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Evaluation of Probiotics Ability to Enhance Population Density, Growth Rate, and Neonates Production of *Moina micrura* in Different Environmental Parameters

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ABSTRACT

Salinity, light intensity, and oxygen concentration are key environmental factors that significantly affect biological processes and the composition and dispersion of *Moina* biomass. Evaluating the effectiveness of probiotic enrichment in improving population density, growth rate, and neonate production can provide valuable details on the effectiveness of probiotics in enhancing the resilience and viability of *Moina micrura* under suboptimal circumstances. The purpose of this research project is to assess the efficacy of two probiotics, *Bacillus pocheonensis* strain S2 and *Lysinibacillus fusiformis* strain A1, in improving the population density, growth rate, and reproductive output in *M. micrura* across various environmental conditions. *Moina micrura* were treated with each probiotic at a volume of 5 x 10^5 CFU/ml under different levels of salinity (0, 2, 4, and 6 ppt), light intensity (800, 1,000, 1,500, and 2,000 lux), and oxygen concentration (80, 70, 60, and 50%). The results indicated that *M. micrura* treated with *L. fusiformis* A1 at 0 ppt attained the highest population density (6 ± 0.90 Ind./ml), growth rate (0.355 ± 0.030 µ), and number of offspring production (5 ± 0.75 Ind./ml). The highest point of population density (5 ± 0.07 Ind./ml), growth rate (0.381 ± 0.002 µ) and

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number of offspring (7 ± 0.41 Ind./ml) of *M. micrura* were obtained while treated with *B. pocheonensis* S2 at light intensity of 1,500 lux. Similarly, the highest population density (5 ± 0.60 Ind./ml), growth rate (0.365 ± 0.190 μ), and offspring production (2 ± 0.25 Ind./ml) of *M. micrura* were observed during enrichment with *B. pocheonensis* S2 at 70% oxygen concentration. Therefore, these results suggested that the optimum conditions for enriching *M. micrura* with *B. pocheonensis* S2 are salinity of 0 ppt, 70% oxygen concentration, and a light intensity level of 1,500 lux.

Keywords: *Bacillus pocheonensis*, enrichment, environmental parameters, growth rate, *Lysinibacillus fusiformis*, *Moina micrura*

INTRODUCTION

Many species in aquaculture can be effectively nourished with live food organisms such as phytoplankton and zooplankton as their initial feed source. These individuals are often called "living capsules of nutrition" due to their rich content of essential macro and micronutrients. Live feed can freely move within the water column and remains consistently accessible to fish and shellfish larvae, whose jerking movements stimulate larval feeding responses (Wikfors, 2004). Live feeds are easily ingested and digested (Rasdi et al., 2020), have no negative effect on water quality (Watanabe et al., 1978) and consist of crucial elements that facilitate growth. Conventional live feed such as copepods, freshwater cladocerans, and rotifers exhibit a significant capacity for reproduction, a capacity to reproduce rapidly, and the ability to live in harsh environments (Neelakantan et al., 1988). Most fish larvae favor cladocerans and have been utilized effectively as starter food in fish farming, hence contributing to the successful production of high-quality larvae (Coronado & Camacho, 2014; Taghavi et al., 2013). *Moina* is one of the largest genera of Cladocera found in North and South America, Australia, China, tropical Asia as well as Eurasia waters (Alonso et al., 2019; Bekker et al., 2016; Wang et al., 2010). *Moina* possesses the capability to adapt to numerous environmental parameters (Rizo et al., 2017) and has been used effectively in inland aquaculture as a live diet for larval fish and shrimp (Gogoi et al., 2016; Saini et al., 2013).

Moreover, *Moina* has been used as a substitute for *Artemia* in the production of red sea bream (Kotani et al., 2016). *Moina* is small, has a short embryonic stage, easy to handle, reproduces easily (Rottmann et al., 2003) and has abundant energy storage (Okunsebor & Ayuma, 2010; Sipaúba-Tavares & Bachion, 2002). *Moina*'s nutritional content varies considerably depending on the stage of their life cycle and the kind of nourishment they get (Gogoi et al., 2016). On average, *Moina* has 50% dry protein content, whereas adults often have a greater fat content (20–27%) than juveniles (4–6%) and their fatty acid content varies depending on the types of media used (Rottmann et al., 2003). However, *Moina*

does not meet the larval fish and crustaceans' requirement for highly unsaturated fatty acids (HUFA) (Kamrunnahar et al., 2019). Hence, enrichment of *Moina* is necessary to further enhance their nutritional values and obtain the optimum level needed for fish growth and survival. However, *Moina* plays a crucial role in the diet of finfish larvae and larger crustaceans. However, the inconsistent availability of *Moina* has been a significant obstacle in hatcheries, leading to the limited ability to produce high-quality fish offspring for the advancement of aquaculture (Kagali et al., 2022). Manipulating microbial community composition has received much attention to improve culture stability and diminish the spreading of detrimental bacteria (Bentzon-Tilia et al., 2016). The use of probiotics has been proven to successfully improve the nutritional value of live feed (Carter, 2015) and subsequently increase survival, stress tolerance (Singh et al., 2019), and growth performance (Pratiwy et al., 2021) of larvae.

