

Original Research

A Study of Environmentally Friendly Recirculating Aquaculture System on Lobster *Panulirus homarus* Nursery

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Abstract

Lobsters' cultivation using floating net cages is associated with several obstacles, such as low survival rates, excessive production expenditures, vulnerability to unpredictable weather, and unregulated feed remnants, which pose a risk of upwelling. Land-based recirculating aquaculture systems (RAS) have been increasingly used to enhance lobster production, as they can maintain optimal water quality in the system and curtail environmental impacts. The present study aimed to identify and evaluate the types of RAS that can provide the best water quality, minimize stress hemolymph responses (Total Hemocyte Count/THC, glucose, total protein, pH), and maximize the productivity of *P. homarus* lobster seeds. The treatments tested in this study included RAS using a biofilter (A), RAS with a combination of biofilter and protein skimmer (B), and RAS with a combination of biofilter and microbubbles (C). The findings revealed that RAS with a combination of biofilter and microbubbles (C) was best than the other treatments, resulting in better water quality, lower stress levels, and finer lobster production performance. Treatment C exhibited lower concentrations of ammonia and higher dissolved oxygen than the other treatments. Additionally, treatment C yielded the most favorable performance in lobster production, as indicated by a survival rate of $75.67 \pm 1.15\%$ and a Specific Growth Rate (SGR) of $1.47 \pm 0.04\%$ per day.

Keywords: RAS, lobster, water quality, biochemical, stress, production

Introduction

The surging demand for seafood commodities has instigated the development of efficient and eco-friendly aquaculture technologies. Among these innovations,

RAS has gained widespread recognition as a pioneering solution. It can enhance water use efficiency, control water quality [1], abate environmental impacts, elevate aquacultural productivity and lower risk of disease outbreak [2].

The global lobster catch data showed a decline from over 300,000 tons in the previous year to 255,000 tons in 2020 [3]. The decrease in environmental carrying

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capacity and the impact of climate change have contributed to the shortage of lobster supply. In addition, the need for more availability of seeds and low survival rates of lobsters have hindered the development of lobster cultivation. Currently, lobster cultivation practices in Indonesia are implemented through floating net cages. Nevertheless, such a cultivation method encounters various impediments, including inadequate survival rates, low growth, high operational cost, an issue with cage fouling, and accumulation of leftover feed on the seabed. Therefore, implementing RAS technology, with its multiple advantages, can be considered a sustainable alternative for developing lobster cultivation.

Previous studies on indoor land-based RAS have investigated the impact of shelter application on lobster growth during the rearing phase [4, 5]. Further research has aimed to determine the optimal ratio of shelter to lobster [6], examine the effects of alkalinity on lobster biochemistry [7], and investigate the impact of higher stocking densities on production performance [8].

The latest advancements in RAS technology, particularly for land-based mariculture, can be implemented by utilizing the principles of biofilters and foam fractionation. The biofilter comprises various microbial communities, such as heterotrophic microbes that degrade organic matter [9, 10] and microbes that transform ammonia into less harmful nitrates [10-13]. Foam fractionation, a water treatment technology, utilizes fine bubbles to separate dissolved and suspended solids in water, particularly in protein (food residue and feces) or metabolic waste from aquaculture activities [14]. Protein skimmers have been predominantly employed in RAS to eliminate organic matter and dissolved inorganic nitrogen and to reduce numerous heterotrophic microorganisms in water [13, 15]. Injectors using the venturi principle can generate microbubbles with a diameter of 0.1-0.3 mm [16]. Microbubbles have been utilized in aquaculture to stimulate the growth of red tilapia [17, 18] and shrimp *Litopenaeus vannamei* [19].

Using RAS in the lobster nursery allows for a better adaptation of each seed to the new environment, resulting in higher-quality seeds and reduced seed mortality. An initial study on the impact of RAS technology on the stress responses of *Panulirus homarus* lobsters during the nursery stage has demonstrated that RAS affects the lobster's hemolymph glucose levels, water quality, and productivity responses [20].

Alterations in the environmental conditions can lead to an elevation in glucose levels in the crustacean hemolymph as a response to stress [5, 7, 21-23]. These studies require further observation to investigate other physiological parameters as stress indicators in utilizing different RAS. THC can assess crustaceans' stress [5, 7, 22-24]. In addition, total protein and hemolymph pH are metabolic variables that can be employed to monitor the stress conditions of crustaceans [7, 25].

Considering the lack of studies on the impact of various RAS applications on nursery water quality and

the biochemical response of the *P. homarus* lobster, it is essential to conduct further research in this area. This study aims to identify and evaluate the types of RAS that can yield optimal water quality, minimize hemolymph stress response (measured through THC, glucose, total protein, and pH), and maximize the productivity of *P. homarus* lobster seeds.

