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Growth Interaction of *Moina* sp. and *Chlorella* sp. for Sustainable Aquaculture

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ABSTRACT

Fish farmers' dependence on costly formulated fish feed has affected their income. The cost of formulated feed is also constantly rising. Efforts to mass-produce the locally available natural resource, namely water flea (*Moina* sp.), were initiated as an alternative to the commercially formulated fish feed. This study evaluates the most suitable growth medium for commercially culturing *Moina* sp. and *Chlorella* sp. and studies the effect of their growth activity on water quality. In this study, the growth behaviour of *Moina* sp. and *Chlorella* sp. was monitored individually before *Moina* sp. was cultured together with *Chlorella* sp. in a growth medium. *Chlorella* sp. was cultured in different mediums (BG-11, Bristol, and organic fertiliser). The first generation of *Moina* sp. took 96 h to mature and begin to reproduce, while the next generation took a

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shorter time (from 24 h to 48 h). The brood size was between five and 15 neonates, while the maximum brood count recorded was eight. The *Chlorella* sp. culture had grown well on Day 5 (555.33 ug/L); thus, it was introduced with *Moina* sp. on Day 6. Although an organic fertiliser medium provided the optimum conditions for *Chlorella* sp. growth, it slightly inhibited the *Moina* sp. growth due to higher ammonia (NH_3) concentration. However, the organic fertiliser medium could sustain *Chlorella* sp. growth while being ingested by *Moina* sp. The growth activity of both species slightly affected the water quality. Meanwhile, the increase in ammonia (NH_3), carbon dioxide (CO_2), and calcium carbonate ($CaCO_3$) was recorded. In conclusion, organic fertiliser is the best medium for *Chlorella* sp. growth, which is the main food source for *Moina* sp. culture.

Keywords: *Chlorella* sp., live feed, *Moina* sp., organic fertiliser, sustainable aquaculture

INTRODUCTION

Live foods are living organisms that can move in water and are always available for fish or larvae, thus stimulating the larval feeding response (Das et al., 2012). Since commercial marine fish culture was developed in the late 1970s, the demand for *Artemia* sp. or brine shrimp cysts has gradually increased from a few tonnes to approximately 800 tonnes per annum (Rasdi & Qin, 2018). It represents approximately 40% of aquaculture feed demand during the early stages. Although applying *Artemia* sp. cysts as feed is simple, several factors are critical for hatching the large quantities needed in larval fish production. These include cyst disinfection or decapsulation before incubation and hatching under the following optimal conditions: constant temperature of 25–28°C, 15–35 parts per thousand (ppt) salinity, minimum pH value of 8.0, near-saturated oxygen levels, maximum cyst densities of 2 g/L, and strong illumination of 2,000 lux (Sorgeloos et al., 2001). The production cost of *Artemia* sp. cysts is relatively high, directly affecting fish production costs. Cheaper diets with the same nutritional values are needed to keep the price of fish competitive in the global market. The lack of suitable live feeds for feeding the fish at various production stages has hampered the industrial development of aquaculture (Das et al., 2012). Most formulated fish pellets are not suitable for feeding aquatic organism larvae. In addition, the high price and rapid consumption of fish pellets place a burden on small-scale fish farmers.

Moina sp., also known as the water flea, is a freshwater crustacean organism smaller than *Daphnia* sp., its closest relative. *Moina* sp. is capable of asexual and sexual reproduction. *Moina* sp. can produce up to 30 neonates asexually for each brood under optimal conditions. Usually, the population is made up entirely of females. The *Moina* sp. diet comprises bacterial, yeast, phytoplankton and decomposing organic matter (Rottmann et al., 2018). *Moina* sp. has a protein content equal to 50% of its dry weight (Rasdi & Qin, 2018). The fat content of adult *Moina* sp. is typically higher than its

juvenile (Conklin & Provasoli, 1977; Rasdi et al., 2020; Rottmann et al., 2018). *Moina* sp. can primarily be found in temporary ponds or ditches. It is smaller in size than its closest relative, *Daphnia* sp. *Moina* sp. has high protein content and is relatively easy to culture. Aside, it is a superior live food compared to *Artemia* sp., which is one of the commonly used live feeds. It is because of its high protein and nutrient content (Loh et al., 2012), thus making it a suitable substitute for *Artemia* in aquaculture hatcheries. The *Moina* sp. was widely utilised as live food in many hatcheries and the maintenance and cultivation of commercially significant ornamental fishes, finfish, crustaceans, teleost, and marine fish culture worldwide (Aguado et al., 2009; Das et al., 2012; He et al., 2001; Fermin, 1991; Fermin & Bolivar, 1994; Ingram, 2009; Poynton et al., 2013). In this study, *Moina* sp. was selected and utilised as a live food to produce sustainable fish feed, particularly for aquaculture production in Tasik Chini. *Moina* sp. cultivation is not as complex as *Artemia* sp. because these freshwater Cladoceras have higher reproduction rates, wide temperature tolerance, and the ability to thrive on phytoplankton and organic wastes; they can thrive in a relatively high content of un-ionised ammonia (Khoo et al., 2013).