Salinity, light, and oxygen are essential ecological elements influencing zooplankton development and reproduction (Nandini & Sarma, 2000; Rose et al., 2002; Sarma et al., 2003). These factors influence zooplankton differently (Peck et al., 2008; Zhang & Baer, 2000). In some cases, light could be a more important factor than salinity (Boolootian, 1963) since it serves as the primary recurring motion of numerous crustaceans, influencing the development, maturity, reproductive processes, and feeding behaviors of aquatic invertebrates (Buikema Jr., 1973; Miliou, 1992). The richness and variety of zooplankton and phytoplankton in the environment are influenced by salinity and diets. As a result, changes in water salinity can manipulate the primary taxonomic composition and ecological activities such as primary productivity, decomposition, nutrient cycles, and functioning of the food web (Yuslan et al., 2021). Different salinity concentrations also directly affect cladocerans' survival, growth, reproduction rate, and fatty acid levels (Rasdi et al., 2019). Oxygen concentration is one of the main limiting factors influencing the growth and reproduction of live feed (Svetlichny & Hubareva, 2002). The metabolic rate of cladocerans is lowered by oxygen deficiency (Wong, 2011) thereby reducing detoxification (Cairns Jr. et al., 1975). Anthropogenic activity has changed the aquatic environment (El-Gamal et al., 2014) and significantly affects the diversity of freshwater species, specifically aquatic organisms that cannot migrate (Rizo et al., 2017).

Thus, understanding these conditions would be beneficial for consistently ensuring the production of high-quality live foods in a controlled environment. This study aims to gauge how probiotics could positively impact the population density, specific growth rate, and production of neonates in *M. micrura* under varying environmental challenges.

MATERIALS AND METHODS

Culture of Probiotic Strains

Two probiotic strains derived from microalgae, specifically *Amphora* sp. and *Spirulina* sp., which were previously isolated, were received from the Fish Health Laboratory, Faculty of Agriculture, Universiti Putra Malaysia (Nur Natasya Ain, 2018). The two strains were identified as *Lysinibacillus fusiformis* strain A1 (GenBank accession number: MK764897) and *Bacillus pocheonensis* strain S2 (GenBank accession number: MK764898). These strains were selected based on their probiotic attributes and strong antagonistic activities toward two marine disease agents, *Vibrio harveyi* and *Vibrio parahaemolyticus* (Rosland et al., 2021) and two freshwater disease agents, *Aeromonas hydrophila* and *Streptococcus agalactiae* (Samat et al., 2021). These probiotic strains were initially cultured on tryptic soy agar (TSA, Millipore®, Germany). After overnight incubation, pure colonies of *L. fusiformis* and *B. pocheonensis* were inserted individually inside a 30 ml inoculum tube of 10 ml tryptic soy broth (TSB, Millipore®, Germany). The cultures were left to incubate for 24 hr at ambient temperature with constant agitation at 250 rpm. The probiotics were harvested through centrifugation at 3,074 x *g* for 10 min. The residue was discharged, and the settling was suspended in sterile distilled water. The probiotics' concentration was measured individually using a UV-1800 spectrophotometer (Eppendorf, Germany) at 550 nm. For experimental use, the end-state proportions of *L. fusiformis* A1 and *B. pocheonensis* S2 were altered to 5×10^5 CFU/ml (Samat, Yusoff, Chong, et al., 2020) within the experimental receptacles.

Chlorella vulgaris Culture

Chlorella vulgaris was cultivated in 1 L conical flasks containing Bold's Basal Medium, which was prepared by referring to Natrah et al. (2007)'s study. Mild aeration was supplied continuously using an air pump. The *C. vulgaris* concentration was ascertained with the aid of an enhanced 0.1 mm Neubauer chamber and viewed using a light microscope as per the subsequent formula:

$$\text{Density, d } \left(\frac{\text{cells}}{\text{ml}}\right) = \frac{\text{Average number of cells per square}}{4 \times 10^{-6}}$$

where, 4×10^{-6} is the sample volume from across the small square area, equal to 0.004 mm^3 (0.2 x 0.2 x 0.1) measured in cm^3 (ml).

Moina micrura Culture

Moina micrura was cultivated in pond water that had been filtered and sterilized (0.45 µm Whatman® fiber glass filters, United Kingdom). The starting culture was kept in a plastic aquarium with a capacity of 2 L and provided with daily feeding of *C. vulgaris* at a concentration of 5×10^4 cells/ml (Munirasu et al., 2016) for two weeks prior to the commencement of the experiment. The concentration of *C. vulgaris* was determined by applying the method and formula described in *C. vulgaris* culture.

