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The effects of recirculating aquaculture system effluent water on the growth of *Moina macrocopa* (Straus)

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Abstract
In the present study, *Moina macrocopa* were grew at four different densities (4, 20, 40 and 80 individuals/50 mL), to investigate the effect of population density on the growth and reproduction using aquaculture effluent throughout 11 days. The organisms in high population densities i.e. 40 and 80 ind./50 mL of aquaculture effluent showed a lower rate of reproduction and population growth compared to those at 4 and 20 ind./50 mL. In the lower densities, *Moina* population increased to the highest densities of 55 (4 ind./50 mL) and 61 individuals (20 ind./50 mL) at the first 5 and 10 days, respectively. In general, two lower densities showed a higher population increment compared to other two tested densities. These results suggest that high population density suppressed the growth in *M. macrocopa*, therefore, lower population densities are recommended for commercial hatchery production using aquaculture effluent.

Keywords: Aquaculture effluent, hatchery, *Moina macrocopa*, population density

1. Introduction
Sustainable development in agriculture is a contemporary global issue. Bio-conversion of liquid and solid wastes of animal origin has immense potential for industrial exploitation. These bio-converted organic products are comparatively safer than chemical products to the humans and environment [9]. Some nutritional contents such as nitrogen and phosphorus are present in high amounts in these liquid and solid organic wastes, these waste materials could be recycled as a renewable energy, rather than eliminated in an unproductive way [19].

Among the zooplankton, *Moina* is the one of the cladocerans that could be mass propagated using organic wastes [9]. In natural habitats, biotic and abiotic parameters such as water quality, quantity and quality level of food available, thermal conditions, inter/intra-competition, predation, and population density are the most important factors that interact in the population growth of the zooplankton [11]. Among these factors, parameters such as population density and food availability are the predominant factors affecting the growth of *M. macrocopa* [11, 21].

Population density determines the production efficiency of *M. macrocopa* in a captive environment. This could be mainly attributed to the high intra-competition of space, food sources, and the accumulation of excretory products, which could eventually lead to a population decline in *Moina* cultivation. Loh *et al*., [17] proposed that fish faeces could be used as a potential culture medium to propagate *M. macrocopa* at a hatchery scale. These fish excretion materials might be one of the alternative ways to mitigate aquaculture impact to the aquatic environment. It also able to reduce the production cost in *Moina* cultivation, simultaneously. Furthermore, *Moina* grew with fish waste materials contained higher nutritional values such as highly unsaturated fatty acids (HUFAs) [18]. These nutrients could subsequently benefit the overall growth performance of fish/shrimp larvae in their early stages. Several studies found that *Moina* could not survive in a high population density under extreme environmental conditions such as high ammonia and total suspended solids [2, 6, 17]. There is also limited information available on the impact of environmental stressors to the growth of *M. macrocopa*, in particularly the maximum stocking capacity of *Moina* in a captive environment. Therefore, this study aimed to investigate the effects of population density on the growth of *Moina macrocopa* using aquaculture effluent collected from a Recirculating Aquaculture System (RAS) system.

2. Materials and Methods
2.1 Experimental set-up
The cladoceran stock, *Moina macrocopa* was obtained from a freshwater hatchery of University Malaysia Terengganu (UMT). The organisms were subsequently maintained with fresh microalgae in the wet lab of University Tunku Abdul Rahman (UTAR) prior to the study. The experiment consisted of 4 culture densities in various Falcon tubes (BD Falcon™, USA), labeled as T1 (4 individuals of *M. macrocopa*/50 mL effluent medium), T2 (20 ind./50 mL), T3 (40 ind./50 mL) and T4 (80 ind./50 mL), and a set of control with the same densities. A total of 48 Falcon

