Original Research

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

Eddy Supriyono[1]*, Kukuh Adiyana[2], Lolita Thesiana[2]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB University), Bogor, Indonesia
[2]Research Center for Fishery, National Research and Innovation Agency (BRIN), Bogor, Indonesia

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±1.15% and a Specific Growth Rate (SGR) of 1.47±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

*e-mail: eddysupriyonoipb@gmail.com

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 cm^3 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 performancedata 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 28.14ºC. 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±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
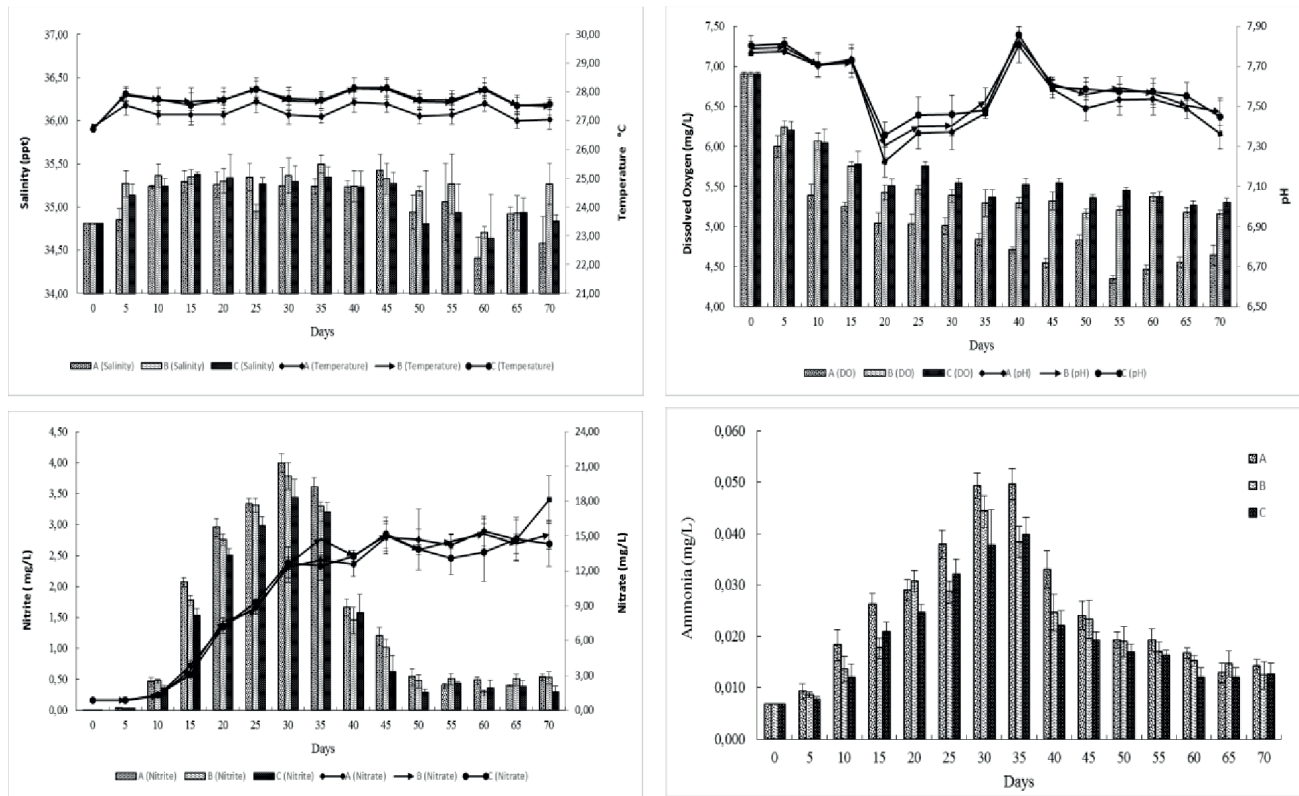
Fig. 1.Water quality conditions during the study: RAS with a biofilter (A), RAS with a combination of biofilter & protein skimmer (B), and RAS with a combination of biofilter & microbubbles (C).
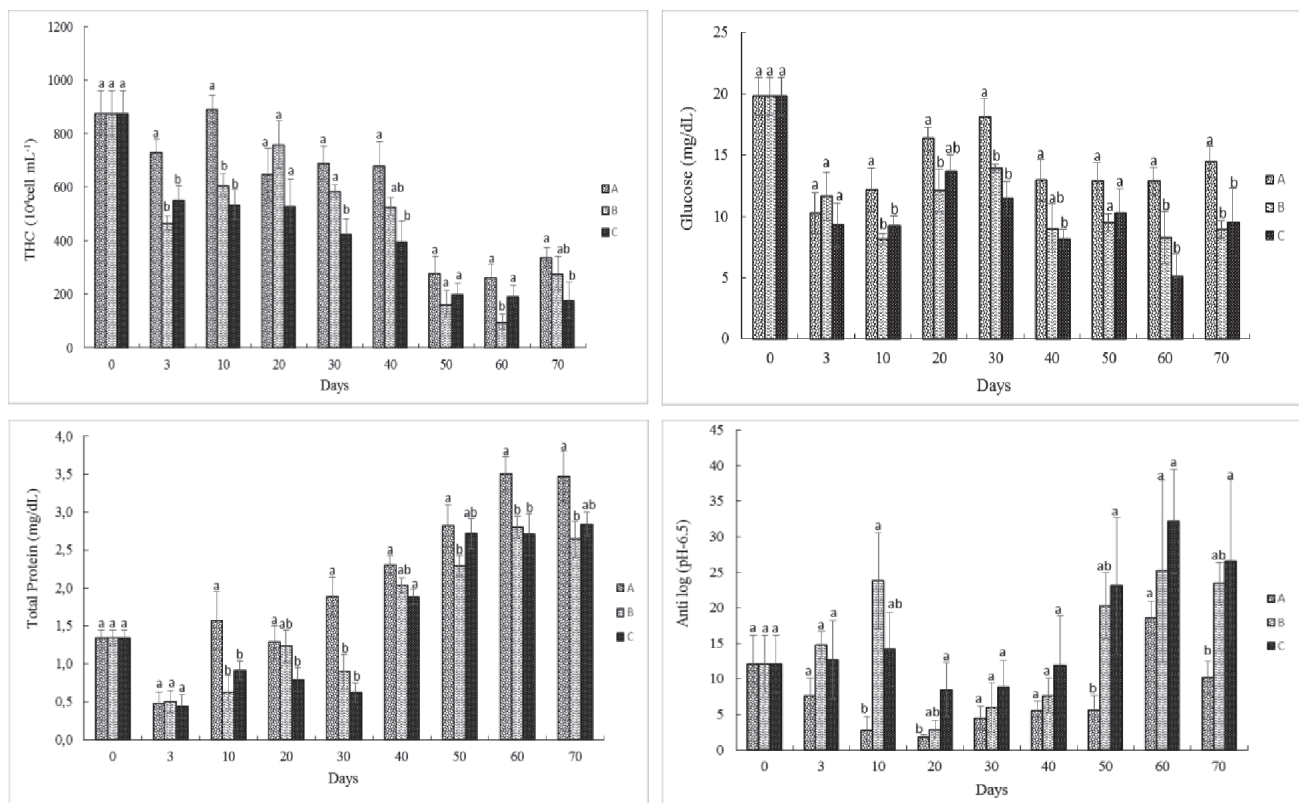

