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Sustainable mass production of *Moina* sp. biomass using semi-open and open outdoor cultivation systems

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Abstract

Due to their small size and high nutritional content, *Moina* sp. is an excellent food source for young fish such as Nile tilapia, African catfish, guppy, and carp. *Moina* sp. is increasingly being utilized as live feed in aquaculture, particularly in hatcheries. However, *Moina* sp. frequently obtained from contaminated environmental sources such as sewage with unsteady production. Thus, in order to use *Moina* sp. as a hygienic and sustainable source of livefeed, in this study, two culture system i.e. semi-open and open culture system, were developed and its production performance were analyzed. The highest production amount per cycle per tank for semi-open system was 733.3 ±251.7g and for the open system the highest production amount was 1463.7 ±348.2g. The mean for total biomass production in the semi-open system monthly was 6.26 ±0.92kg. Meanwhile, for the open culture system, almost five fold more production was recorded during the culture period which was 33.5 ±8.92kg. Based on the production performance of both culture system per metric tonne (MT) within the ten-month period of study, the total *Moina* sp. biomass produced per month per MT was almost comparable. In this study, the productions of both systems per tank per cycle in each system were consistent. The open culture system recorded significantly higher production of *Moina* sp. per culture cycle per tank compared to the semi-open system. The overall total productivity of each culture system every month, on the other hand, fluctuated slightly. Both culture system could be used to mass produced *Moina* sp. The study's findings were crucial for the prospective mass production and sustainable of *Moina* sp as live feed in aquaculture.

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Introduction

Moina sp. is a genus of freshwater crustacean increasingly used in aquaculture as live feed in various hatcheries. *Moina* sp. is commonly used as a live feed for freshwater fish, especially in the early stages of their development (Adeyemo *et al.*, 1994; Suminto & Nugroho 2019). Among the species used as live feed are *M. dubia* (Adeyemo *et al.*, 1994), *M. micrura* (Edeh *et al.*, 2021), and *M. macropa* (Suksomnit *et al.*, 2011; Islam *et al.*, 2017). *Moina* sp. are small and easy to culture, making them an ideal food source for fry, juveniles and young fish.

The small size and high nutritional value of *Moina* sp. make them an ideal food source for juvenile fish and other aquatic animals (Kadar *et al.*, 2014; Saputra *et al.*, 2018). *Moina* sp. can be fed with a variety of diets, including algae, yeast, and commercial dry feeds. *Moina* sp. are also easy to culture and can be produced in large quantities, making them a cost-effective alternative to other live feeds like brine shrimp or daphnia. The use of live feeds like *Moina* sp. can help to improve the survival and growth rate of fish in aquaculture operations. Furthermore, *Moina* sp. is a popular live food species for ornamental fish, especially for smaller fish like tetras, killifish, and bettas. They are small, easy to culture, and can provide a natural source of food for ornamental fish (De Silva *et al.*, 2011; Kwon *et al.*, 2013; Rathnayake *et al.*, 2016. *Moina* sp. provides essential nutrients like protein, fats, and vitamins. Ornamental fish tend to be more active and interested in live food, as opposed to dried or frozen food (Srikrishnan *et al.*, 2017; Rasdi *et al.*, 2020; Eiras *et al.*, 2022).

Moina sp. can be cheaply found in filthy and contaminated environmental sources such as sewage ponds or local drainage systems that are especially prone to pathogenic bacteria. Obtaining *Moina* sp. from such a polluted environment poses a significant danger of the existence of disease-carrying organisms, which might introduce diseases and further disrupt fish fry production activities. Furthermore, *Moina* sp. output is frequently low, restricted, and unpredictable, making it insufficient to fulfill the expanding demand of the fish fry hatchery and ornamental fish sector.

Thus, in order to use *Moina* sp. as a hygienic and sustainable source of livefeed, our study has developed two culture system namely, semi-open and open culture system. In this study we aimed to compare the production of each culture system in producing *Moina* sp. biomass.

Material and methods

Moina sp., organic culture fertilizer and microalgae species

Moina sp. used in this study was previously isolated from a fish culture pond located in Perak, Malaysia. *Moina* sp. stock culture obtained from Livefeed Laboratory, Fish Nutrition Unit, Fisheries Research Institute Glami Lemi, Negeri Sembilan. The stock culture was cultured in controlled and hygienic conditions to ensure that it was free from pathogen contamination. *Moina* sp. was put on Sedgwick-Rafter counting chamber slides and viewed under a compound light microscope (Olympus biological microscopes, CX23) to determine the culture density. The organic fertilizer, FRIGL-M1, used comprised of rice bran, corn flour, hydrated lime, urea and triple super phosphate (TSP) and the composition were as mentioned in the manual by Hanan et. al (2019) (Table 1). All chemicals were purchased from Chemiz (Malaysia).