tubes including controls were prepared for the experiment. The experiment was performed in 6 replications for each treatment. Culture media was prepared from non-filter effluent water collected from Nile Tilapia, *Oreochromis niloticus* recirculating aquaculture system (RAS), the RAS consisted of 5 tanks and 20 fishes of each tank. Based on cross examination, the aquaculture effluent used for this study contained a mixture of 80% fish feaces, approximately 10% uneaten feed and 5–10% protein materials such as fish skin and debris. The water quality parameters in the RAS system e.g. total hardness, carbonate hardness, dissolved oxygen (DO), pH, temperature, total ammonia nitrogen (NH_3-N), nitrite nitrogen (NO_2-N) and nitrate (NO_3-N) were determined using water quality test kits (JBL GmbH & Co., Germany), and Hach colorimeter (DR890, Hach, USA) according to the Standard Methods for the Examination of Water and Wastewater [1]. The non-filter effluent (DO: 4±1 mg/L; NH_3-N: 0.44±0.15 mg/L; NO_2-N: 0.10±0.05 mg/L; NO_3-N: 23.32±11.12 mg/L) contained no algae was used to determine the effect of density in ***M. macrocopa***. For control treatment, de-chlorinated tap water (DO: 3±1 mg/; NH_3-N: 0 mg/L; NO_2-N: 0.01±<0.001 mg/L; NO_3-N: 1.37±0.05 mg/L) was used as a comparison. During the study, culture polyether tubes were un-capped to allow permeable of oxygen into the culture media. Average ambient temperature was recorded at 29±1°C and the natural photoperiod at 12 h light: 12 h dark. Culture media in the Falcon tubes were replenished twice a week.

2.2 *Moina macrocopa* quantification

For *Moina macrocopa* quantification, sterile microclothes (Calbiochem, Merck, Germany) was used to cover the mouth of the Falcon tubes, and the culture media containing *M. macrocopa* were decanted slowly into Petri dishes. *M. macrocopa* was collected by rinsing the microclothes gently with 5 mL culture medium using a glass pipette. This procedure was repeated again until all *M. macrocopa* were completely removed from the tubes. *M. macrocopa* were then transferred into different Petri dishes for quantification. The counting was carried out using a Tally counter under a dissecting microscope (10x to 40x magnification). After quantification, live *M. macrocopa* were returned to the culture vessels, and the dead organisms were discarded. The experiment was carried out for 11 days.

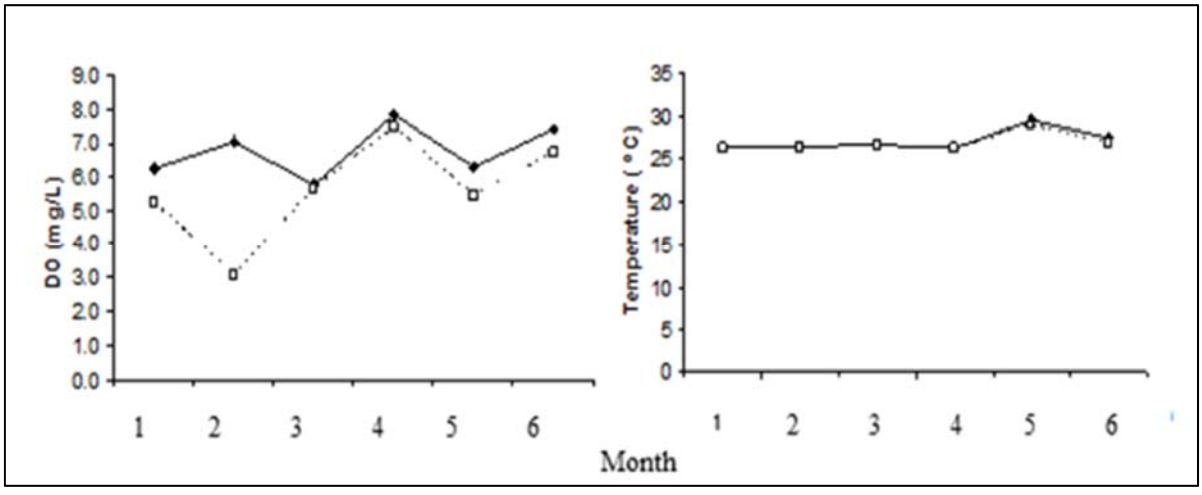
2.3 Statistical Analysis

All data was analyzed using Minitab version 13 (Minitab Inc., USA). The data was firstly tested by the Kolmogorov-Smirnov test (Normality) and Levene's test (homogeneity-of-variance) [5] prior to the one-way ANOVA and *Post Hoc* Tests (Tukey's tests). Data was tested to compare the differences between population density, culture media, and experiment duration. Statistical significance among the different treatments was accepted at $p < 0.05$ or $p < 0.001$.