Experimental Design

The impacts of different salinities (0, 2, 4, and 6 ppt) (Sarma et al., 2006), light intensities (800, 1,000, 1,500, and 2,000 lux) (Serra et al., 2019), and oxygen concentrations (80, 70, 60, and 50%) (Svetlichny & Hubareva, 2002) on *M. micrura* with probiotics enrichment were observed. Two separate experimental setups were designed. The first experiment determined the population growth of *M. micrura*, while the second experiment evaluated the production of neonates throughout their lifetime. All treatments were run with triplicates.

Salinity

For experiment one, ten one-day-old *M. micrura* were allocated 50 ml of Falcon tube comprising 40 ml of sterilized freshwater. On day one, a probiotic was added at a concentration of 10^5 CFU/ml, and *Moina* was then fed with *C. vulgaris* once a day at 10^4 CFU/ml. The cultures were maintained for 12 days at the desired salinity level (0, 2, 4, 6, and 8 ppt) at 25°C and were exposed to 12 hr light and 12 hr dark. The salinity level was adjusted using sodium chloride (NaCl, Millipore®, Denmark). The concentrations of NaCl were determined using a refractometer (ATAGO, Japan). Water exchange and re-supplementation of probiotics were done once every two-day interval. The *M. micrura* population density was measured daily by extracting 1 ml of the well-mixed culture and analyzing the sample within a Petri dish. The formula below was employed to compute the population growth rate:

$$\text{Population growth rate, } \mu = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1}$$

where, X_1 = the number of *M. micrura* at the beginning of the selected time interval; X_2 = the number of *M. micrura* at the end of the selected time interval; t_1–t_2 = the selected time (in days).

For experiment two, five one-day-old *M. micrura* were allocated in a 50 ml Falcon tube with 40 ml of purified sterile freshwater. Similar conditions were applied to the first experiment. The presence of neonates was monitored in the morning every day and discharged after the count. The experiment continued until the natural death of all *M. micrura* occurred.

Light Intensity

For experiment one, ten newly hatched *M. micrura* were allocated into a 50 ml Falcon tube containing 40 ml of sterilized freshwater. On day one, each probiotic was added individually at 10^5 CFU/ml, and *M. micrura* was given microalgae *C. vulgaris* once a day at 10^4 CFU/ml. The cultures were maintained for 12 days at different light intensities (600, 800, 1,000, 1,500, and 2,000 lux) at 25°C and experienced a cycle of 12 hr of light followed by 12 hr of darkness. The light intensity levels were adjusted using a digital lux meter (LX1020B, Redmark Industry, Malaysia). The light-intensity experiment was carried out in a dark room, and an adjustable lamp was used as the light source. Water exchange and re-supplementation of probiotics were

done every two days. The daily evaluation of *M. micrura* population density involved extracting 1 ml from the thoroughly mixed culture and scrutinizing the sample within a Petri dish. The population's growth rate was determined using the identical section salinity formula.

For experiment two, just one day old, five newly hatched *M. micrura* were placed into a 50 ml Falcon tube containing 40 ml of sterilized freshwater. Similar conditions were applied to the first experiment. The presence of neonates was monitored in the morning every day and discharged after the count. The experiment continued until the natural demise of all *M. micrura*.

Oxygen Concentration

For experiment one, ten *M. micrura* that were one day old, were allocated in a 50 ml Falcon tube consisting of 40 ml of sterilized freshwater. On day one, each probiotic was added individually at 10^5 CFU/ml, and *M. micrura* was nourished with microalgae of the species *C. vulgaris* once a day at 10^4 cells/ml. The cultures were sustained for 12 days at different oxygen concentrations (80, 60, 50, 40, and 30%) at 25°C and subjected to a 12-hr light and 12-hr dark cycle. The oxygen concentrations were determined using a dissolved oxygen meter (YSI 550A, USA). Water exchange and re-supplementation of probiotics were done every two days. Each day, the population density of *M. micrura* was assessed by extracting 1 ml from the well-mixed culture and analyzing the specimen within a Petri dish. The population growth rate was determined using the identical formula under section salinity.

For experiment two, five one-day-old *M. micrura* were allocated in a 50 ml Falcon tube containing 40 ml of sterilized freshwater. Similar conditions were applied to the first experiment. The presence of neonates was monitored in the morning every day and discharged after the count. The experiment continued until all the *M. micrura* died naturally.

Statistical Analysis

All the data was analyzed using a one-way analysis of variance (ANOVA). Multiple comparison tests (Tukey's test) at the 0.05 probability level were performed to identify a significant difference between treatments. All statistical tests were performed using IBM SPSS statistic V27.0 software.