Table 1. The composition of organic fertilizer used, FRIGL-M1 for mass cultivation of *Moina* sp.

Ingredient	Weight (w/v) (g/1000 L)
Rice bran	600
Corn flour	200
Hydrated lime, $Ca(OH)_2$	30
Urea	100
Triple super phosphate (TSP)	100

Microalgae, *Scenedesmus obliquus* GL03 (https://www.ncbi.nlm.nih.gov/sra/SRX17206935) was used for the cultivation *Moina* sp. (Hanan *et al.*, 2022). The microalgae were also obtained from the culture collection of Livefeed Laboratory, Fish Nutrition Unit, Fisheries Research Institute Glami Lemi, Negeri Sembilan. The microalgae was previously isolated from the freshwater riverine of Glami Lemi River, Jelebu, Negeri Sembilan, Malaysia. Microalgae cell density was calculated using Neubauer chamber and was observed under a compound light microscope (Olympus biological microscopes, CX23).

Culture system set-up

Both the semi-open culture system and open culture system consisted of up of ten units of fiberglass tank or cement pond each with a capacity of one metric tonnes (MT) and five metric tonnes (MT) respectively. Each unit has an (size) water inlet and (size) outlet. The system was also inclusive of two reservoir tank (capacity), a blower, electrical, drainage pipe, waste water tank and waste water treatment tank. A retractable roof was a component of the semi-open system but was absent from the open system.

Fig. 1. *Moina* sp. cultivation system FRI Glami Lemi A) semi-open culture system in B) open culture system.

Culturing Moina sp. biomass

For the semi-open system, as many of 10 units of fiberglass tanks (size: 1.2m x 3.0m) were used. Water was aerated for 3 days and filtered using a 40 µm nylon filter prior to stocking it in tanks in order to dipose of other zooplankton competitors. Strong aeration was introduced for 72 hours in 10 different aeration spots. The organic fertilizer, FRIGL-M1, was poured into the 1000L culture tank accordingly and was mix well. Every tools and equipments used in the study were soaked in a chlorine liquid beforehand. The fiberglass tank were inoculated with 20 L of *S. obliquus* at the density 1x10^6 cells/ml. the microalga was left to bloom after three days, the tank was inoculated with 40 g *Moina* sp. starter stock. The culture pond was allowed to grow for within seven days. The culture pond was aerated for 24 hours before harvesting.

For the open system, 10 units of cement ponds (size: 3.0m x 6.1m) were used. Water was aerated for 3 days and filtered using a 40µm nylon filter prior to stocking it in tanks in order to dipose of other zooplankton competitors.

Strong aeration was introduced for 72 hours in 10 different aeration spots. The organic fertilizer, FRIGL-M1, was poured into the 5000 L culture pond accordingly and was mix well. Every tools and equipments used in the study were soaked in a chlorine liquid beforehand. The culture pond were inoculated with 20 L of *S. obliquus* at the density 1x10^6 cells/ml. the microalga was left to bloom after three days, the tank was inoculated with 200 g *Moina* sp. starter stock. The culture pond was allowed to grow for within five days. The culture pond was aerated for 24 hours before harvesting.

Measuring Moina sp. production

The wet weight measurements were used to determine total production of *Moina* sp. for each cycle per tank or pond. Microalgae cell density was calculated using Neubauer chamber and was observed under a compound light microscope (Olympus biological microscopes, CX23). The production of each tank or pond unit in each system in a particular month was added to calculate the overall Moina sp. production per system per month. The total *Moina* sp. production per system per month per metric tonne (MT) was determined by adding the production of each tank or pond unit in each system monthly and divided with the system in MT capacity per month.

Water quality

Analysis of physico-chemical parameters were carried out for each experimental tank or pond. Physicochemical parameters pH, salinity, temperature, and dissolved oxygen (DO) were recorded weekly in water samples of experimental tanks and ponds using electric analysers (YSI-ProPlus, Yellow Springs Instruments Inc., OH, USA; pH-100 meter, LICHEN Sci-Tech, Co., Ltd., Shanghai, China). The chemical parameters of water quality for total ammonia (NH_3) and nitrite (NO_2) were determined using spectrophotometer (model HACH, DR 2800). The water quality was monitored on weekly basis.