3. Results

3.1 RAS system water quality

Dissolved oxygen (DO) levels of the recycled influent water were recorded at 3.06 to 7.46 mg/L, which were slightly lower than DO (5.78 to 7.84 mg/L) in the fish rearing tanks (Figure 1). The pH levels of the effluent (waste water) and influent water (treated water) were found to be in the range of 7.20 to 7.69, which was relatively constant throughout the 6-month culture period (Figure 1). Water temperature did not fluctuate much neither in the culture water nor the treated water. The lowest temperature was recorded at 26.2°C, while the highest was at 29.5°C (Figure 1). Total water hardness and alkalinity (carbonate hardness) of the culture system were shown in the Table 1. These two parameters were slightly below the recommended range, for instance total water hardness for fish farming is suggested between 10 to 400 mg/L, while alkalinity should fall within 10 to 100 mg/L [27]. The NH_3-N in the effluent was ranged from 0.04 to 0.60 mg/L, while the influent water had a significantly lower level at 0.04 mg/L (Figure 1). On the other hand, NO_2-N level in the fish rearing tanks was ranged from 0.006 to 0.071 mg/L (Figure 4.1), which were considerably safe for the cultured fishes (suggested concentration <0.1 mg/L) based on US Environmental Protection Agency [27]. However, NO_3-N level was slightly higher in both effluent and influent water due to intensive stocking density of the fish (averaging 300 g per liter culture water). Nevertheless, no mortality of the Tilapia was found during the culture period. For the effluent water, the NO_3-N level was ranged from 10.0 to 58.9 mg/L, while, for the influent water, it was recorded from 9.4 to 60.9 mg/L (Figure 1). This parameter was above the recommended range of the standard water quality (< 3.0 mg/L) suggested by EPA [27].



