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Land-Based Poly-Eco-Aquaculture of Abalone and Seaweed in a Small Scale Recirculating System Using a Recycled Freezer Container

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Abstract：To minimize environmental problems associated with aquaculture, we wanted to develop an abalone and seaweed polyculture approach in a small scale recirculating aquaculture system housed in an air-conditioned recycled freezer container. We conducted two experiments; each used two recirculating systems. Each system consisted of two biofilters and two abalone culture tanks. Each abalone culture tank contained three plastic baskets for abalone. In the first experiment one of the systems also incorporated a protein skimmer (PS) to evaluate its effects on water quality and abalone growth. In the second experiment, the same system was incorporated with both a PS and a seaweed culture tank (PSS) to evaluate their combined effects on water quality and abalone growth. The abalone stocking density was 20 individuals (average weight 5.3 ± 0.08 g and 8.7 ± 1.9 g in the first and second experiment) per basket. Pelleted artificial feed was supplied six days per week at 2.3% of abalone body weight per day. The pH, dissolved oxygen (DO), total inorganic nitrogen (TIN), total inorganic phosphorus (TIP), and bacterial abundance were monitored daily. The duration of first and second experiments were 87 and 70 days. DO concentration was significantly higher in the system with the PS. An opposite trend was observed in TIN concentration and bacterial abundance. PS had no effect on pH or TIP. PSS influenced water quality parameters and bacterial abundance similar to PS except TIP, which was greater in the system with PSS than without. Treatment effects on growth, feed consumption, and FCR were similar in both experiments. Abalone consumed less feed and had significantly higher FCR and lower growth rates in the control. However, feed consumption, FCR and growth rate of abalone were comparatively better in the PSS system than in the PS system. The PSS system was not only better for abalone growth, but also produced an additional crop in the form of seaweed. The system did not discharge waste. Therefore, future abalone culture systems can be focused on this model. However, more research is necessary before extrapolating results to an industrial level.

Key words：poly-eco-aquaculture, abalone, seaweed, recirculating system

Introduction

Abalone is one of the most expensive seafoods (Qi *et al.,* 2010). Because of high demand and price, wild abalone fisheries in many regions are declining as a result of over-exploitation (Alcantara and Noro, 2006; Taylor and Tsvetnenko, 2004). Decreased wild catches, combined with increasing demand for abalone have accelerated the development of their aquaculture (Coote *et al.,* 2006). Abalone aquaculture began in Japan over 60 years ago (Hone and Flaming, 1997). Since then, abalone aquaculture

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has been developing with the goal of maximizing production and minimizing cost. However, in most cases, aquaculture impacts the surrounding ecosystem through habitat destruction, waste discharge, and pathogen invasions (Barg, 1992; Wu, 1995; Naylor *et al.,* 2000). Therefore, awareness is growing in the society about the negative impacts on marine ecosystems, which puts increasing demand on environment friendly aquaculture.

In Japan, abalone is generally cultured in coastal floating net cages and in land-based flowthrough systems (Alcantara and Noro, 2006; Pereira and Rasse, 2007). In conjunction with floating net cages, farmers face many obstacles related to predation, poaching, weather conditions and offshore mariculture regulation. In addition, floating net cages discharge waste directly into the marine environment. Although land-based flowthrough systems reduce some obstacles related to predation, poaching and weather conditions (Leonard, 1993), they also discharge high nutrient-rich waste directly or indirectly into coastal waters (Hall *et al.,* 1992; Islam, 2005). Moreover, land-based flowthrough systems need a large area of land, which is often difficult to find in a country like Japan. Another critical problem for abalone culture in land-based flowthrough systems is maintaining optimum water temperature especially during the winter season (Nie *et al.,* 1996; Troell *et al.,* 2006). These problems reduce the economic success of land-based flowthrough systems.

To minimize environmental problems and the control of water temperature, a new approach to abalone aquaculture was proposed: a small scale recirculating aquaculture system housed in an air-conditioned recycled freezer container. Such a system should enable treatment of wastewater and allow complete environmental control. The system could confer ecological and economic advantages by reducing the amounts of water, energy and land use required. Comparatively little space and costs would be required compared to flowthrough systems. Importantly, systems could be established far from expensive coastal areas and use artificial seawater prepared from sea salt and freshwater.