Material and Methods

Experimental Design

The study employed three treatments with three replications, which were RAS using a biofilter (A), RAS with a combination of biofilter and protein skimmer (B), and RAS with a combination of biofilter and microbubbles (C). Each treatment was conducted in two plastic rearing tanks measuring 1.2 x 0.9 x 1.0 m. *P. homarus* lobster seeds used in the study had an average weight of 2.14 ± 0.04 g and a carapace length of 11.24 ± 0.07 mm. The lobster seeds were reared at a density of 100 lobsters m^{-3} , with the shelter made from PVC pipes. The shelter volume to bottom tank surface area ratio was $7900 \text{ cm}^3 \text{ m}^{-2}$ [5]. The biofilter consisted of a series of vertically arranged plastic boxes measuring 56 x 56 x 85 cm in total dimension. The filter media included zeolites, activated carbon, bio-foam, and green mat arranged in layers from bottom to top. Each filter, protein skimmer, and microbubble had a pump capacity of 2000 L $hour^{-1}$. The acrylic protein skimmer used in the study measured 25 x 20 x 60 cm, while the microbubbles were generated using the injector on the pump. The diameter of the microbubbles was measured by flowing a mixture of microbubbles and water through an observation box with a thin configuration (10 x 10 x 5 mm). In this configuration, the bubble population will look less dense and clearer. The image is then taken using a digital camera with backlighting. Furthermore, the image is processed with image processing software to obtain the bubble size and population, as detailed in [26]. The microbubbles produced in this measurement have a diameter of 0.0400 mm to 0.2770 mm. During the 70-day study, the lobsters were fed trash fish at 10% of their body weight [5].

Sampling

Daily measurements of water quality parameters, including dissolved oxygen (DO) temperature, pH, and salinity were conducted using the YSI Multi-parameter Model 556. Water sampling for water quality parameters, namely NH_3 , NO_2 , and NO_3 , was carried out every five days and analyzed using the spectrophotometric method [27]. Lobster biometry, which included total weight and carapace length, was recorded every ten days [7]. Hemolymph biochemicals, namely THC, glucose, total protein, and pH, were recorded on days 0, 3, 10, and every seven days after that. THC analysis was conducted

using a hemocytometer [28], while glucose and total protein levels were analyzed using spectrophotometry [29-30]. Hemolymph pH was analyzed using a Horiba-LAQUAtwin pH meter. The following formulas were utilized to calculate the Hemolymph biochemical parameters:

THC (cell mL⁻¹) = average number of cells counted x dilution factor x (volume of large squares counted)⁻¹

Glucose (mg dL⁻¹) = 100 x ΔA Sample x (ΔA standard)⁻¹

Total protein (mg dL⁻¹) = 200 x ΔA Sample x (ΔA standard)⁻¹

Statistical Analysis

The hemolymph biochemical and lobster production performance data collected in this study were subjected to statistical analysis using Minitab 16.1.1 Statistical Software. Statistical analysis involved ANOVA with an F test at a confidence level of 95%. The Tukey method determined the differences between treatments if the results were significant. Descriptive analysis was employed to analyze the water quality data recorded throughout the study.

Results and Discussion

Water Quality

The utilization of distinct water treatment units in the RAS significantly affected the water quality conditions during lobster cultivation, as evidenced by the findings presented in Fig. 1. The dissolved oxygen (DO) concentration ranged from 4.34 to 6.90 mg L⁻¹, which meets the requirement of fish culture that demands DO concentration higher than 3 mg L⁻¹ [31]. The measurement results indicated that treatment C (RAS with a combination of biofilter and microbubble) exhibited a higher DO concentration than the other treatments. The utilization of microbubbles facilitated the long-term water saturation and maintained the DO concentrations during the study [32]. The microbubbles with a size of ≤ 0.1 mm remained stable without merging between bubbles; thus, their solubility in water could be sustained for a longer period. Conversely, bubbles with a size of > 0.1 mm tended to ascend more rapidly and eventually spread on the water surface [33]. Sufficient dissolved oxygen (DO) concentration in water will lead to optimum metabolic processes, increase growth, and suppress the production of stress hormones [8].