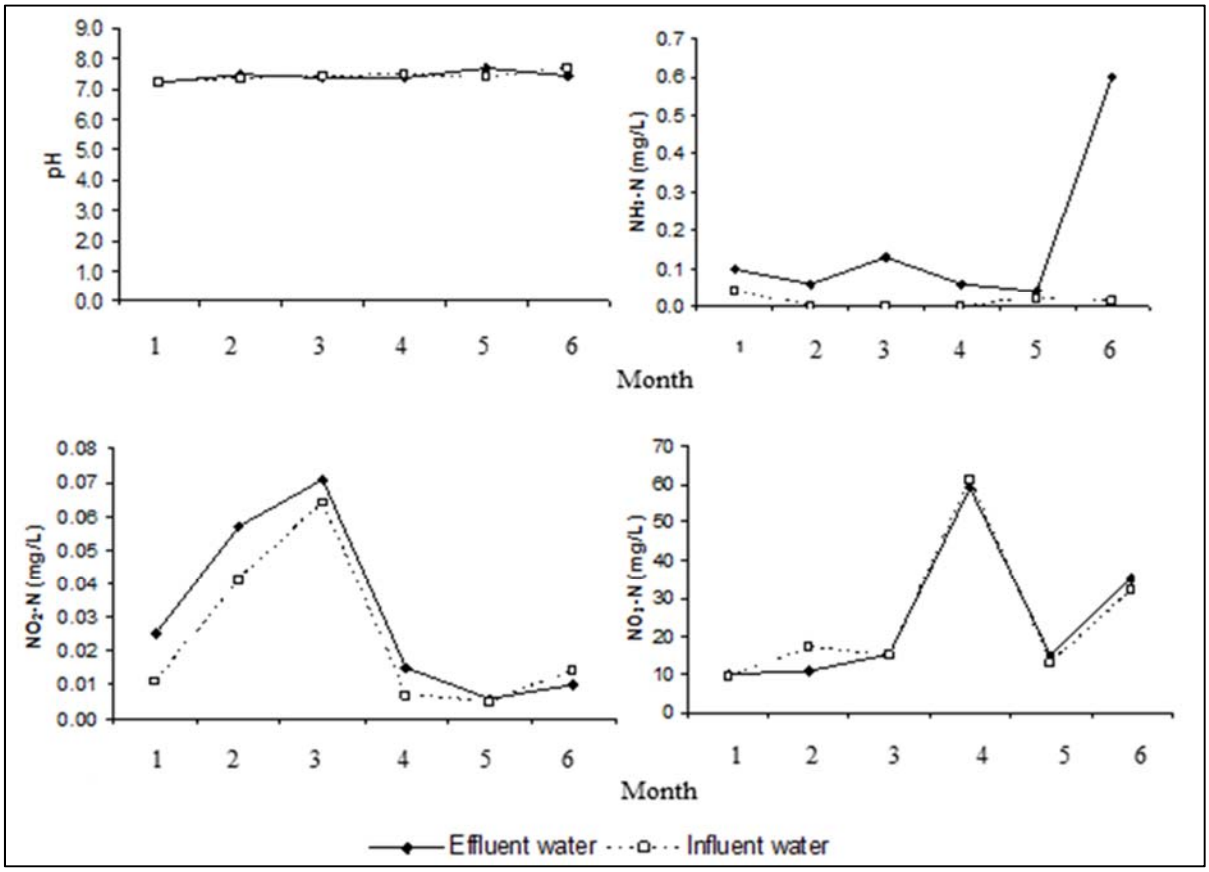


Fig 1: Water quality in the effluent and influent water of the Nile Tilapia (n = 100) RAS system within 6 month culture period.

Table 1: Total water and carbonate hardness of the effluent and influent water

	Effluent water		Influent water	
Month	**Total hardness**	**Carbonate hardness**	**Total hardness**	**Carbonate hardness**
	(GH, °d)	(KH, °d)	(GH, °d)	(KH, °d)
1	7.0 -8.0	6.0	7.0 - 8.0	6.0
2	7.0 - 14.0	6.0	7.0 - 14.0	6.0
3	>14.0	6.0	14.0 - 21.0	6.0
4	>14.0	6.0 - 10.0	>14.0	3.0 - 6.0
5	7.0 - 8.0	6.0	>14.0	6.0
6	>14.0	10.0	>4.0	6.0

3.2 Effects of effluent water on the population growth of *M. macrocopa*

Figure 2 shows the comparison of population densities of *M. macrocopa* in the control and treatment culture for 11 days. Population density of *M. macrocopa* cultured with aquaculture effluent (T1) was increased significantly ($p < 0.05$) (Table 2) on day-3 compared to the control (Figure 2). The population densities of *M. macrocopa* in the control culture were relatively constant with low population density (4 individuals of *Moina macrocopa*/ 50 mL effluent medium). A positive growth trend in T1 was observed in the effluent culture (Figure 2), whereby the population densities increased gradually from day-0 to day-4. A total number of 40 individuals were observed at day-4 to day-5. The population densities reached their peak, recorded at 55 individuals of *M. macrocopa* at day-5 (Figure 2). The population in T1, however, decreased gradually after the peak and slightly increased at day-8, before it declined again at day-10. Similar growth trend was observed at T2, the *M. macrocopa* grew slowly for the first 2 days in the effluent culture. The numbers dropped slightly on day-3 and day-4. However, the *Moina* population started to increase after day-4, with an exponential scale ($p < 0.05$, Table 2) to day-5, and then increased again at a much slower rate later today-8 (Figure 3). The population continued to increase and reached to the peak density (61 ind.) at day-8. The population density then dropped ($P < 0.05$; Table 2) at the end of the experiment. On the other hand, the number of *Moina* in control treatment was decreased from day-1 to day-6. An absolute mortality was noticed at day-10 (Figure 3). In T3, the population density of *M. macrocopa* showed an increment (4 ind.) at day-1, and started to decrease to 23 individuals until day-5 (Figure 4). The population started to increase again until day-8 (42 ind.) before it declined to 4 individuals at day-11. A drastic effect was observed in the control treatment, whereby, no survivors were recorded from