Humans regularly use microalgae as a food source and in hatchery production for commercial fish and shellfish (Kay & Barton, 1991). These microscopic organisms are still consumed as food supplements, and their products are also used for different purposes like dyes, pharmaceuticals, animal feed, aquaculture, and cosmetics (Safi et al., 2014). Microalgae, such as *Chlorella* sp., *Dunaliella* sp., *Scenedesmus* sp., and *Spirulina* sp., are frequently utilised algae. *Chlorella* sp. is a collection of aquatic organisms that lack complex cell structures and are often found in plants (Slade & Bauen, 2013). *Chlorella* sp. can also be defined as prokaryotic or photosynthetic eukaryotic microorganisms with unicellular or compact multicellular structures capable of rapid reproduction and survival in harsh environments (Mata et al., 2010). In terms of nutrient content, *Chlorella* sp. has a protein content of appropriately 70% and a higher beta-carotene concentration than broccoli (Kay & Barton, 1991). *Chlorella* sp. has valuable components with potential in the food industry due to its high content of macro and micro components and low-calorie count. *Chlorella* sp. contains various vitamins and minerals, including calcium and magnesium, in addition to the basic nutrients.

As fish feed prices rise rapidly, identifying a reliable food source for livestock and a sustainable system that can produce affordable and sustainable food sources is critical. Given the correlation between *Chlorella* sp. and *Moina* sp., as well as their ability to reproduce in the laboratory, it is crucial to investigate the growing linkages between these two organisms and assess their potential. Therefore, this study aims to evaluate *Moina* sp. and *Chlorella* sp. growth. The suitable growth medium for both organisms was determined, and the effect of their growth activity on water quality was also evaluated. At the end of this

study, a suitable medium for *Moina* sp. and *Chlorella* sp. growth could be formulated.

MATERIALS AND METHODS

Organism and Culture Medium

The cultured *Chlorella* sp. was a gift from the Fisheries Research Institute Glami-Lemi. *Moina* sp. was obtained directly from a pet shop which sells wild *Moina* sp. Meanwhile, *Chlorella* sp. was cultured using two types of culture medium: BG-11, Bristol, and an organic fertiliser mix (Table 1). All chemicals were purchased from Chemiz (Malaysia).

Table 1
Composition for BG-11, Bristol, and organic fertiliser

Ingredient	Media		
	BG-11 (g/L)	Bristol (g/L)	Organic fertiliser (g/10 L)
Sodium nitrate ($NaNO_3$)	1	0.250	-
Magnesium sulphate ($MgSO_4$)	0.513	0.075	-
Dipotassium phosphate (K_2HPO_4)	0.250	0.075	-
Calcium chloride ($CaCl_2$)	0.058	0.250	-
Ammonium chloride (NH_4Cl)	0.050	-	-
Iron (III) chloride ($FeCl_3$)	0.003	-	-
Rice bran	-	-	0.60
Corn	-	-	1.20
Urea	-	-	0.10
Triple super phosphate	-	-	0.10
Agricultural lime	-	-	0.05

Culturing *Moina* sp. Biomass

Adult *Moina* sp. with neonate was placed in a separate container that contained water. New neonates were produced, and those less than 24 hours old were isolated and used as new adults. The new neonates were placed in test tubes that contained 20 ml of dechlorinated tap water to assess the first reproduction day, number of reproductions, and lifespan of *Moina* sp. The neonates were fed daily with 1 ml of *Chlorella* sp. cultivated in BG-11. When the neonates matured (adult *Moina* sp.) and started to reproduce, the average number of neonates produced (brood size) was determined, and they were isolated from the mother. This procedure was repeated to determine the brood number and female life expectancy (Martínez-Jerónimo & Gutierrez-Valdivia, 1991). This experiment was carried out in 10 replicates.

Culturing *Chlorella* sp. Under Laboratory Culture Condition

Chlorella sp. was cultured using a batch culture system (Laing, 1991). First, it was cultured in an initial culture volume of 10 mL and gradually transferred or subcultured in a larger culture volume (10 L). At Stage 1 of cultivation (small volume), *Chlorella* sp. was grown using BG-11 (1 ml). Then, it was to prepare as feed for *Moina* sp. and a subculture for a larger culture volume to be cultivated with *Moina* sp.