Materials and Methods

System design: We conducted two experiments, each with two recirculating systems housed in a recycled freezer container (4.3 × 1.9 × 1.9 m) at the Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan. Each recirculating system consisted of two biofilters (100 and 200 L) and two abalone culture tanks (200 L each). A schematic diagram of the systems is presented in Figure 1. One of the systems incorporated a PS to evaluate the effects of it on water quality and abalone growth (first experiment). The same system was incorporated with both a PS and seaweed culture tank to evaluate the effects of the combination on water quality and abalone growth (second experiment). Initially, 20 g of seaweed (*Ulva pertusa*) was stocked in the seaweed tank. Seaweed was partially harvested when it' s biomass exceeded 100 g.

The biofilter tanks were continuously aerated from the bottom through 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 six plastic baskets. An air-conditioner (1.1 kwh) was used to maintain the desired water temperature (19.2 ± 0.8℃ for both experiments). Artificial sea salt was obtained from Marine-Tech Ltd., Japan and mixed with freshwater up to desire salinity (32.9 ± 0.2 ppt). Water circulation during both experiments was maintained at 43 L/minute. The first experiment was conducted for 87 days between March and August 2009 and the second experiment for 70 days between October and December 2009 with zero water exchange.

Abalone stocking and Management: Twenty hybrid abalone (*Haliotis discus hannai* × *H. sieboldii*) averaging 5.3 ± 0.08 g and average shell length 3.5 ± 0.8 cm (first experiment) and 8.7 ± 1.9 g and average shell length 4.0 ± 0.2 cm (second experiment) were stocked in each plastic basket. All abalone were collected from the Kosumo Ocean Ranching Co. Ltd., Japan. Abalone were fed pelleted feed containing 34% crude protein, 4% fat, 1.6% calcium, 1% phosphorous and 20% total mineral six days per week at 2.3% of body weight daily

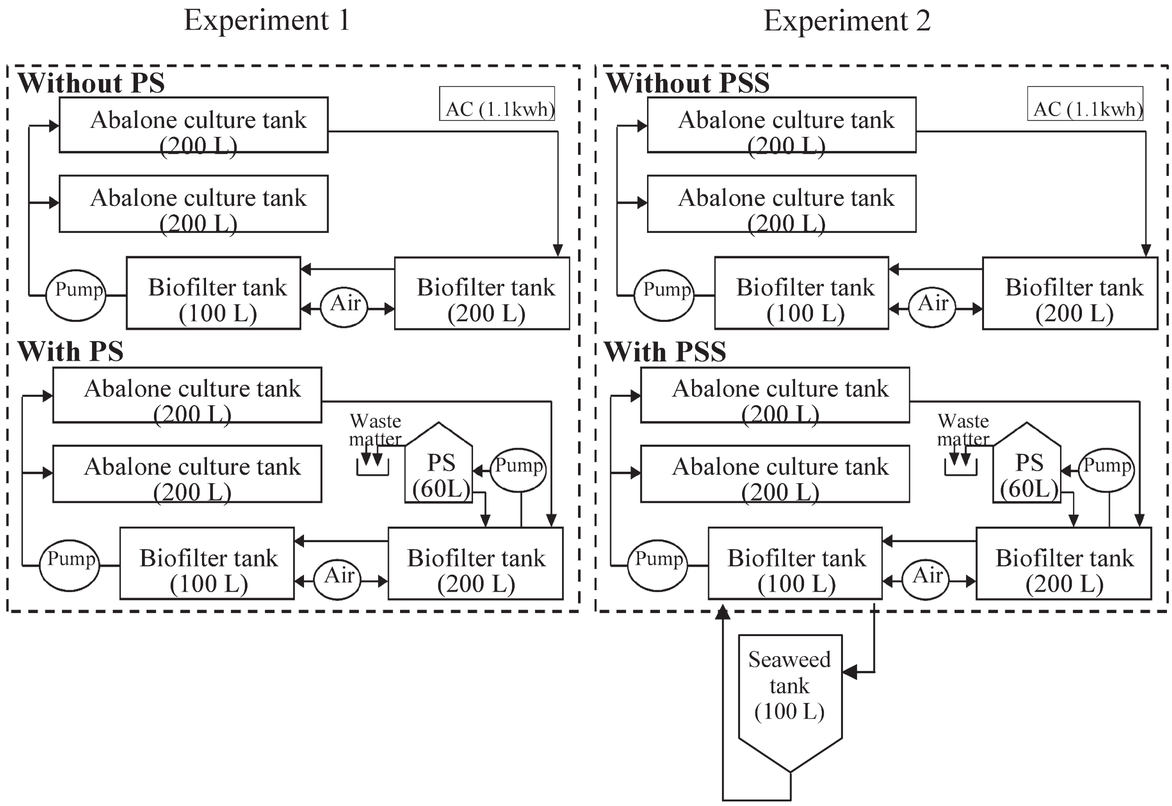

Fig. 1. Schematic diagram of recirculating systems used in experiment 1 and 2. Dotted line indicates container (4.3 × 1.9 × 1.9 m), PS indicates protein skimmer and PSS indicates protein skimmer plus seaweed.

starting the day after stocking until the day prior to harvesting. Feces and uneaten feed were collected next day of feeding.