The optimum pH values for clawed and spiny lobsters were 7.8-8.2 and 8.0-8.5, respectively [34]. Throughout the study, the pH value for all treatments was 7.3-7.8, which satisfies the optimum requirements for lobster cultivation. The water temperature conditions in all treatments were appropriate for lobster growth, as the range was between 26.71 to 28.14°C. However, treatments B and C exhibited higher water temperatures

than treatment A. The elevated water temperature in treatments B and C resulted from the heat produced by the submersible pump in the protein skimmer and microbubbles. The water salinity remained stable at 33.4-37.4 ppt in treatments A, B, and C throughout the study. The suitable salinity and range for *P. ornatus* and *P. Homarus rearing* are 34 \pm 0.1 ppt [35] and 25-35 ppt [36], respectively.

The ammonia levels ranged from 0.006-0.049 mg L⁻¹ during the study, indicating that the ammonia concentration tended to increase until the 35th day. The elevation of ammonia levels implies the accumulation of feed residues and feces. Nitrifying bacteria needed time to grow on the surface area of biofilter media, resulting in an incomplete nitrification process at the study's outset. A surge in ammonia levels in seawater RAS happens over 30 days, indicating that the nitrification process in the biofilter has yet to be established [37]. This phenomenon could be attributed to insufficient nitrifying bacteria in the media filter to perform nitrification.

Biological processes, such as nitrification, and physical approaches, such as air stripping, can degrade ammonia in water [38-39]. Foam fractionation, which utilizes an air-stripping mechanism, is commonly used to remove ammonia from the water. Small-sized microbubbles have a greater ability to remove ammonia in water. Microbubbles with a size of 0.60 mm can remove up to 89.35% of ammonia in water [40]. During the study, treatment C, equipped with microbubbles, had the lowest ammonia level. The microbubbles used in treatment C were 0.0400 mm - 0.2770 mm, allowing the ammonia stripping process to function effectively.

During the study, the nitrite level was positively correlated with ammonia concentration, which increased until the 35th day. The high nitrite level indicates that the conversion of ammonia to nitrate did not occur correctly due to a low number of nitrifying bacteria at the study's outset. The RAS system with microbubbles (treatment C) showed the lowest nitrite concentration, it is likely due to the higher solubility of oxygen and ammonia removal by microbubbles. Nitrite levels for lobster cultivation in RAS should be less than 5 mg L⁻¹, while nitrate levels are suggested to be less than 100 mg L⁻¹ [3, 34]. Overall, this study's nitrite and nitrate levels still meet the water requirements for lobster cultivation.

Hemolymph Biochemical

The use of different RAS treatments led to varying water quality conditions that affected the lobster's hemolymph biochemical, as shown in Fig. 2. The THC and glucose levels in treatment C were lower and more stable compared to treatments A and B. It occurred because the ammonia levels produced in treatment C during the study were lower than those in treatments A and B. Exposure to high levels of ammonia can alter the expression of certain proteins in lobsters, specifically