Statistical analysis

Data were presented as mean ± standard deviation (SD). T test was used to test the statistically significant differences between two groups data of an independent variable using GraphPad-Prism 6.0. P-value <0.05 was considered as statistically significant.

Result and discussion

Results

In this study, the total production of *Moina* sp. using two different culture system were analysed. Fig. 2 showed the microscopic observation of *Moina* sp. and microalgae, *Scenedesmus obliquus* GL03, used as feed source in the mass cultivation of the livefeed. *Moina* sp. is regarded as a potential alternative ideal livefeed source for fish seeds especially for freshwater species (Saputra *et al*., 2018).

The genus *Scenedesmus* has been used in a number of studies to promote the growth of Moina (Nandini *et al*., 2004; Nandini *et al*., 2012; Chen *et al*., 2015). Ovie & Egborge (2002) reported the cell densities of Scenedesmus sp.(1.5×10^6 cells ml^{-1}) was satisfactory micro-alga food for *M. micrura*. The species S. obliquus is abundant in several countries, presents high tolerance at climatic variations at different values of pH, a high growth rate, and contains 40 to 56% of protein (dry mass) (Rocha *et al*., 2019). Afify *et al*., 2018 evaluated the suitability of *S. obliquus* and found that the species could be a source of essential (Thr, Val, Met, Ile, Leu, His) and non-essential (Arg, Ala, Pro, Asp) amino acids. In addition to that, the ease of cultivation and robustness of some strains of *Scenedesmus* makes them more appropriate for sustainable large-scale production (Pribyl *et al*., 2015).

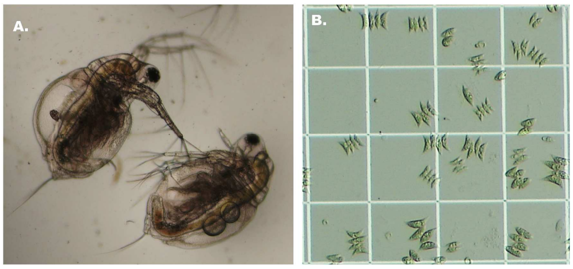

Fig. 2. This fig. showed the microscopic observation of A) *Moina* sp B) *Scenedesmus obliquus* GL03 under compound light microscope at 40x and 100x magnification respectively.

This study has developed two culture systems, semi-open and open culture systems, to utilize *Moina* sp. as a hygienic and sustainable source of livefeed. Table 2 showed the total weight production *Moina* sp. per tank per culture cycle in each of the system. Based on the data observed, the production per tank in the semi-open system and open system were considered consistent within the three culture cycle observed. The highest production amount for semi-open system was 733.3 ±251.7g and for the open system the highest production amount was 1463.7 ±348.2g. The open culture system recorded significantly higher production of *Moina* sp. per culture cycle compared to the semi-open system.

Table 2. Total weight (g) production *Moina* sp. per tank per culture cycle in each semi-open system and open system.

	Semi-open system (g)				Open system (g)			
	C1	C2	C3	Mean	C1	C2	C3	Mean
Tank 1	800.0	700.0	560.0	686.7±120.6	1220.0	1330.0	1371.0	1307.0 ±78.1*
Tank 2	700.0	1000.0	500.0	733.3±251.7	1861.0	1212.0	1318.0	1463.7 ±348.2*
Tank 3	560.0	500.0	1100.0	720.0±330.5	1430.0	1467.0	1174.0	1357.0 ±159.6*

C: Cycle, Significant difference of mean (*) was determined at p-value less than 0.05 ($p<0.05$)

The semi-open system exhibited the greatest mean density per tank per culture cycle in this study, at 1.8 ± 0.6 x10^4 ind/L, whereas the open culture system exhibited the highest mean density per tank per culture cycle, at 7.0 ±1.6 x10^3 ind/L (Table 2). Both systems likewise displayed nearly constant density over all three cycles per tank. The density recorded in the semi-open system was coherent as reported by Ovie & Egborge 2002) for *M. micrura* (1.1 x10^4 ind/L).The *Moina* sp density reported in the open culture system was almost comparable as reported by Shidik *et al*. (2021) in his study which was 6.770 ± 0.5 x10^3 ind/L (Shidik *et al*., 2021). The Moina sp. density recorded was also consistent as reported by Rahman *et al*. (2003) which was 6.505 x10^3 ind/L.

Table 3. Mean density *Moina* sp. per tank per culture cycle in each semi-open system and open system.