RESULTS

Salinity

The effects of different salinities on population density, population growth rate, and neonates' production of *M. micrura* throughout the culture period were presented in Figures 1, 2A, and 3A, respectively. Results showed that *M. micrura* treated with *L. fusiformis* A1 at 0 ppt obtained the maximum population density (6 ± 0.90 Ind./ml) on day eleven of culture (Figure 1A). Similarly, *M. micrura* treated with *L. fusiformis* A1 at 0 ppt reached the peak growth rate (0.355 ± 0.030 µ), significantly higher ($p < 0.05$) contrasted with the control group having akin salinity concentration (Figure 2A). Additionally, treatment with *L. fusiformis* at 0 ppt produced the highest number of offspring (5 ± 0.75 Ind./ml, Figure 3A) compared to other treatments

at all salinity concentrations (Figure 3A). Meanwhile, *M. micrura* was treated with both probiotic strains at 6 ppt and died on the 5th day of the experiment (Figure 1D), whereby no population growth and neonate production were observed. Regardless of salinity concentrations, enrichment of *M. micrura* with either *L. fusiformis* A1 or *B. pocheonensis* S2 showed a higher growth rate and neonate production compared to the control group. However, the data were not statistically significant for some treatments ($p > 0.05$) (Figures 2A and 3A).

Light Intensity

Results of the effects on different light intensity levels for population density, growth rate, and neonate production of *M. micrura* were presented in Figures 2B, 3B, and 4 respectively. The ultimate peak population density of *M. micrura* was obtained at 1,500 lux when treated with *B. pocheonensis* S2 (5 ± 0.07 Ind./ml) on day ten of culture (Figure 4C). Similarly, the highest growth rate (0.381 ± 0.002 µ) was obtained at 1,500 lux when enhanced with *B. pocheonensis* S2 (Figure 2B). Although the utmost neonate's production (7 ± 0.41 Ind./ml) was observed during enrichment with *B. pocheonensis* S2 at 1,000 lux, the data showed no significant difference compared to other treatments ($p > 0.05$) (Figure 3B).

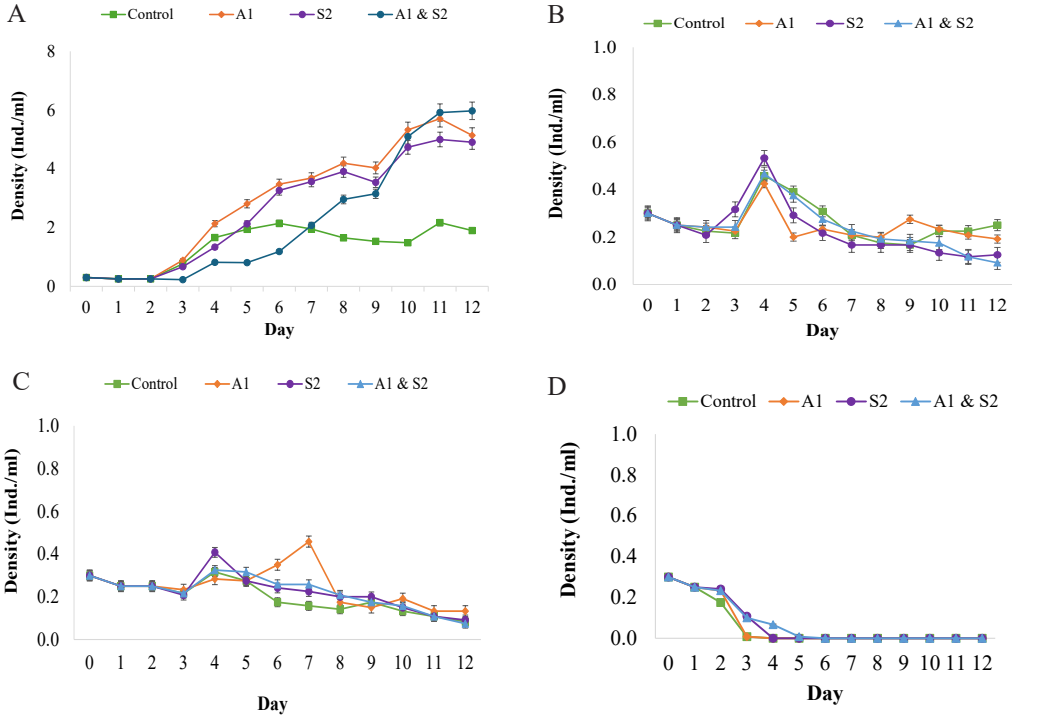
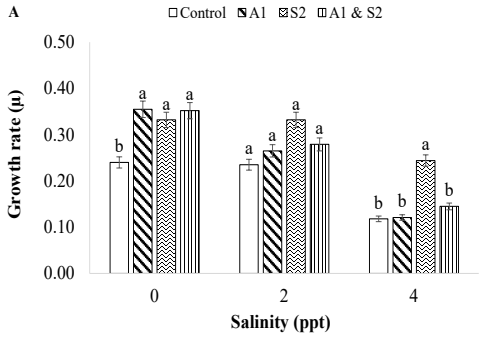
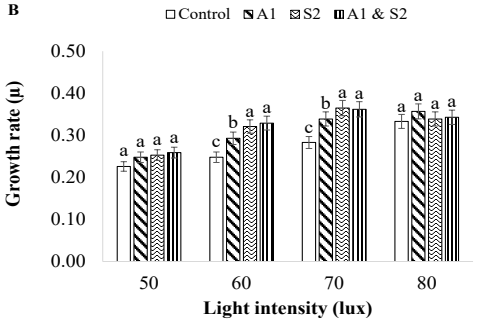