Fig. 2. Hemolymph of lobster biochemical contents: RAS using a biofilter (A), RAS with a combination of biofilter & protein skimmer (B), and RAS with a combination of biofilter & microbubbles (C). The use of distinct lowercase letters in the graph indicates significant difference ($p < 0.05$).

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

Production performance	RAS Treatment		
	Biofilter	Biofilter & Skimmer	Biofilter & Microbuble
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).

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

References

1. PETTERSSON S.J., LINDHOLM-LEHTO P.C., PULKKINEN J.T., KIURU T., VIELMA J. Effect of ozone and hydrogen peroxide on off-flavor compounds and water quality in a recirculating aquaculture system, Aquacultural Engineering, Volume **98**, 102277, **2022**.

2. KAMALI S., WARD V.C.A., RICARDEZ-SANDOVAL L. Dynamic modeling of recirculating aquaculture systems: Effect of management strategies and water quality parameters on fish performance, Aquacultural Engineering, **99**, 102294, **2022**.

3. FAO, The state of world fisheries and aquaculture. Towards blue transformation. Rome, FAO, **2022**.

4. SUPRIYONO E., PRIHARDIANTO R.W., NIRMALA K. The stress and growth responses of spiny lobster *Panulirus homarus* reared in recirculation system equipped by PVC shelter. AACL Bioflux, **1**, 147, **2017**.

5. SUPRIYONO E., THESIANA L., ADIYANA K. Biochemical response and production performance of spiny lobster *Panulirus homarus* seed reared with different shelter materials. Indian Journal of Fisheries, **69** (3), 59, **2022**.

6. DJAI S., SUPRIYONO E., NIRMALA K., ADIYANA K. Total hemocyte count and hemolymph glucose concentration response of spiny lobster *Panulirus homarus* on ratio of shelter. Jurnal Ilmu dan Teknologi Kelautan Tropis, **9**, 125, **2017** [In Indonesian].

7. SUPRIYONO E., SOELISTYOWATI D.T., ADIYANA K., THESIANA L. The effects of alkalinity on production performance and biochemical responses of spiny lobster *Panulirus homarus* reared in a recirculating aquaculture system. Israeli Journal of Aquaculture, **74**, 1, **2022**.

8. SUBHAN R.Y., EDDY S., WIDANARNI, DANIEL D. Grow-out of spiny lobster *Panulirus* sp. with high stocking density in controlled tanks. Jurnal Akuakultur Indonesia, **17**, 53, **2018** [In Indonesian].

9. SONG C., ZHOU Y., LI X., DONG R., SONG X., HUANG Z., DONG D. Interaction among biofilter microbiome, fecal metabolome and water quality and regulation of sewage discharge in the recirculating aquaculture system of Apostichopus japonicus, Journal of Environmental Management, **330**, 117100, **2023**.

10. HÜPEDEN J., WEMHEUER B., INDENBIRKEN D., SCHULZ C., SPIECK E. Taxonomic and functional profiling of nitrifying biofilms in freshwater, brackish and marine RAS biofilters. Aquac. Eng., **90**, 102094, **2020**.

11. BARTELME R.P., MCLELLAN S.L., NEWTON R.J. Freshwater recirculating aquaculture system operations drive biofilter bacterial community shifts around a stable nitrifying consortium of ammonia-oxidizing archaea and comammox Nitrospira. Front. Microbiol, **8** (101), 1, **2017**.

12. TIMMONS M.B., GUERDAT T., VINCI B.J. Recirculating Aquaculture, 4th ed., Ithaca Publishing Company: Ithaca New York, USA, **2018**.

13. AALTO S.L., SYROPOULOU E., DE JESUS GREGERSEN K.J., TIIROLA M., BOVBJERN PEDERSEN P., PEDERSEN L. Microbiome response to foam fractionation and ozonation in RAS. Aquaculture, **550**, 1-9, **2022**.

14. GESTO M., GREGERSEN K.J.J., PEDERSEN L.F. Effects of ozonation and foam fractionation on rainbow trout condition and physiology in a small-scale freshwater recirculation aquaculture system, Aquaculture, **557**, 738312, **2022**.

15. RANJAN R., MEGARAJAN S., XAVIER B., RAJU S.S., GHOSH S., GOPALAKRISHNAN A. Design and performance of recirculating aquaculture system for marine finfish broodstock development, Aquacultural Engineering, **85**, 90, **2019**.