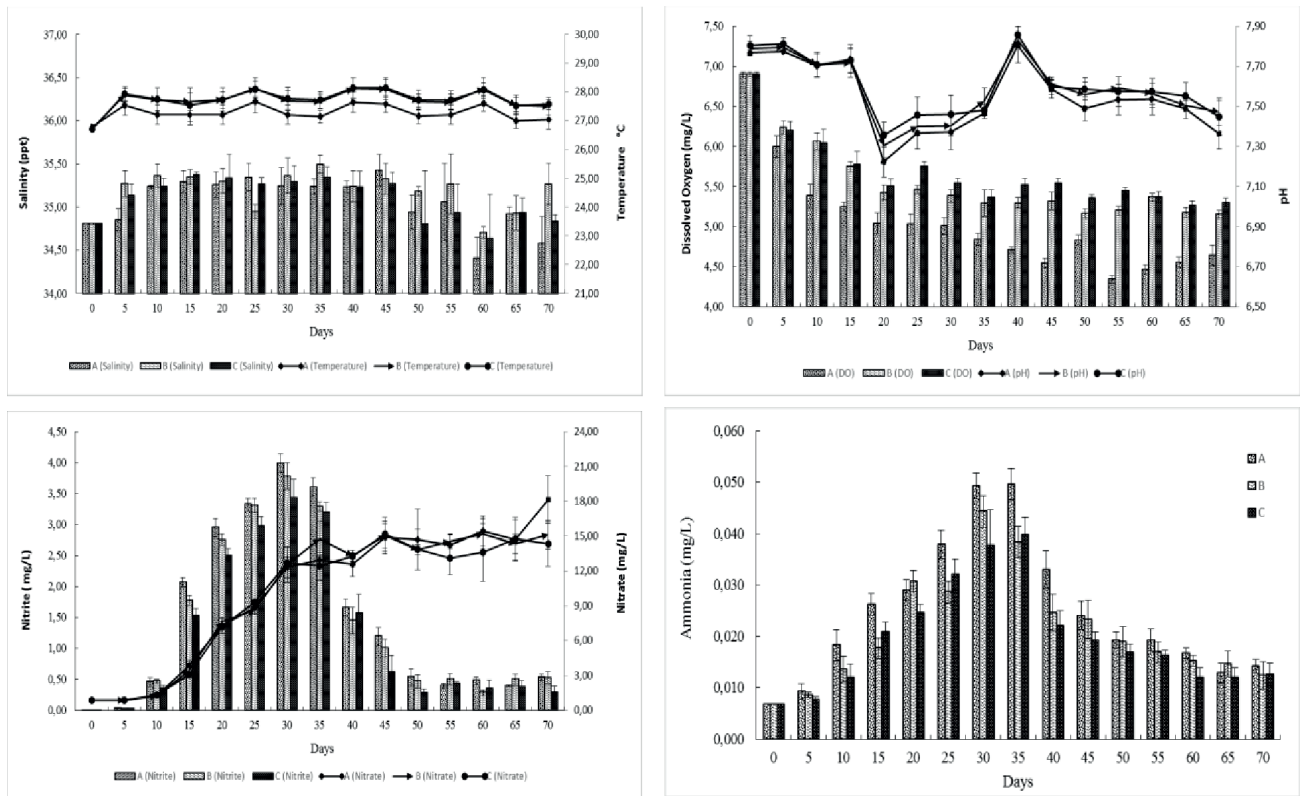


Fig. 1. Water quality conditions during the study: RAS with a biofilter (A), RAS with a combination of biofilter & protein skimmer (B), and RAS with a combination of biofilter & microbubbles (C).