Figure 1. Differences in *Moina micrura* population density under varying salinity levels: (A) = 0 ppt, (B) = 2 ppt, (C) = 4 ppt, and (D) = 6 ppt. *Note*. Four different treatments have been done in each salinity level: Control (no probiotic added), A1 (*Lysinibacillus fusiformis*), S2 (*Bacillus pocheonensis*), A1 and S2 (*Lysinibacillus fusiformis* combined with *Bacillus pocheonensis*); Vertical bars = Standard errors of the means (n = 3)





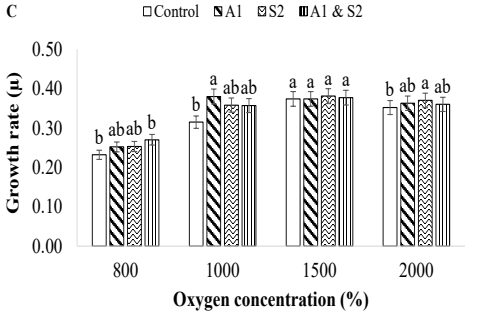
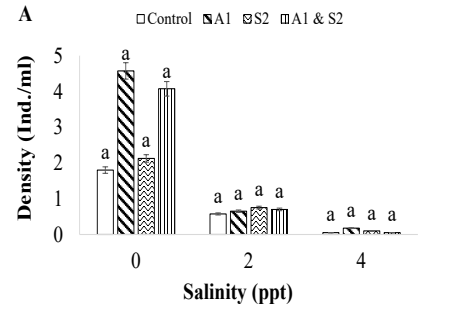
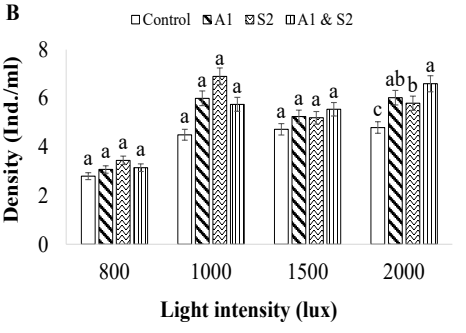


Figure 2. Specific growth rate (µ) of *Moina micrura* in different environmental parameters: (A) salinity (ppt), (B) light intensity (lux), and (C) oxygen concentration (%)

Note. Four different treatments have been done in each environmental parameter: Control (no probiotic added), A1 (*Lysinibacillus fusiformis*), S2 (*Bacillus pocheonensis*), A1 and S2 (*Lysinibacillus fusiformis* combined with *Bacillus pocheonensis*); Vertical bars = Standard errors of the means (n = 3); Different letters show significant differences among treatments ($p < 0.05$)





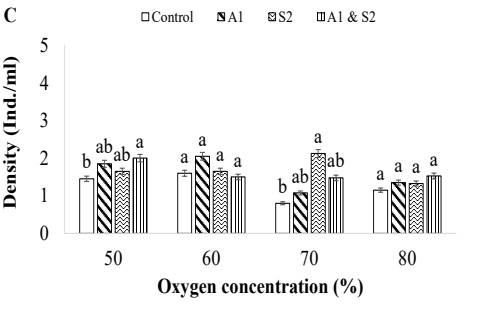


Figure 3. The number of neonates produced by *Moina micrura* in different environmental parameters: (A) salinity (ppt), (B) light intensity (lux), and (C) oxygen concentration (%)

Note. Four different treatments have been done in each environmental parameters level: Control (no probiotic added), A1 (*Lysinibacillus fusiformis*), S2 (*Bacillus pocheonensis*), A1 and S2 (*Lysinibacillus fusiformis* combined with *Bacillus pocheonensis*); Vertical bars = Standard errors of the means (n = 3); Different letters show significant differences among treatments ($p < 0.05$)