Water Quality Analysis: Dissolved oxygen (DO), pH and all inorganic nitrogen and phosphorus species in the abalone culture baskets were monitored daily between 1000 and 1100 hours from the day before stocking until the day of harvest. Digital meters were used to measure DO and pH. Inorganic nitrogen and phosphorus species were quantified using a HACH DR/2000 spectrophotometer (HACH Co., Loveland, Colorado USA).

Total Bacterial Load Assessment: The bacterial loads were estimated following the same schedule used to determine water quality. Water samples were collected in sterile glass bottles from each basket for transport to the laboratory. Total count water testers (Millipore S.A.S. 67120 Molsheim, France) and an incubator were used to estimate total numbers of bacteria/ml of water.

Abalone Harvesting: All abalone were individually measured (both length and weight) at the end of the experiment. Growth in length was calculated for each basket using the formula: *Growth in length* $(\mu m/day) = (L_f - L_i)/T$, where T is in days, L_f is the length at the end of the experiment and L_i is the length at the beginning of the experiment. Weight measurements were used to calculate specific growth rates (SGR, % body weight day^{-1}), using the formula of Day and Fleming (1992): $SGR = [\ln WT_f - \ln WT_i] \times 100/T$, where WT_f is the average final weight (g), WT_i is the average initial weight (g), and T is the duration of the experiment (days). Food conversion ratio (FCR) was calculated from feed consumption and growth in weight using formula,: *FCR = feed intake (g)/weight gain (g).*

Data Analysis: Data were analyzed using SAS version 8 (SAS Institute, Inc., Cary, North Carolina USA). A t-test was performed to compare main fixed treatment effects (first experiment: PS vs. no PS; second experiment: with PS and seaweed vs. without PS and seaweed) on different growth parameters. A repeated measure ANOVA was used to examine temporal changes in water quality parameters and heterotrophic bacteria abundance for each treatment. The assumptions of normality and homogeneity of variance were tested. The data were transformed to satisfy normality if required. Differences were considered significant at $P \leqq 0.05$.

Results

Effects on Water Quality Parameters and Bacterial Abundance: Table 1 shows the mean, maxima, minima and ANOVA results for pH, DO, total inorganic nitrogen (TIN), total inorganic phosphorous (TIP) and total bacterial abundance. First and second experiments showed similar treatment effects on water quality parameters and bacterial abundance except TIP. In the first experiment, PS significantly influenced DO, TIN and bacterial abundance, but had no impact on pH and TIP (Table 1). DO concentration was significantly higher in the system with PS than without. An opposite trend was observed in TIN concentration and bacterial abundance. PS increased DO concentration by 7% and decreased TIN concentration by 15% and bacterial abundance by 60%. In the second experiment, PSS increased DO concentration by 21% and decreased TIN concentration by 15%, TIP concentration by 22 % and bacterial abundance by 49%. All water quality parameters and bacterial abundance also varied significantly over time in both experiments (Table 1). However, in the first experiment, there was no interaction effect of experimental period and PS on any water quality parameter except TIN concentration and bacterial abundance. In the second experiment, there was no interaction effect of experimental period and PSS on any water quality parameter or bacterial abundance.

Effects on Growth, Feed Consumption, FCR and Survival of Abalone: Treatment effects on growth in length, SGR, feed consumption and FCR were similar in both experiments when compared with the control in each system (Figure 2). Abalone consumed less feed and had significantly higher FCR and lower growth rates (greater shell growth and higher specific growth rate) in the control system than in the other system. However, abalone feed consumption, FCR and growth rate were comparatively better in the PS plus seaweed system (second experiment) than in the PS system (first experiment). Treatment effect on abalone survival was not significant (*P>0.05*) in either experiment.

Table 1. Water quality parameters in abalone tanks of experiments 1 and 2 based on one-way repeated measure ANOVA. Ranges are presented in parentheses.