For Stage 2 of cultivation in the larger volume (10 L), *Chlorella* sp. was cultivated in three different mediums: BG-11, Bristol, and organic fertiliser. The cultures were exposed to continuous fluorescents illumination at 22°C to achieve the maximum growth rate and aerated by using an air pump (0.03 MPa, 50l/min, 220-240 V, 35W). It was to prevent sedimentation of the algae and ensure that all cells of the population were equally exposed to light and nutrients. Gas exchange between the culture medium and the air was simultaneously improved. *Chlorella* sp. culture was not bacteria-free; however, all culture mediums and liquids were autoclaved at a temperature of 120°C for 15 min. Algal biomass that was tested for its nutrient content was centrifuged and refrigerated.

Chlorophyll A Concentration

Chlorophyll A concentration of *Chlorella* sp. biomass cultured in the 10 L culture volume was recorded daily until it was ready to be fed to *Moina* sp. and continued until all *Chlorella* sp. was consumed by *Moina* sp. The following equation calculated the specific growth rate of the *Chlorella* sp.:

$$\text{Specific growth rate, } \mu = \text{Ln}\,(N_2 / N_1)/(t_2 - t_1)$$

where μ is the specific growth rate, and N_1 and N_2 are biomass at Time 1 (t_1) and Time 2 (t_2), respectively (Krishnan et al., 2015).

Chlorophyll A of *Chlorella* sp. biomass of more than 10 L was measured using YSI 600 OMS V2 Sonde (Xylem Analytics, USA) along with temperature and salinity. *Chlorella* sp. biomass (10 L) was moved to room temperature before *Moina* sp. was inoculated. Experiments were carried out in three replicates.

Moina sp. Weight

The initial wet weight of *Moina* sp. before being inoculated into 10 L of algae culture was determined. *Moina* sp. biomass was collected using a fine mesh sieve/strainer (<400 μm), which was also used to drain excess water simultaneously and weighted using a laboratory analytical scale. All measurements were carried out in triplicates.

Water Quality Tests on *Chlorella* sp. Culture

Several water parameter tests were carried out on the *Chlorella* sp. cultures to see if there were changes in water quality during *Chlorella* sp. growth and after *Moina* sp. inoculation. Water parameter test kits from (HACH, Malaysia) were used to check the water quality. The parameter test kits included acidity and alkalinity, carbon dioxide (CO_2), unionised ammonia, and

nitrite test kits. In addition, YSI-600-OMS V2 Sonde (Xylem Analytics, USA) was used to check the salinity, temperature, and dissolved oxygen (DO).

Statistical Analysis

All analysis was done in triplicate. Data were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Growth and Reproduction of *Moina* sp.

The Cladocera species, especially *Moina* sp., were extensively studied worldwide concerning the effects of food abundance, growth, and reproduction parameters (Burak, 1997; He et al., 2001; Rasdi, Ikhwanuddin et al., 2021). In addition, other literature has addressed *Chlorella* sp. to be used as a primary food source for *Moina* sp. (Das et al., 2012; Shidik et al., 2021). Therefore, in this study, *Chlorella* sp. was used as feed for the *Moina* sp. growth. Figure 1 shows the *Moina* sp. count after a single neonate was fed daily with 1 ml of *Chlorella* sp. for six days.