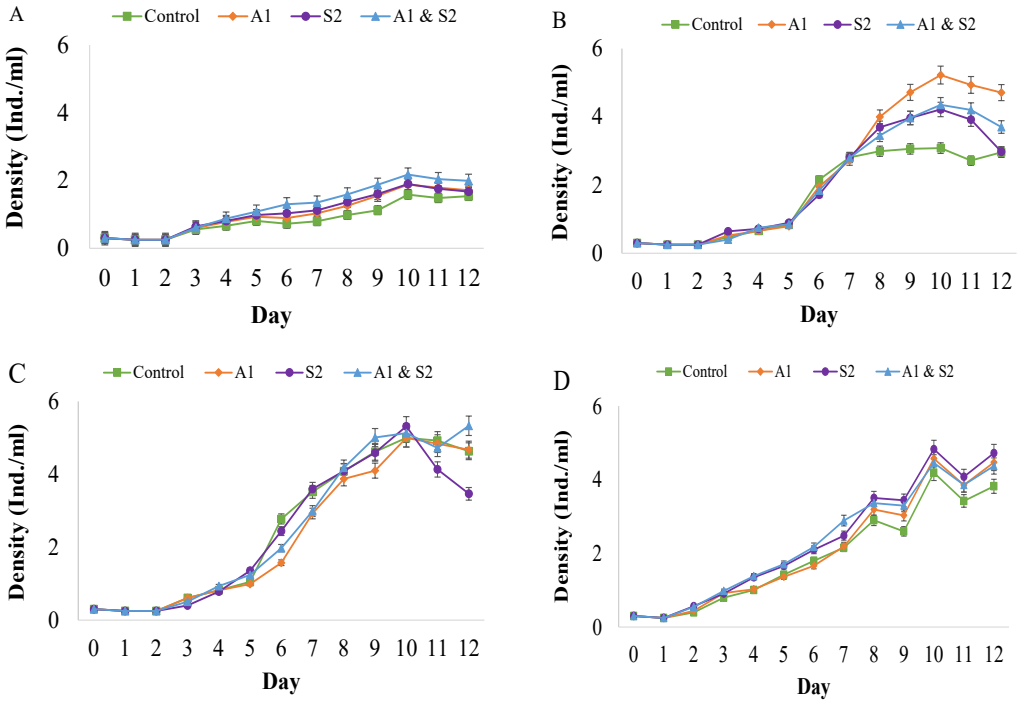


Figure 4. Shifts in the population density of *Moina micrura* in response to diverse light intensity levels: (A) = 800 lux, (B) = 1,000 lux, (C) = 1,500 lux, and (D) = 2,000 lux

Note. Four different treatments have been done in each intensity level: Control (no probiotic added), A1 (*Lysinibacillus fusiformis*), S2 (*Bacillus pocheonensis*), A1 and S2 (*Lysinibacillus fusiformis* combined with *Bacillus pocheonensis*); Vertical bars = Standard errors of the means (n = 3)

Oxygen Concentration

The effects of different oxygen concentrations on the population density, growth rate, and neonate production of *M. micrura* throughout the process of enrichment with probiotics were presented in Figures 2C, 3C, and 5, respectively. Results showed that the enrichment with *B. pocheonensis* S2 at 70% oxygen concentration had a significant outcome. The top maximum population density (5 ± 0.60 Ind./ml, Figure 5C), growth rate (0.365 ± 0.190 µ, Figure 2C), and offspring production (2 ± 0.25 Ind./ml, Figure 3C) of *M. micrura* were observed in the same treatment. Moreover, the growth rate and offspring production of *M. micrura* enriched with *B. pocheonensis* S2 at 70% oxygen concentration exhibited a notable increase compared to the control group (Figures 2C and 3C).

DISCUSSION

Although *Moina* is considered an important live feed for the larval rearing of most freshwater species, they are not readily obtainable from natural habitats in commercial-scale quantities (Loh et al., 2009). The larvae of red sea bream (*Pagrus major*) and kuruma shrimp (*Marsupenaeus*

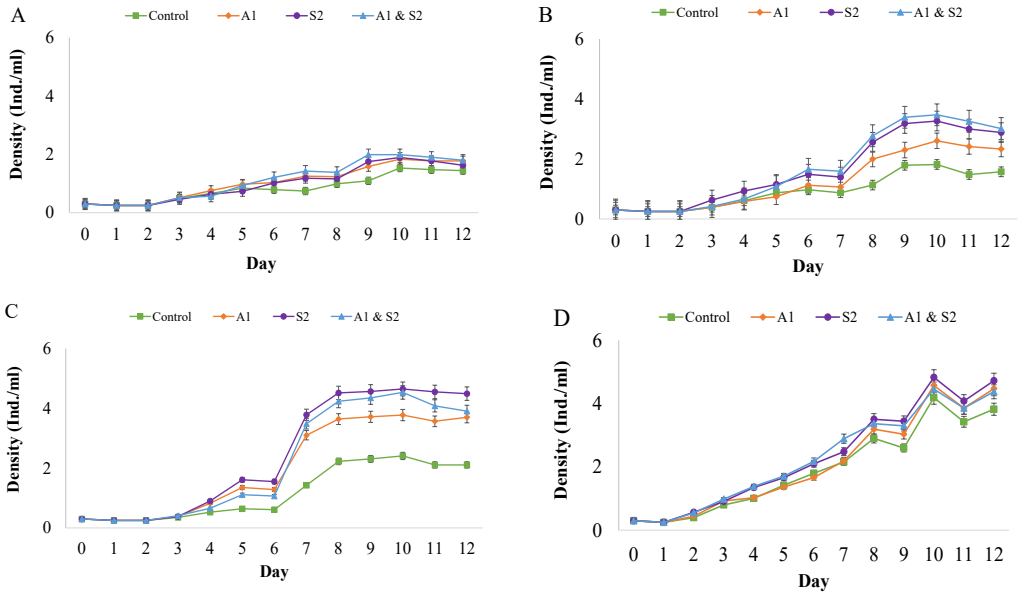


Figure 5. Changes in population densities of *Moina micrura* in varied oxygen concentrations: (A) = 50%, (B) = 60%, (C) = 70%, and (D) = 80%