16. JUWANA W.E., WIDYATAMA A., DINARYANTO O., BUDHIJANTO W., INDARTO, DEENDARLIANTO. Hydrodynamic characteristics of the microbubble dissolution in liquid using orifice type microbubble generator. Chem. Eng. Res. Design, **141**, 436, **2019**.

17. HERIYATI E., RUSTADI R., ISNANSETYO A., TRIYATMO B., ISTIQOMAH I. Microbubble aerator test and harvest target prediction based on oxygen consumption of red tilapia (*Oreochromis* sp.). AACL Bioflux, **14**, Issue 5, **2021**.

18. HERIYATI E., RUSTADI R., ISNANSETYO A., TRIYATMO B., ISTIQOMAH I., DEENDARLIANTO D., BUDHIJANTO W. Microbubble Aeration in A Recirculating Aquaculture System (RAS) Increased Dissolved Oxygen, Fish Culture Performance, and Stress Resistance of Red Tilapia (*Oreochromis* sp.). Trends in sciences, **19** (20), 6251 **2022**.

19. LIM Y.S., GANESAN P., VARMAN M., HAMAD F.A., KRISHNASAMY S. Effects of microbubble aeration on water quality and growth performance of *Litopenaeus vannam*ei in biofloc system. Aquac. Eng., **93**, 1, **2021**.

20. PRAMA E.A., SUPRIYONO E., NIRMALA K., ADIYANA K. Impact of nursery using different physical filtration system on hemolymph glucose level and survival rate of spiny lobster juvenile *Panulirus homarus*. Jurnal Ilmu dan Teknologi Kelautan Tropis, **9** (2), 569, **2017** [In Indonesian].

21. ZHANG C., WANG X., WANG C., SONG Y., PAN J., SHI Q., QIN J., CHEN L. Gamma-aminobutyric acid regulates glucose homeostasis and enhances the hepatopancreas health of juvenile Chinese mitten crab (*Eriocheir sinensis*) under fasting stress. General and Comparative Endocrinology, **303**, 113704 , **2021**.

22. POWELL A., COWING D. M., ERIKSSON S. P., JOHNSON M. L. Stress response according to transport protocol in Norway lobster, *Nephrops norvegicus*. Crustacean Research, **46**, 17, **2017**.

23. SUPRIYONO E., RASUL BUDIARDI T., HASTUTI Y.P., ADIYANA K., THESIANA L. A study on the effect of different colours of culture tanks in nursery, on the production performance, biochemical composition of flesh and physiological responses of whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture Research, **52**, 4086, **2021**.

24. JARRED L. KNAPP, LUTZ AUERSWALD, LOUWRENS C. HOFFMAN, BRETT M. MACEY.Effects of chronic hypercapnia and elevated temperature on the immune response of the spiny lobster, *Jasus lalandii*. Fish & Shellfish Immunology, Volume **93**, 752, **2019**.

25. DAY R.D., FITZGIBBON Q. P., GARDNER C. The impact of holding stressors on the immune function and haemolymph biochemistry of Southern Rock Lobsters (*Jasus edwardsii*), Fish & Shellfish Immunology, Volume **89**, 660, **2019**.

26. XU R., WANG Y., LI Z. Exploration of particle technology in fine bubble characterization. Particuology, **46**, 109, **2019**.

27. APHA, Standard methods for the examination of water and wastewater. 22nd edition., American Public Health Association, Water Environment Federation, APHA Pr: Washington DC, USA, **2012**.

28. BLAXHALL P.C., DAISLEY K.W. Routine haematological methods for use with fish blood. Journal of Fish Biology, **5**, 577, **1973**.

29. WEDEMEYER G.A., YASUTAKE W.T. Clinical methods for the assessment of the effects of environmental stress on fish health. Department of the Interior Fish and Wildlife Service: Washington DC, USA, **1977**.

30. LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. Protein Measurement with the Folin Phenol Reagent. Journal of Biological Chemistry, **93**, 265, **1951**.