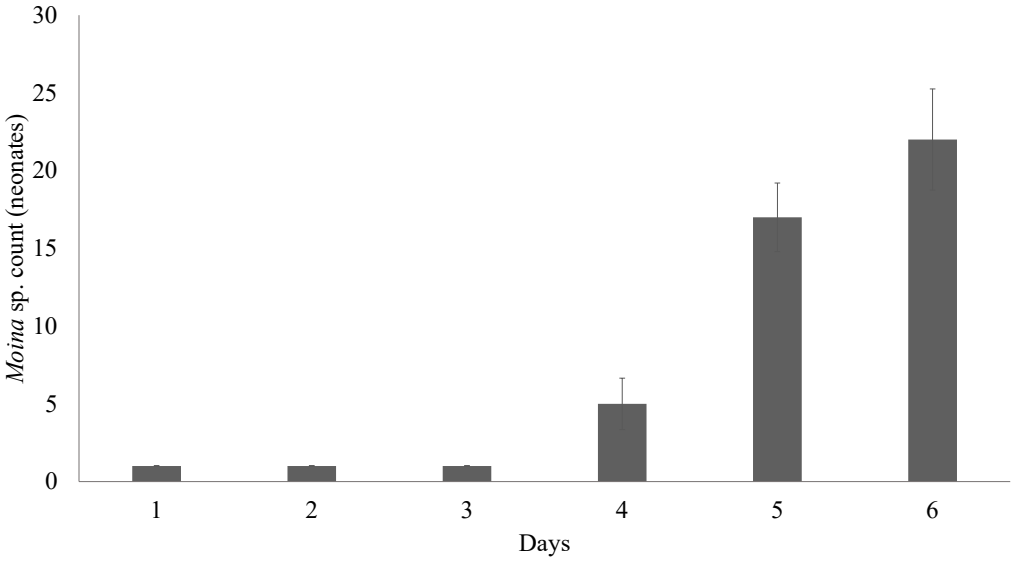

Figure 1. *Moina* sp. count. A neonate is fed 1 ml of *Chlorella* sp. and counted every day for six days to know the sex maturity and first reproduction time. Neonate-turned-adult and new neonates are counted together for the first and subsequent reproduction. The error bar represents the standard deviation of the results

Moina sp. feds *Chlorella* sp. took as early as four days to mature and began to produce brood. Subsequent broods are produced every 24 h after the first reproduction (First brood number). Figure 2 shows the brood size of each reproduction from a single

neonate after the first reproduction. The second reproduction shows the average highest brood size produced (15 neonates) and continues up to eight reproductions, with the brood size ranging from four to ten neonates on each reproduction. As reported previously, the *Moina* sp. reproduction activity ranges from 6 to 12 reproductions (Kamrunnahar et al., 2019; Martínez-Jerónimo & Gutierrez-Valdivia, 1991).

Effect of Medium on the *Chlorella* sp. Growth

Quality and quantity of food are the most important factors in determining biomass production by *Moina* sp. (Rasdi et al., 2020, Rasdi, Yuslan et al., 2021; Sipaúba-Tavares et al., 2014). To mass produce *Moina* sp., an adequate supplement of microalgal must be provided. For usage in aquaculture, a microalgal strain must meet various criteria, such as ease of culture, lack of toxicity, high nutritional value with correct cell size and shape, and a digestible cell wall to make nutrients available (Hemaiswarya et al., 2011; Rasdi, Yuslan, et al., 2021). Different kinds of literature have addressed *Chlorella* sp. as a primary food source for *Moina* sp. (Das et al., 2012; Shidik et al., 2021). Therefore, the growth of *Chlorella* sp. in different growth mediums was observed to identify the most suitable growth medium to be used and if there was any effect on *Chlorella* sp. growth. Figure 3 shows the chlorophyll A concentration in *Chlorella* sp. growth in different mediums.

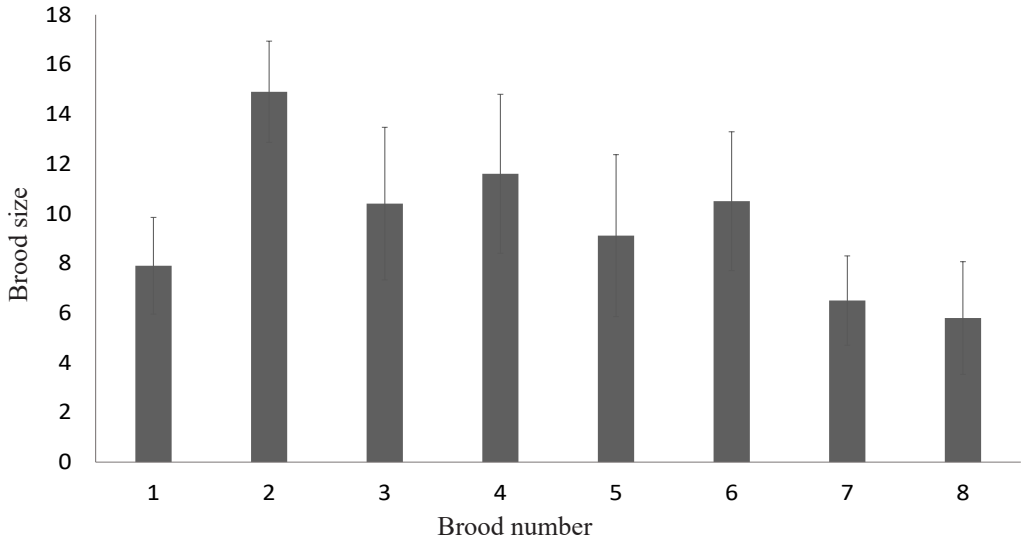

Figure 2. Average brood size and brood number (lifespan) of *Moina* sp. Each generation of juvenile *Moina* sp. produced from single adult *Moina* sp. is calculated and separated from the mother. Brooding *Moina* sp. is fed by 1 ml of *Chlorella* sp. The error bar represents the standard deviation of the results