Note. Four different treatments have been done in each oxygen concentration: Control (no probiotic added), A1 (*Lysinibacillus fusiformis*), S2 (*Bacillus pocheonensis*), A1 and S2 (*Lysinibacillus fusiformis* combined with *Bacillus pocheonensis*; Vertical bars = Standard errors of the means (n = 3)

japonicus) were reared successfully using *Moina* in small-scale experiments (Kamrunnahar et al., 2019; Nakamoto et al., 2008). However, mass production of *Moina* is unstable and often results in culture crashes. Although organic waste such as animal manure has been used as the source of nutrients in *Moina* culture, the risk of pathogen transfer is greater with these diets (Kagali et al., 2022; Kamrunnahar et al., 2019). Moreover, the nutritional content of *Moina* is unfavorable for small fish and shellfish. Therefore, introducing probiotics may be a potential solution to address these limitations by stabilizing the culture conditions and improving the reproductive capacity of *Moina*.

This study indicated that each of the probiotics, *L. fusiformis* and *B. pocheonensis*, had the ability to enhance the growth and production of *Moina*. The influence of a diet on the population growth of zooplankton can be evaluated based on its population density (Peña-Aguado et al., 2005). By improving the quality and quantity of the feed, the reproductive capacity and duration can be manipulated to enhance *Moina*'s population density and growth (Damayanti et al., 2020). The nutritional content of *Moina* can be altered through enrichment by taking advantage of primitive feeding characteristics (Samat, Yusoff, Rasdi, et al., 2020; Singh et al., 2019). Probiotics have been proven to increase the variety of microbial organisms present in live

feed (Jiang et al., 2019). The population and dispersion of *Moina* are affected by seasonal change, the number and size of the eggs, sexual development, changes in temperature, salinity, and light, as well as other environmental parameters (Ramírez-Merlano et al., 2013; Samat et al., 2022).

The focus of this research was to analyze the potential of probiotics to tolerate high salinity stress. Increased salinity can cause osmotic stress, reducing somatic development, reproduction, and survival and delaying sexual maturation in cladocerans (Santangelo et al., 2008). This study showed that *M. micrura* enriched with 10^5 CFU/ml of *L. fusiformis* have higher population density, growth rate, and neonates' production at 0 ppt salinity. As indicated by (Yuslan et al., 2021), for another species of *Moina*, *M. macrocopa* had achieved the highest survival and growth rate ever recorded for an environmental salinity of 0 ppt. Moreover, according to (Santangelo et al., 2008), salinity above 2 ppt negatively affected the reproduction of *M. micrura*, which could be due to osmoregulation pressure whereby the organism was unable to adapt its physiology as the salinity level increased. A study also showed that increased salinity concentration towards *Daphnia magna* and *Daphnia longispina* can affect their production performance (Toruan, 2012). Changes in water salinity may cause osmotic disequilibrium in zooplankton dwelling in freshwater-marine interface zones (El-Gamal et al., 2014; Harris et al., 2000). These findings suggested that the growth and production of *Moina* are undoubtedly affected by the increase in salinity levels. Enrichment with probiotics was unable to enhance *Moina* tolerance in a high salinity environment.

Although several studies on cladocerans have been published, most information was limited to the genus *Daphnia*. Comparatively limited studies have so far investigated the impact of light intensity on *Moina* culture. In recent research, *M. micrura* enriched with *B. pocheonensis* has greater population density, growth rate and neonate production at 1,500 lux of light intensity. For *D. magna*, the amount of neonate production was negatively affected by increasing light intensity, while it was positively correlated to survival rate (Wonkwon et al., 2019). Additionally, since the effectivity of probiotics is marginally influenced by light intensity, photosynthetic microalga probably was the main cause of reduced population growth of *M. micrura* in our experiment, as lower light intensity conditions reduced food availability (Vijverberg, 1989).

Because of its fast potential growth, *Moina* is being used for live feed in larvae and juvenile finfish worldwide (*Moina* is much smaller compared to *Daphnia* and contains 70% higher protein) (He et al., 2001). *Moina* is frequently utilized as a substitute meal for *Artemia* in both larval fish and shrimp cultures. *Moina micrura* has also been discovered to be an intriguing alternative to *Artemia* for increasing *Macrobrachium rosenbergii* production (Kang et al., 2006). It seems necessary to enrich the nutritional content of *Moina* as live feed to enhance their

nutritional value, particularly in terms of important fatty acids. *Moina* has nourished various aquatic species, including catfish *Clarias macrocephalus*, different types of catfish, shrimp, and larvae of red sea bream (Nakamoto et al., 2008). Enrichment of live food with probiotics aids their survival and proliferation within the components of the live food, allowing them to be successfully transferred into the hosts (Hai, 2015). Probiotic bacteria have the dual potential to improve the nutrient content of live food by delivering vital nutrients like vitamins or non-organic components that are absent from the diet. Additionally, they can increase the live food's population density while inhibiting the growth of harmful pathogens (Douillet, 2000).