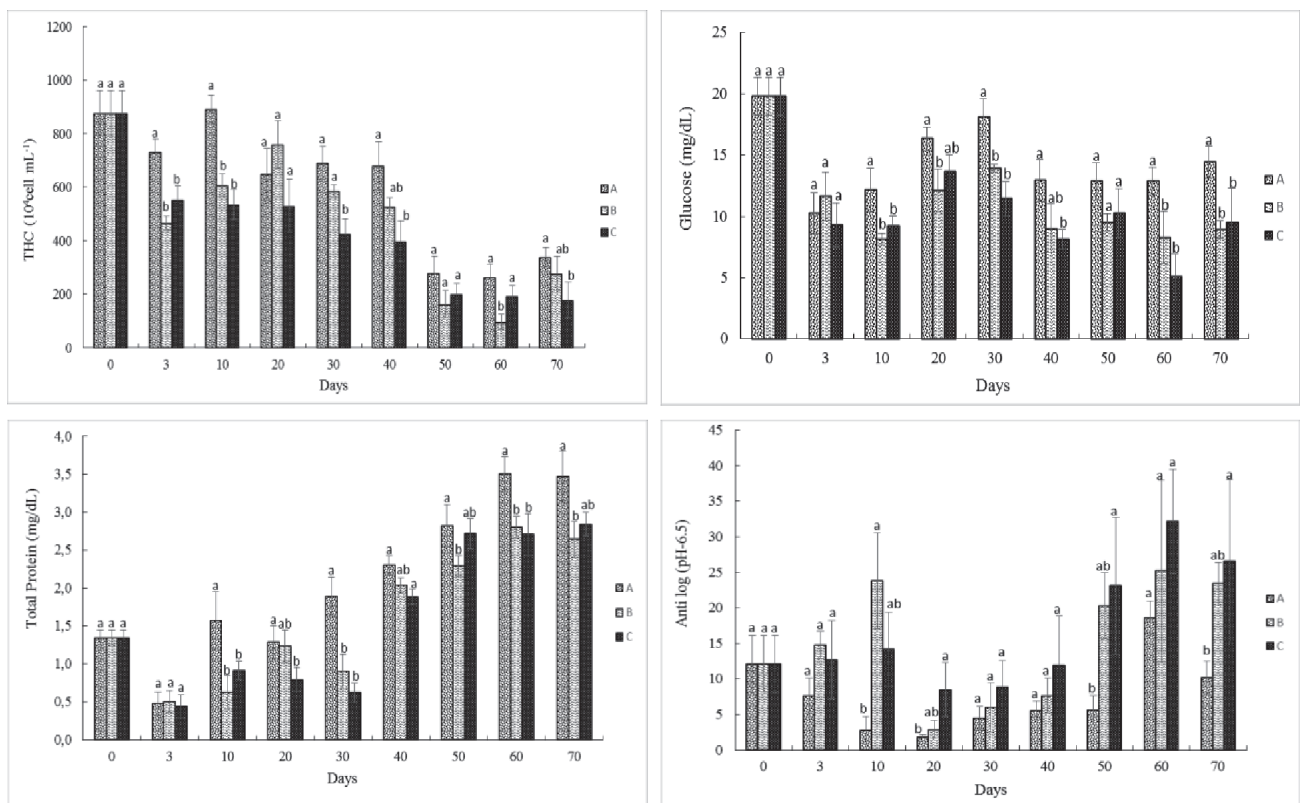


Fig. 2. Hemolymph of lobster biochemical contents: RAS using a biofilter (A), RAS with a combination of biofilter & protein skimmer (B), and RAS with a combination of biofilter & microbubbles (C). The use of distinct lowercase letters in the graph indicates significant difference (p<0.05).

Table 1. The production performances of *P. Homarus* after cultured 70 days in different RAS.

Production performance	RAS Treatment		
	Biofilter	Biofilter & Skimmer	Biofilter & Microbubble
Final weight (g)	5.47±0.11 ^b	5.81±0.15 ^a	5.95±0.17 ^a
Final carapace length (mm)	18.51±0.04 ^b	18.87±0.06 ^a	19.12±0.16 ^a
Survival Rate (%)	67.00±2.00 ^b	73.33±1.53 ^a	75.67±1.15 ^a
SGR _w (% day ⁻¹)	1.35±0.03 ^b	1.44±0.03 ^{ab}	1.47±0.04 ^a
SGR _{CL} (% day ⁻¹)	0.72±0.00 ^b	0.74±0.00 ^a	0.76±0.01 ^a
FCR	8.36±0.27 ^a	7.99±0.21 ^a	8.29±0.17 ^a

^{a-b} Significant difference between treatment

those related to stress response, amino acid metabolism and detoxification [41].

Additionally, the increased glucose concentration in hemolymph can be directly affected by several stress hormones, including neuropeptide crustacean hyperglycemic hormone (CHH), mandibular organ inhibitory hormone (MOIH), and neuropeptide molting inhibitory effect hormone (MIH) [8, 42].

The protein present in the hemolymph of crustaceans is a colloidal form of amino acids found in plasma. Hemolymph protein levels can fluctuate due to environmental changes. On the third day of the study, there was a decrease in total protein concentration, followed by a decrease in glucose and THC concentrations as lobsters started adapting to the new environment. However, from day three until the end of the study period, there was a gradual increase in the lobster's total protein concentration, indicating an increase in total hemolymph protein content with lobster growth. This increase in total protein metabolic activity was in response to increased lobster homeostasis [7], which is necessary for oxygen transportation. Treatment C showed the lowest and most stable trend in total protein content compared to other RAS treatments.

Hemolymph pH is a measurable parameter for determining the stress levels in crustaceans. Several studies have been conducted on the effect of various factors, including holding stressor and transportation of *Jasus edwardsii* [25, 43], ocean acidification in *Chionoecetes bairdi* [44], and differences in alkalinity in *P. Homarus* [7], on the hemolymph pH parameter. Treatment A exhibited the lowest hemolymph pH value, which reached 6.77. The acidic hemolymph pH value in treatment A is a stress response to the increased ammonia concentration in the environment. Overall, the hemolymph pH levels in treatments A and B are acidic compared to treatment C, indicating that using RAS with C treatment resulted in lower stress levels than other treatments.

Lobster Production Performance

The productivity of various types of RAS can be evaluated by examining the survival rate and specific growth rate (SGR) of the tested lobsters during the study period. Based on Table 1, treatment C showed the highest SGR weight (SGR_w) and SGR carapace length (SGR_{CL}) at 1.47±0.04% day⁻¹ and 0.76±0.01% day⁻¹, respectively. These findings are superior to the SGR_w values of *P. cygnus* and *P. Homarus* fed wet feed, which was 1.25±0.03% day⁻¹ [45] and 1.29±0.02% day⁻¹ [5], respectively. Similarly, the SGR_{CL} value was better to the previous *P. homarus* puerulus nursery study, which 0.73%±0.01 day⁻¹ [5]. The RAS systems that applied filters and microbubbles demonstrated the highest survival rate of 75.67±1.15%. Maintaining environmental quality and reducing stress levels can enhance the lobsters' immunological abilities against disease and promote better growth and survival [5, 34].

Conclusions

According to the findings of this study, treatment C (RAS incorporating both biofilter and microbubbles) yielded the highest productivity of *P. homarus* lobster seeds compared to the other treatment. At the same time, it maintains the best water quality and minimizes the hemolymph stress response (measured by THC, glucose, total protein, and pH).

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Conflict of Interest

The authors declare no conflict of interest.

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