After inoculation, *Chlorella* sp. grew well in the BG-11 medium, followed by Bristol medium and organic fertiliser. Chlorophyll A reached its maximum concentration of 552.5 ± 1.7 ug/L after 48 h in biomass cultured using BG-11 medium and 72 h in biomass cultured by Bristol medium and organic fertiliser. Although *Chlorella* sp. cultured in fertiliser showed a lag phase on Day 1, it has a significantly higher growth rate than the others. It indicated that the organic fertiliser not only had the basic nutrients required to culture *Chlorella* sp. but also had more macronutrients and micronutrients, such as potassium, zinc, iron, and copper, to help facilitate *Chlorella* sp. growth (Bhosale & Vijayalakshmi, 2015; Prasanthi et al., 2017). From Day 4 onwards, all cultures reached the maximum growth number. This phase occurred due to the depletion of nutrients in the medium (Krishnan et al., 2015). In this stationary phase, there was an equal rate of cell division and dying cells. Therefore, culture at this stage was suitable as a starter culture to produce a continuous supply of *Chlorella* sp. (Sánchez-Bayo et al., 2020).

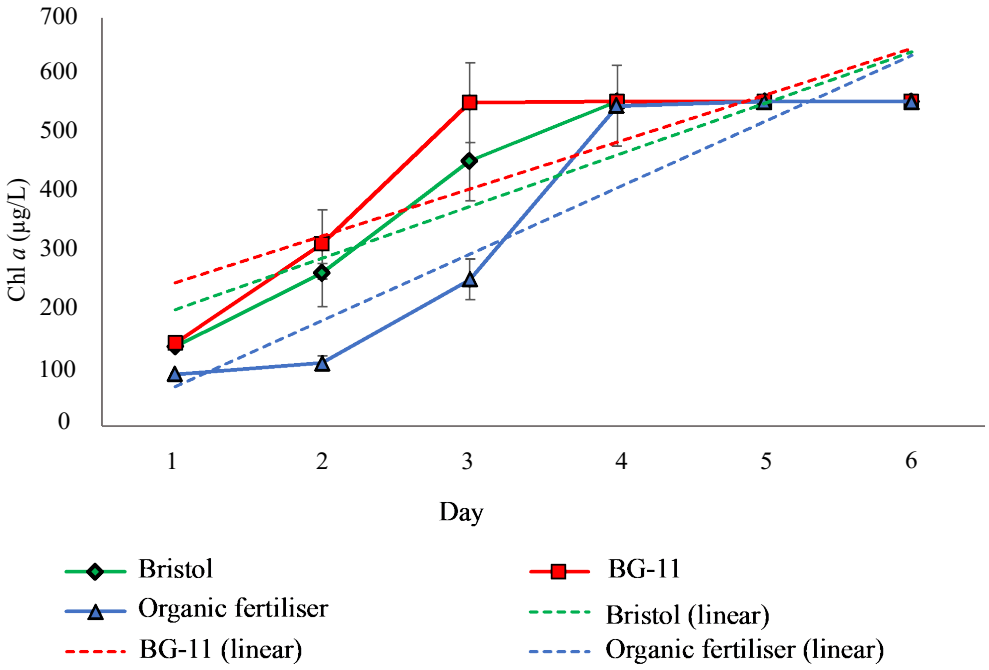

Figure 3. The concentration of chlorophyll A in *Chlorella* sp. culture cultured in different growth mediums at 22°C, 24 h of illuminated light and aeration for five days

The Growth of *Moina* sp. in Different Media

A single mature neonate (72 h old) was introduced into the six-day-old medium, which contained well growth chlorophyll A. Figure 4 shows the growth of *Chlorella* sp. from Day 1 until Day 6 and changes that occurred when *Moina* sp. was introduced on Day 6.

Results showed that the growth of *Chlorella* sp. in BG-11 and Bristol started to decrease, respectively, after 24 h and 48 h of *Moina* sp. introduction and depleted on Day 10. Meanwhile, the concentration of *Chlorella* sp. in the organic fertiliser medium was maintained until Day 15 and depleted on Day 17. The ability of *Chlorella* sp. to remain at the same concentration until Day 15 indicated that organic fertiliser could supply adequate nutrients (Ratomski & Hawrot-Paw, 2021) throughout the growth of *Chlorella* sp. for 15 days. Further tests from previous research findings on nutrient concentration effects (nitrogen and phosphorous) showed that low nitrogen concentrations could also stimulate algal growth (Blinova et al., 2015).