	Semi-open system ($x10^4$ ind/L)				Open system ($x10^3$ ind/L)			
	C1	C2	C3	Mean	C1	C2	C3	Mean
Tank 1	1.9	1.7	1.3	1.6±0.3*	5.8	6.4	6.6	6.3±0.4
Tank 2	1.7	2.4	1.2	1.8±0.6*	8.9	5.8	6.3	7.0±1.6
Tank 3	1.3	1.2	2.6	1.7±0.7*	6.8	7.0	5.6	6.5±0.8

C: cycle, Significant difference of mean (*) was determined at p-value less than 0.05 ($p<0.05$)

This research further evaluated each culture system's production performance on a monthly basis over a 10-month period from January to October in 2022.Throughout the study period, for the semi-open culture system, the total production of *Moina* sp. biomass was the highest in both May and August (7.2kg), and the lowest in March (4.2kg) (Fig. 3). The mean for total biomass production in the semi-open system monthly was 6.26kg ±0.92.

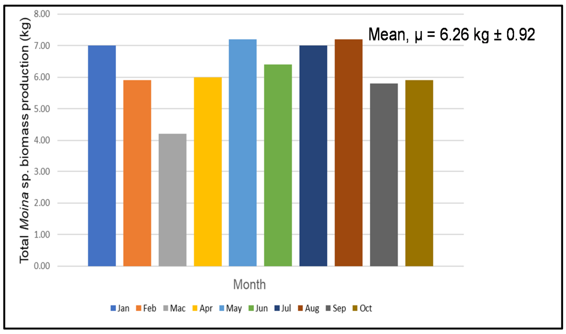

Fig. 3. Total *Moina* sp. monthly biomass (wet weight) (kg) production per semi-open system.

Meanwhile, for the open culture system, almost five fold more production was recorded during the culture period. The production of *Moina* sp. biomass in the system was highest in September (46.0kg) and was the lowest in April (18.2kg) (Fig. 4). The mean for total biomass production in the open system monthly was 33.5kg ±8.92.

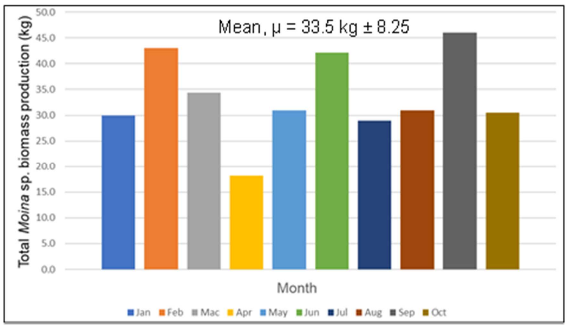

Fig. 4. Total *Moina* sp. monthly biomass (wet weight) (kg) production per open system.

Based on the production performance of both culture system per metric tonne (MT) within the ten-month period of study, the total *Moina* sp. biomass produced in each system per month per MT was almost comparable (Fig. 5). The mean total production per system monthly was 6.3kg ±0.9 and 6.7kg±1.6 for the semi-open system and open system respectively. The temperature for both culture system was around 26 up to 28°C. The pH was kept consistently at the range of 7 to 8, the ensuring good level of dissolved oxygen (>3.0mg/L) in the system. The acceptable salinity range for *Moina* sp ranged from 0.00 to 0.04 ppt. *Moina* sp. might readily perish if exposed to salinities more than 3.0 ppt. Total dissolved ammonia and phosphate levels were controlled within an acceptable range for *Moina* sp. cultivation.

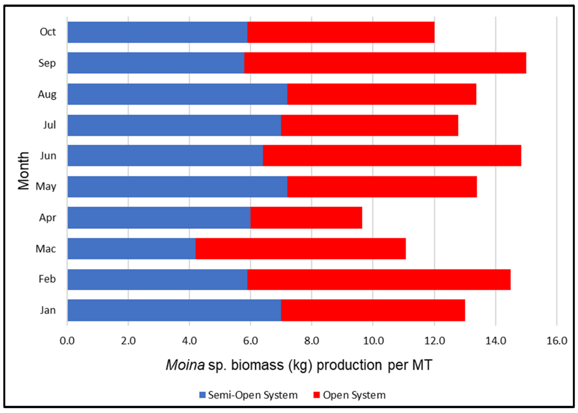

Fig. 5. Comparison of monthly *Moina* sp. biomass (wet weight) (kg) production per system per MT.