Variable	Experiment 1 (Effects of PS)					Experiment 2 (Combined effects of PSS)				
	Significance (*P* value)			Treatment means ± 95% confidence intervals		Significance (*P* value)			Treatment means ± 95% confidence intervals	
	PS	Time	PS×Time	Without PS	With PS	PSS	Time	PSS×Time	Without PSS	With PSS
pH	NS	*	NS	8.14 ± 0.03 (7.66–8.35)	8.16 ± 0.03 (7.71–8.34)	NS	**	NS	7.84 ± 0.02 (6.20–7.91)	7.94 ± 0.02 (5.90–7.97)
DO (mg/L)	**	**	NS	6.6 ± 0.04 (6.0–7.8)	7.1 ± 0.04 (6.2–8.1)	**	**	NS	6.03 ± 0.10 (4.69–8.24)	7. 30 ± 0.05 (5.71–8.54)
TIN (mg/L)	*	**	**	4.38 ± 0.23 (2.11–4.93)	3.73 ± 0.20 (1.61–4.34)	**	**	NS	19.13 ± 0.90 (4.30–34.22)	16.21 ± 0.84 (2.41–20.12)
TIP (mg/L)	NS	*	NS	0.22 ± 0.01 (0.10–0.41)	0.20 ± 0.01 (0.08–0.37)	**	*	NS	3.34 ± 0.46 (0.39–5.40)	2.59 ± 0.29 (0.29–5.85)
Bacteria Abundance ($\times 10^5$ CFU/mL)	*	*	*	2.64 ± 0.62 (0.001–6.60)	1.03 ± 0.28 (0.001–4.30)	*	**	NS	6.20 ± 1.18 (0.001–12.90)	3.18 ± 0.97 (0.001–7.20)

* $P \leq 0.05$; ** $P < 0.01$; NS, not significant; PS, protein skimmer; PSS, protein skimmer plus seaweed; Time, effects of sampling period; TIN, total inorganic nitrogen; TIP, total inorganic phosphorous.

Discussion

Heating is a common method of maintaining water temperature in recirculating systems (Nie *et al.,* 1996; Masser *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, we used an insulated container and an air conditioner (AC), which successfully maintained optimum water temperature (range 18–20℃; mean: 19.2 ± 0.8℃) in both experiments without significant differences between treatments. The study suggests