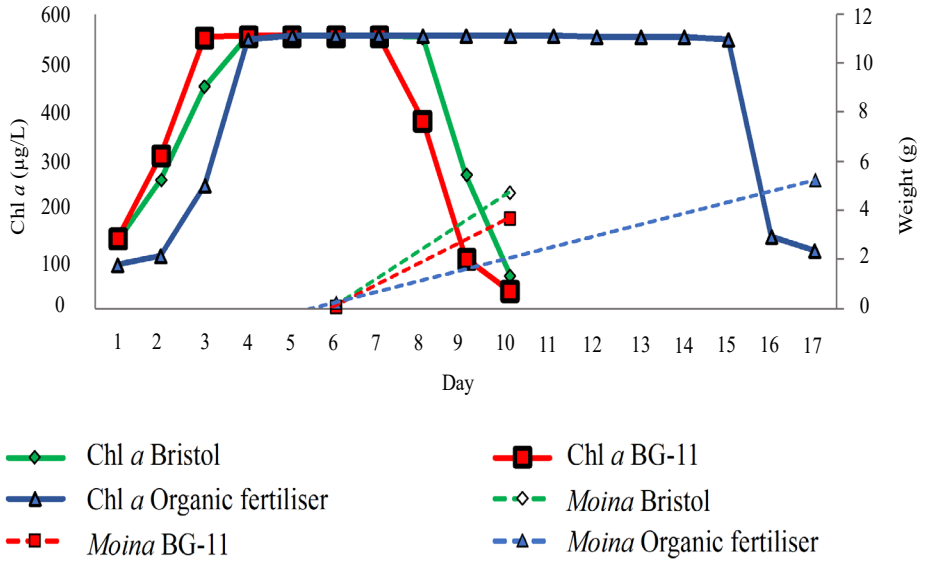

Figure 4. Changes in the concentration of chlorophyll A and wet weight (g) of *Moina* sp. straight line indicated the *Chlorella* sp. concentration changes while the dashed line represents the *Moina* sp. wet weight changes when inoculated on the sixth days of *Chlorella* sp. growth and final weight once *Chlorella* sp. biomass fully consumed

Of all *Moina* sp. growth in different mediums, *Moina* sp. growth in organic fertiliser showed the highest final weight (5.24 g), followed by *Moina* sp. growth in Bristol (4.73 g) and the lowest in BG-11 (3.69 g). Although *Moina* sp. in BG-11

started to feed on *Chlorella* sp. faster, it had the lowest final weight than the other two *Moina* sp. in different mediums. These differences probably happened due to differences in medium composition and thus influenced the growth, reproduction, and metabolism of *Moina* sp. and *Chlorella* sp. The presence of organic ingredients supported *Chlorella* sp. growth and *Moina* sp. as a nutrition source (Shidik et al., 2021). It was reported that the feed's protein and amino acid concentrations directly affected the fecundity and population growth of *M. macrocopa* (Mubarak et al., 2017). It was also reported that the high fertility and reproductive rate of Cladocera could be obtained when its food source had a high carbon/nitrogen ratio (Khoo et al., 2013). In addition, the higher growth rate of *Chlorella* sp. in an organic fertiliser medium (Figure 3) indicated that it could be a source of food supply for *Moina* sp. for a longer period. The highest final weight of *Moina* sp. was due to the adequacy of *Chlorella* sp. to ensure the growth and development of the brood generation produced.

Changes in Water Quality During *Moina* sp. Growth

Moina sp. is known to survive in poor water quality, especially in an oxygen-poor environment (Rottmann et al., 2018). However, there is not much to know about the chemical or physical requirements for *Moina* sp. Therefore, in this study, changes in water quality during *Moina* sp. growth were evaluated: Un-ionised ammonia (NH_3) level, salinity, dissolved oxygen (DO), average carbon dioxide (CO_2) content, and average calcium carbonate ($CaCO_3$) total alkalinity. The results are presented in Figure 5.

Organic fertiliser had a higher concentration of NH_3 (0.01098 mg/L) than the other two mediums used (Figure 5a), which were at 0 mg/L, respectively. Compared to the nutrient composition in BG-11 and Bristol medium that was in liquid form, organic fertiliser was composed of a solid substrate that would break down in the *Chlorella* sp. inoculated water, providing nutrients for *Chlorella* growth. Therefore, decomposition occurred in the *Chlorella* sp. biomass and inevitably released a much higher concentration of NH_3 (Shidik et al., 2021). The increase in NH_3 concentration in all mediums indicated that the growth activity of *Chlorella* sp. and *Moina* sp. unionised ammonia (NH_3) values in the *Chlorella* sp. cultured using organic fertiliser increased to 0.0015 mg/L on Day 3 and remained on the lag phase until Day 12. The high concentration of NH_3 might explain the higher growth rate of *Chlorella* sp. in organic fertiliser compared to the other mediums in the first four days (Figure 4).

