable alternative to land-based, flow-through systems. However, more research is needed to further increase the efficiency of this system.

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