Discussion

Moina sp. is frequently used in aquaculture and aquatic research as live feed or food for a range of aquatic species. Several freshwater fish larval species, including tilapia, catfish, trout, and bass, may be fed *Moina* sp. during their early stages of development (De Silva *et al.*, 2011; Kadhar *et al.*, 2014; Islam *et al.*, 2017; Edeh *et al.*, 2021). *Moina* sp. can be fed to shrimp larvae, including marine shrimp (Penaeidae), freshwater shrimp (e.g., *Macrobrachium* sp.), as a dietary supplement and some species of crayfish and crabs (Alam *et al.*, 1993; Suksomnit *et al.*, 2021).

Because of their nutritional composition, size, and simplicity of cultivation, *Moina* sp. provides various advantages as live feed. *Moina* sp. has a high concentration of vital elements such as proteins, lipids, vitamins, and minerals (Shidik *et al.*, 2021). Their makeup gives them an ideal meal for many aquatic creatures, particularly during their larval phases, when they experience fast growth and development (Islam *et al.*, 2017; Edeh *et al.*, 2021). *Moina* are quite simple to culture, requiring only minimal equipment and an awareness of their needs (Rahman *et al.*, 2003). They can be produced in both outdoor and indoor culture systems, making them suitable for aquaculturists of varied degrees of expertise. *Moina* cultures may typically be kept alive for longer periods of time than other live feed species (Saputra *et al.*, 2018). Their capacity to adapt to changing climatic circumstances leads to their continual availability as live feed. In rare occasions, *Moina* sp. is utilized as a substitute to *Artemia*, particularly when Artemia is scarce or expensive. This can assist minimise reliance on a single live feed source and increase overall aquaculture operation resilience.

Moina sp. may be found at a low cost in unclean and polluted environments such as sewage ponds or local drainage systems, which are particularly prone to harmful bacteria. Obtaining *Moina* sp. from such a contaminated environment exposes to a considerable risk of disease-carrying organisms, which might bring illnesses and affect fish fry production operations further (Chang *et al.*, 2019). Furthermore, the output of *Moina* sp. is usually poor, limited, and unpredictable, making it unable to meet the growing demand of the fish fry hatchery and ornamental fish sectors.

Phytoplankton cultures such as *Scenedesmus* sp., *Chlorella* sp., and *Nannochloropsis* sp. can provide a natural food supply for *Moina* sp (Chen *et al.*, 2015). Microalgae supply important resources, such as vitamins and minerals, to let *Moina* sp. thrive (Nandini *et al.*, 2004; Nandini *et al.*, 2012; Composted materials and organic waste can all help to improve nutrition levels in *Moina* sp. cultures (Rahman *et al.*, 2003; Shidik *et al.*, 2021). These materials gradually release nutrients and can promote the growth of microorganisms that act as food for *Moina* sp. Nitrogen and phosphorus can be supplied via inorganic fertilisers such as ammonium nitrate, potassium nitrate, and monoammonium phosphate.

We developed two culture systems in this study that produce sustainable and hygienic *Moina* sp. as a source of livefeed in aquaculture. Good hygiene and management practises are critical for effective *Moina* sp. cultivation. Maintaining a clean and regulated environment helps to avoid the growth of harmful bacteria and maintains ideal circumstances for *Moina* culture's productivity and well-being. Maintaining a hygienic culture setting is critical for the health and development of your *Moina* culture. It may establish an appropriate and productive environment for your *Moina* sp. culture by following certain hygiene practises while remaining paying attention to its demand.

Conclusion

In this study, we successfully developed a cultivation system for producing consistent and high yields of *Moina* sp. in a hygienic environment. The productions of both systems per tank per cycle in each system were consistent. The open culture system recorded significantly higher production of *Moina* sp. per tank per culture cycle compared to the semi-open system. However, the overall total productivity of each culture system every month, fluctuated slightly. Both culture system could be used to mass produced *Moina* sp. The semi-open production system is appropriate for livefeed monthly requirements less than 10kg, while the open production system is appropriate for larger scale larviculture activity with monthly livefeed demand of more than 30kg. The study's findings were crucial for the prospective mass production and sustainable of *Moina* sp as livefeed in aquaculture.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Recommendation(S)

The data and information gathered from this experiment were critical in scaling up the production of *Moina* sp. as livefeed for continuous application in larviculture. Future prospective research that might be conducted is to further develop strategies for producing high-density and super-high density *Moina* sp. culture methods at an affordable cost. Furthermore, focus should be made on the biosecurity of high-density *Moina* sp. cultivation in order to ensure that the livefeed produced does not contain pathogens and to reduce the possibility of pathogen contamination.

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