Chlorella sp. could adapt to the high NH_3 concentration (Tam & Wong, 1996) on Day 4 after inoculation because there was no decrease in chlorophyll A concentration, albeit with a low reproduction on Day 1. The increased NH_3 level (Figure 5a) after the introduction of *Moina* sp. indicated that nitrogenous waste was produced during *Moina* sp. growth. The prolonged growth of *Moina* sp. in organic fertiliser for up to 12 days as compared to other mediums

(five days, respectively) was due to the prolonged growth and reproduction rate of *Chlorella* sp. It was also reported that when the NH_3 concentration was above 0.013 mg/L, the growth and reproduction activity of *Moina* sp. seemed to be affected as *Moina* sp. population could be seen in high yield on Day 4 of inoculation in *Chlorella* sp. biomass cultured by BG-11 and Bristol mediums. However, when the NH3 concentration reached 0.236 mg/L, it would kill the *Moina* sp. completely instead of affecting its reproduction and growth rate (He et al., 2001). *Chlorella* sp. and *Moina* sp. kept growing and number simultaneously up to Day 10 until the consumption rate of *Chorella* sp. by *Moina* sp. was higher than the *Chlorella* sp. reproduction (Figure 4). The last reproduction of *Moina* sp. was recorded on Day 12, probably due to the shortage of food supply as the *Moina* sp. could adapt to environmental stress.

In this study, *Chlorella* sp. was cultured in three different mediums with salinity values of 0.05 ppt to 0.015 ppt on the day of *Moina* sp. inoculation (Figure 5b). *Chlorella* sp. cultivated using organic fertiliser had a higher salinity value than BG-11 and Bristol mediums. This high salinity value corresponded to the high concentration of unionised ammonia NH_3, which slowed down the *Chlorella* sp. (Fakhri et al., 2017; Goto et al., 2018). However, all mediums had a salinity value below the limit of 0.5 ppt for proper Cladocera growth, although the optimal growth was suggested at 0 salinity (Yuslan et al., 2021) and limit at 2 ppt (Rasdi & Qin, 2018). Therefore, it was concluded that 0.015 ppt salinity value has no significant effect on *Moina* sp. growth.

Dissolved oxygen during *Moina* sp. inoculation in organic fertiliser medium was lower (7.09 mg/L) than BG-11 (11.71 mg/L) and Bristol (11.95 mg/L) mediums. Dissolved oxygen is an indicator of photosynthetic activity by microalgae. Low dissolved oxygen indicates problems with microalgal growth and possibly dissolved oxygen consumption by heterotrophic microorganisms (Morales et al., 2018). The decreased level of dissolved oxygen when *Moina* sp. was inoculated indicated the respiration activity of *Moina* sp. (Murakami et al., 2020). Meanwhile, dissolved oxygen levels in BG-11 and Bristol mediums decreased until all *Moina* sp. stopped reproducing (Day 5). Dissolved oxygen in the organic fertiliser medium started to increase on Day 5. Increased dissolved oxygen levels indicated that the *Chlorella* sp. growth rate was higher than *Moina* sp. consumption and respiration rate (Kazbar et al., 2019). These changes were caused by high NH_3 concentration, which inhibited *Moina* sp. growth and reproduction. However, on the Day 8 of *Moina* sp. growth, the dissolved oxygen concentration started to decrease. It indicated that *Moina* sp. could adapt to the environmental stress and started to reproduce, thus causing the increased consumption rate and respiration rate of *Moina* sp.

The increase in carbon dioxide rate was higher in the organic fertiliser medium than in other mediums after inoculation of *Moina* sp. This result was consistent with the changes in the dissolved oxygen concentration (Figure 5c). Although algae growth requires a source of carbon

dioxide (Slade & Bauen, 2013), the low photosynthesis rate of *Chlorella* sp. during the first four days caused a higher carbon dioxide concentration due to the *Moina* sp. respiration (Santoso et al., 2020). Therefore, it is viewed that high carbon dioxide does not necessarily increase the growth rate (Rashid et al., 2014). In addition, carbon dioxide was also reported to be produced partly from the decomposition of organic fertiliser and partly from the carbonate-bicarbonate system (Ventura & Enderez, 1980).