Moreover, introducing probiotics directly into the cultivation water carries a risk due to their susceptibility to contamination by microorganisms (Sun et al., 2013). Moreover, using live food as a carrier is an optimal approach, as probiotics in seawater have a short survival period (Gatesupe, 2008). Live food persists within the rearing water for multiple hours prior to consumption. Thus, encapsulated bacteria must possess the ability to endure within the live food for a duration sufficient for the larvae to engage in feeding (Pintado et al., 2010). The multiplication of bacteria during the initial growth phase of fish larvae's gut microflora is intricately linked to the bacteria in the live food. As a result, the bioencapsulation approach of enriching live food with probiotics enables the controlling of the microbial community in the live food. It may result in improved development and viability of fish and crustacean larvae (Olafsen, 2002). The traditional application of antibiotics for minimizing bacterial diseases is debatable, and in certain circumstances, it has lost its effectiveness in addressing such infections (Defoirdt et al., 2011).

In recent times, the popularity of using dietary intake of nutritional supplements like probiotics for the management or treatment of illnesses has increased (Hoseinifar et al., 2018). In certain circumstances, administering probiotics to the target host's gut via probiotic enrichment through a bioencapsulation technique of using zooplankton as a live food is an intriguing initiative (Gomez-Gil & Roque, 2000). Studies have indicated that encapsulating probiotics within live food can enhance zooplankton's growth, population density, and reproductive rate (Le et al., 2017; Planas et al., 2004).

Even though most of the probiotics treated in *M. micrura* were not significantly different among the treatments, they remained higher compared to the control group. *M. micrura* enriched with probiotic *B. pocheonensis* S2 has maximum population density, growth rate and neonates' production in light intensity and oxygen concentration parameters, while *M. micrura* enriched with *L. fusiformis* A1 has the highest population density, growth rate and neonates' production in 0 ppt salinity. *Moina micrura* enriched with *L. fusiformis* shows the finest result than control treatment in population density, growth rate and neonate

production at pH 8 (Babitha Rani et al., 2006). Synbiotic enrichment of *Artemia* (*Pediococcus acidlactici* at 700 mg/L and fructooligosaccharide at 100 mg/L) substantially enhanced fish development, diversity of microorganisms, ability to handle stress, and immune responses (Lobo et al., 2018). Studies have shown that feeding fish larvae with *Artemia* enriched with *Shewanella putrefaciens* can increase n-3 HUFA levels (Sun et al., 2013). Copepod enrichment with any one of lyophilized *Bacillus clausii* or *Bacillus pumilus* at 10^6 CFU/ml for 3 hr increased fish larval development, survival, and favorable gut microbiota (Green, 1956). It provides strong evidence that the probiotics were successfully incorporated into the live feed culture, thus validating their efficacy in enhancing the experimental conditions.

In this research endeavor, the enrichment of *M. micrura* with *B. pocheonensis* S2 at 70% oxygen concentration showed the highest population density, growth rate, and offspring production. Similarly, Svetlichny and Hubareva (2002) found that low oxygen level negatively affects the growth rate and reproduction of *M. micrura*, and the study suggested that low oxygen levels reduced the locomotion efficiency of *M. micrura*, which consequently reduced their filtration rate. Furthermore, a study reported a reduction in growth and egg production, increased egg abortion, alteration of feeding behavior, and reduction in feeding rate of cladoceran *Daphnia* spp. when exposed to a lower oxygen concentration of less than 0.002% (Jiang et al., 2019). Therefore, the enrichment of *M. micrura* with probiotics in 70% oxygen concentration assisted in improving the population density, growth rate, and neonates' production of *M. micrura*.

CONCLUSION

The population density, population growth rate, and neonates' production of *M. micrura* enriched with probiotics surpass those in the control treatments. Both probiotics have proved to be partially effective in conferring benefits to *M. micrura*. The current study unveiled that the most effective performance was observed with *B. pocheonensis* S2 at improving the population density, population growth rate, and neonates' production of *M. micrura* under diverse environmental parameters. This study's findings demonstrated that probiotics could play a role in enhancing *M. micrura*'s ability to withstand conditions slightly higher or lower than the ideal range. Furthermore, the results suggested that the most effective enrichment for *M. micrura* is achieved with *B. pocheonensis* S2 at 0 ppt, with 70% oxygen concentration, and at the light intensity level of 1,500 lux. This study gathered important data that would be valuable for the mass production of *M. micrura* through probiotic enrichment since this species holds promise as a viable alternative source of live food during the larviculture of many fish and crustacean species.

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