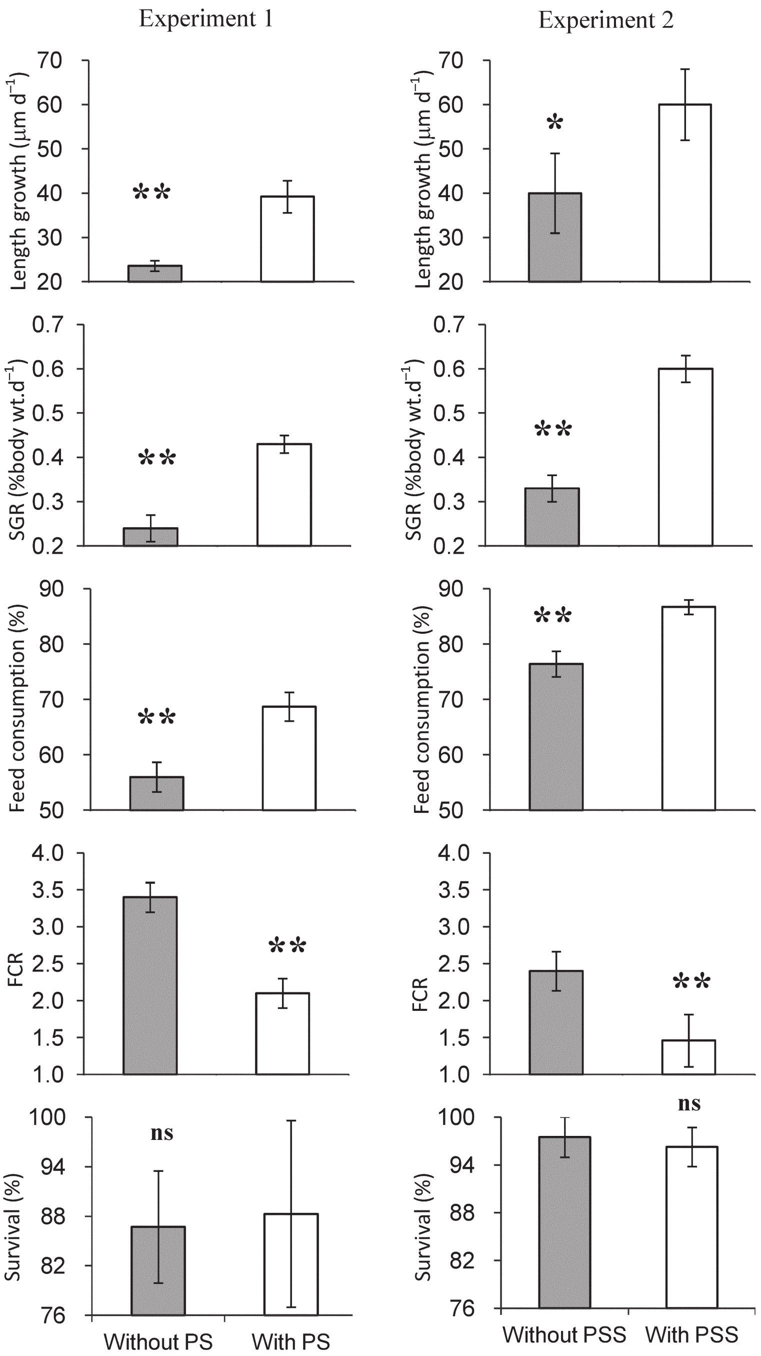

Fig. 2. Growth, feed consumption, FCR and survival in experiments1 and 2 based on t-test. Data are mean D 95% confidence intervals. Asterisk ($^*P≦0.05$; $^{**}P<0.01$) represents significant treatment difference and ns represents no significant treatment difference.

that an insulated recycled container and an air conditioner can be used to maintain suitable water temperature in recirculating systems for abalone aquaculture.

In the present study, protein skimming reduced waste matter and subsequently aerobic bacterial decomposition, resulting in lower TIN concentrations in the system with a PS alone or a PS along with seaweed (Sugawara and Kadowaki, 2006; Rahman *et al.,* 2008). Protein skimming reduced organic waste and bacterial abundance, resulting in less decomposition, lower TIN concentrations and higher DO concentrations in system with PS or PS and seaweed compared with the control systems. The lower decomposition and TIN and higher DO can also explain why the abundance of bacteria in the water was lower in system with PS or PS and seaweed than in systems with neither. The influence of PS and seaweed on DO concentration was more pronounced than the influence of PS alone on DO concentration. The reason might have been due to the addition of the seaweed culture tank with a PS. Seaweed produces oxygen, which increased dissolved oxygen concentration in the system with seaweed (Kadowaki, 2004).

DO and TIN concentrations may have been the most significant environmental factors influencing abalone feed consumption, FCR and growth in the present study. All of these growth parameters were higher in the two systems that had a PS. There are no previous studies comparing the effects of a PS on abalone food consumption and growth in recirculating systems, but Sano and Maniwa (1962) reported decreased food consumption and growth of *Haliotis discus hannai* with increasing TIN concentration. Similar effects of TIN on feed consumption and growth were also observed in greenlip abalone (*H. laevigata*) by Harris *et al.* (1998). Previous studies have suggested frequent water exchange as a method for managing TIN concentrations in traditional land-based abalone culture (Capinpin *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.

Addition of a PS skimmer and seaweed culture tank resulted in comparatively better abalone growth than the abalone growth in a recirculating system with only a PS. This result concurs with Kadowaki (2004), who reported better seaweed and fish growth near a seaweed culture area than away from the seaweed culture area. According to him, seaweed utilize inorganic nitrogen and phosphorus, inhibit pathogenic bacteria and produce oxygen. All of these influence abalone growth. Abalone growth is extremely slow and often varies with size and age (Steinarsson and Imsland, 2003; Naidoo *et al.,* 2006). Although several previous studies have examined the growth of *H. discus hannai* (e.g., Neori *et al.,* 2000; Wu *et al.,* 2009; Qi *et al.,* 2010), none have focused on growth of the hybrid used in this study. However, we observed comparatively higher growth (SGR = 0.60 %/day) in the system with a PS and seaweed if we compare that SGR with those from other studies. Neori *et al.* (2000) reported a SGR for 44.2 mm *H. discus hannai* fed on *Ulva* sp. at 0.34%/ day. Wu *et al.* (2009) observed SGR 0.24–0.33%/day for 23.4–34.9 mm *H. discus hannai* fed *Gracilaria lemaneiformis* and *Laminaria japonica.* Qi *et al.* (2010) observed a very low SGR range of 0.11–0.12%/day in 7.6 cm *H. discus hannai* fed various seaweed diets.

Conclusion

The PS with seaweed system not only improved abalone growth, but also produced an additional crop in the form of seaweed. The system also did not discharge waste. Therefore, future abalone culture systems can be focused on the described system. However, more research (optimization of abalone density and optimization of seaweed biomass) is necessary before extrapolating the results to an industrial level.

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