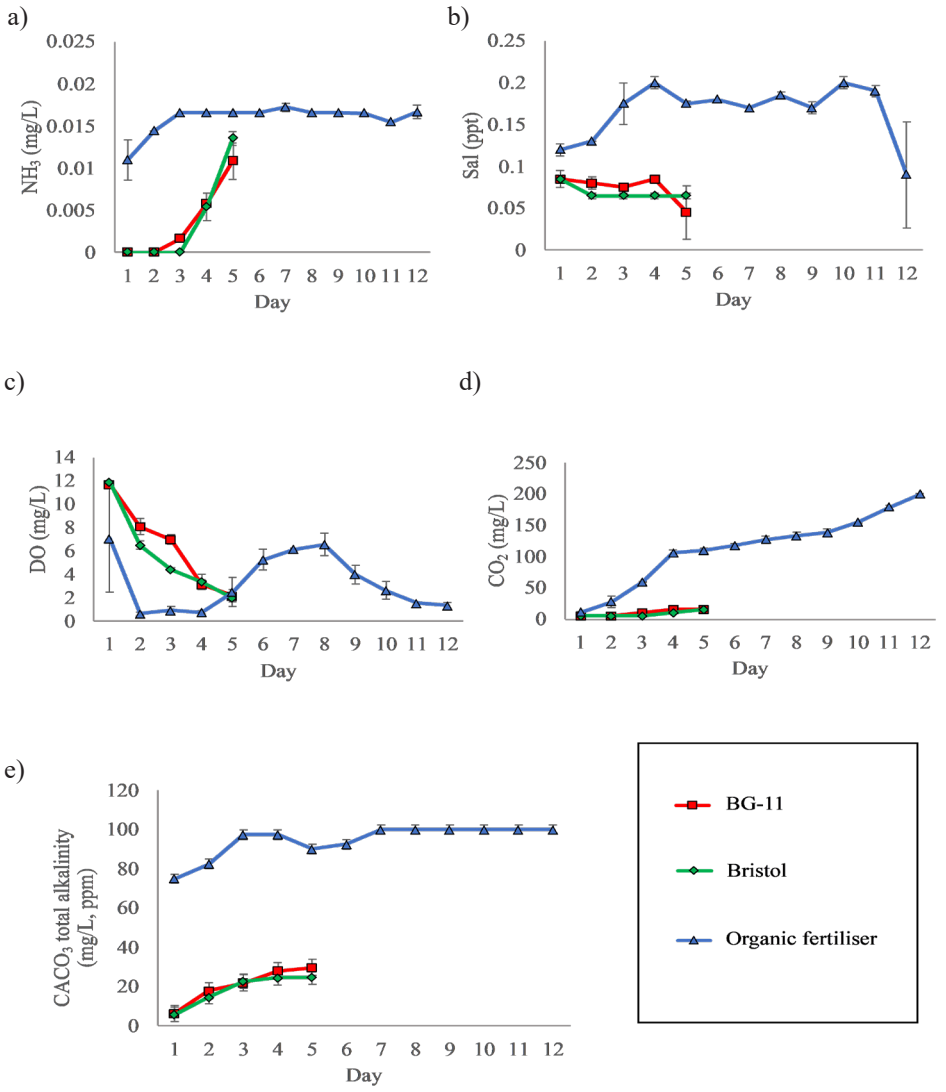

Figure 5. Water quality changes on three different mediums used to culture *Chlorella* sp. and *Moina* sp.: Day 1 indicated the first day of *Moina* sp. inoculation and the sixth day of *Chlorella* sp. cultivation. (a) Un-ionised ammonia level (NH_3); (b) Salinity of *Chlorella* sp. Culture (Sal); (c) Dissolved oxygen (DO); (d) Average carbon dioxide content (CO_2); and (e) Average $CaCO_3$ total alkalinity.

Organic fertiliser medium had higher $CaCO_3$ total alkalinity (19.6 ± 7.5 mg/L) than other mediums used, which only had a trace amount. It was due to agricultural lime ($CaCO_3$), an organic fertiliser ingredient, stabilising the pH value (Kar, 2016). However, when carbon dioxide was produced as a photosynthesis product, the dissolved carbon dioxide produced carbonic acid, which dissolved $CaCO_3$ (Qian et al., 2021). As a result, the concentration of carbon dioxide increased (Figure 5d), and the alkalinity also increased (Figure 5e). In addition, the high alkalinity (80 mg/L $CaCO_3$) might be the other factor which affected the *Chlorella* sp. growth on Day 1, as the limit for optimum *Chlorella* sp. growth was 63 mg/L $CaCO_3$ based on hardness (Kim & Kim, 2014).

CONCLUSION

This research showed that *Moina* sp. started to mature and reproduce after 72 h. The usage of different mediums affected the *Chlorella* sp. growth, whereby organic fertiliser medium showed the highest growth rate. *Moina* sp. reproduction in a *Chlorella* sp. biomass cultured using organic fertiliser showed a longer reproduction time, possibly due to growth inhibition by high NH_3 concentration and the rate of solid decomposition of the nutrient substrate in the culture. *Moina* sp. growth activity in all mediums used could slightly affect water quality but within the acceptable range. It was found that the most important factor determining the survival, longevity, and growth of *Moina* sp. was the medium used. *Moina* sp. could adapt to environmental stresses when the food supply was adequate. This study concludes that organic fertiliser with higher NH_3 content is more suitable for *Chlorella* sp. optimal growth and supports the growth of *Moina* sp.

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