day-6 onwards (Figure 4). The population density of *M. macrocopa* in T4 reached its peak density at the day-1 (after inoculation), recorded with 108 individuals. However, both population densities in the effluent and control treatments decreased drastically after that. At the end of the experiment, only 2 organisms were observed in T4 culture medium, no organism was found in the control started at day-8 (Figure 5). Statistical analysis showed that all population densities were found to be significantly influenced ($P < 0.05$) by the population densities and days (Table 3). However, there was no significant difference ($P > 0.05$) in the population density among the groups (T1, T2, T3 and T4) on day-8 and day-11 (Table 3).

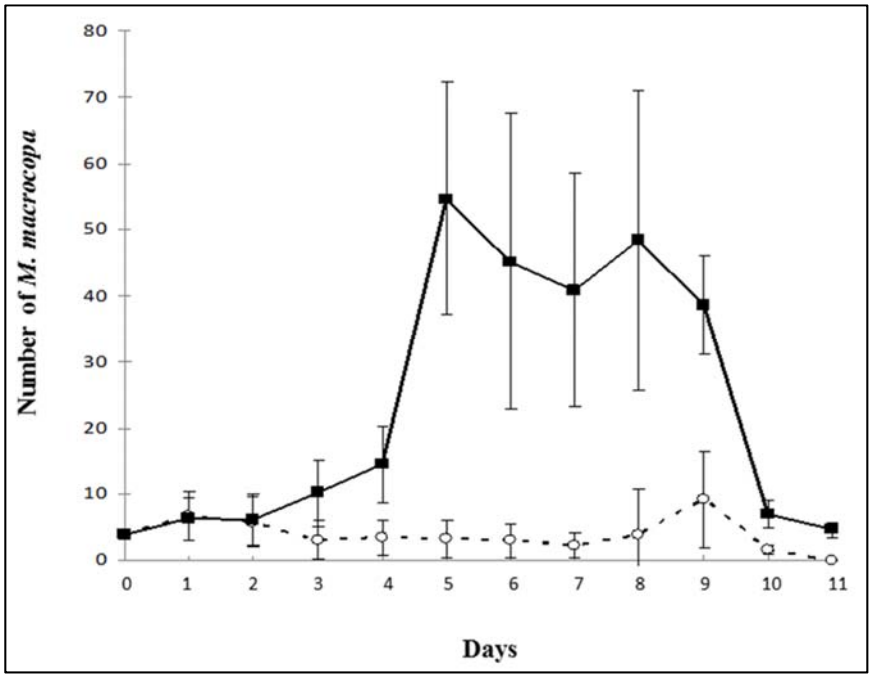


Fig 2: Population growth of *Moina macrocopa* at the initial stocking density of 4 ind. per 50 mL culture media. ■ indicates wastewater effluent (RAS); ○ indicates control (de-chlorinated water). Error bars show mean ± of the standard deviation.