31. WHANGCHAI N., KLAHAN R., BALAKRISNAN D., UNPAPROM Y., RAMARAJ R., PIMPIMOL T. Development of aeration devices and feeding frequencies for oxygen concentration improvement in 60-tones freshwater recirculating aquaculture and biofloc ponds of Asian seabass (*Lates calcarifer*) rearing. Chemosphere, volume **307** part 3, 135761, **2022**.

32. DAHRAZMA B., NAGHEDINIA A., GORJI H.G., SAGHRAVANI S.F. Morphological and physiological responses of *Cucumis sativus* L. to water with micro-nanobubbles. J. Agr. Sci. Tech., **21**, 181, **2019**.

33. TAKAHASHI M. Potential of Micro-Bubbles in Aqueous Solutions: Electrical Properties of the Gas-Water Interface. J. Phys. Chem. B, **109**, 21858, **2005**.

34. RADHAKRISHNAN E.V., PHILLIPS B.F., ACHAMVEETIL G. Lobsters: Biology, Fisheries and Aquaculture. Springer Nature Singapore Pte Ltd: Singapore, **2019**.

35. UY T., FITZGIBBON Q.P., CODABACCUS B.M., SMITH G.G. Thermal physiology of tropical rock lobster (*Panulirus ornatus*); defining physiological constraints to high temperature tolerance. Aquaculture, **569**, 739357, **2023**.

36. CHEN H., PAN J., WANG Y., QIAO Y., HAN F., XU C., FARHADI A., LI E. Growth, health status and gut microbiota of the scalloped spiny lobster (*Panulirus homarus*) at different salinities. Aquaculture, **562**, 738779, **2023**.

37. THESIANA L., PAMUNGKAS, A. Performance test of recirculating aquaculture system (RAS) technology to water quality condition in spiny lobster *Panulirus homarus* nursery. Jurnal Kelautan Nasional, **10** (2), 65, **2015** [In Indonesian].

38. KINIDI L., TAN I.A.W., WAHAB N.B.A., TAMRIN K.F.B., HIPOLITO C.N., SALLEH S.F. Recent development in ammonia stripping process for industrial wastewater treatment. International Journal of Chemical Engineering, 1-14, **2018**.

39. ZANGENEH A., SABZALIPOUR S., TAKDATSAN A., YENGEJEH R.J., KHAFAIE M.A. Ammonia removal form municipal wastewater by air stripping process: An experimental study. South African Journal of Chemical Engineering, **36**, 134, **2022**.

40. AN S., JIN Q. Significant removal of ammonia nitrogen in low concentration from aqueous solution at low pH by advanced air stripping. Environmental Science and Pollution Research, **28**, 35113, **2020**.

41. REN X., JIA S., GAO B., ZHOU Q., XU Y., LIU P., LI J. Application of proteomics and metabolomics to assess ammonia stress response and tolerance mechanisms of juvenile ornate rock lobster *Panulirus ornatus*. Science of The Total Environment, **837**, 155751, **2022**.

42. VASUDEVAN S., RAJENDRAN S. Thermal stress induced hyperglycemia in the blue swimmer crab, *Portunus pelagicus*. Journal of Thermal Biology, **100**, 103076, **2021**.

43. SIMON C. J., MENDO T. C., GREEN B. S., GARDNER C. Predicting transport survival of brindle and red rock lobsters *Jasus edwardsii* using haemolymph biochemistry and behaviour traits. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, **201**, 101, **2016**.

44. MESECK S.L., ALIX J.H., SWINEY K.M., LONG W.C., WIKFORS G.H., FOY R.J. Ocean Acidification Affects Hemocyte Physiology in the Tanner Crab (*Chionoecetes bairdi*). PLoS One. **11** (2), e0148477, **2016**.

45. JOHNSTON D., MELVILLE-SMITH R., HENDRIKS B., MAGUIRE G.B., PHILLIPS B. Stocking density and shelter type for the optimal growth and survival of western rock lobster *Panulirus cygnus* (George). Aquaculture, **260**, 114, **2006**.