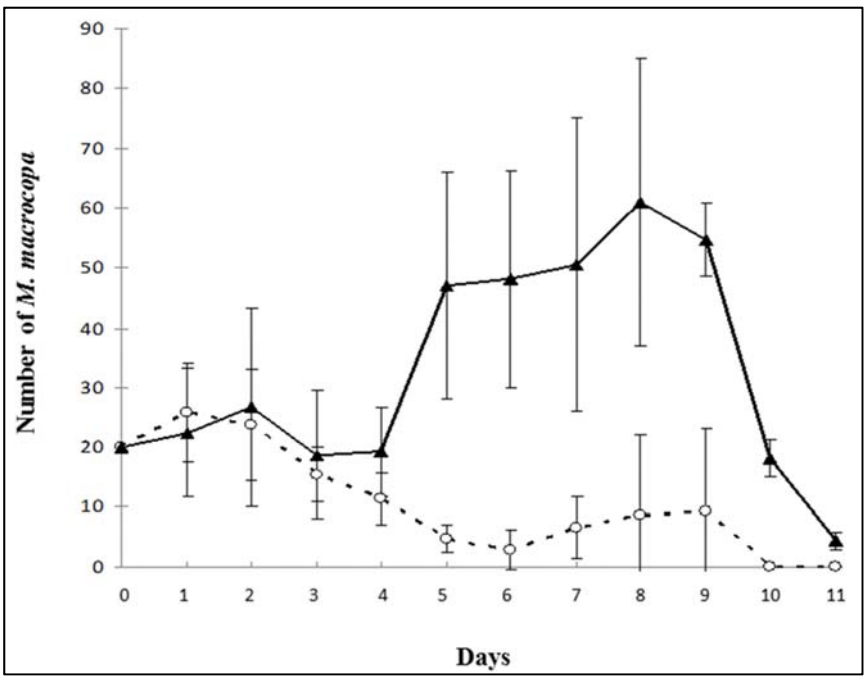


Fig 3: Population growth of *Moina macrocopa* at the initial stocking density of 20 ind. per 50 mL culture medium. ▲ indicates wastewater effluent (RAS); ○ indicates control (de-chlorinated water). Error bars show mean ± of the standard deviation.

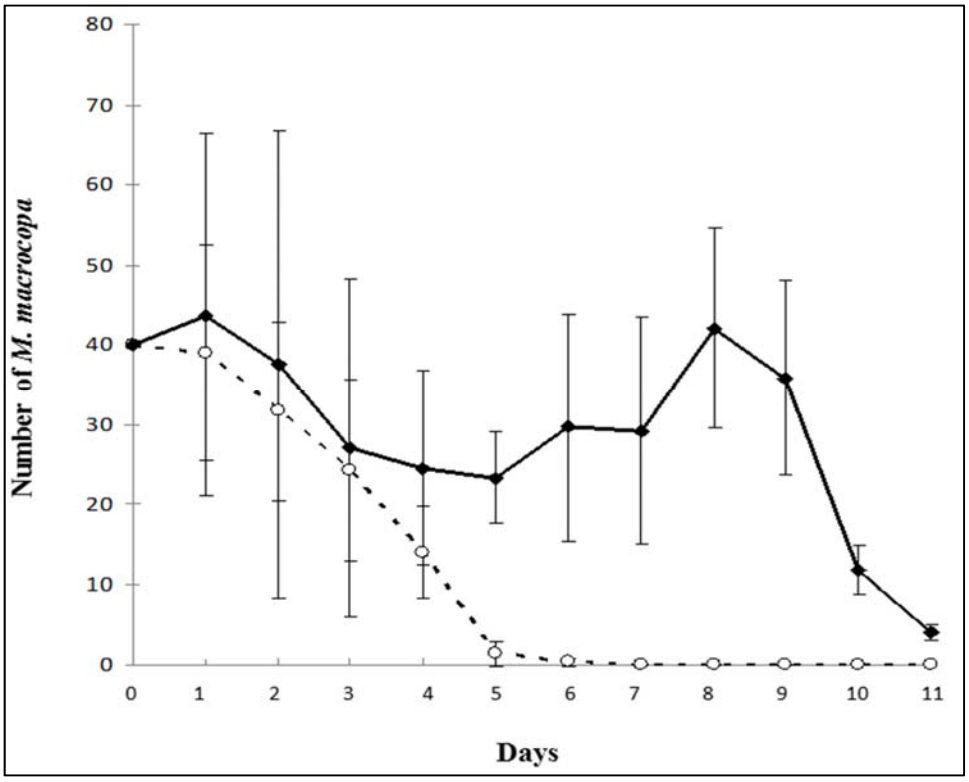


Fig 4: Population growth of *Moina macrocopa* at the initial stocking density of 40 ind. per 50 mL culture medium. —◆— indicates wastewater effluent (RAS); –○– indicates control (de-chlorinated water). Error bars show mean ± of the standard deviation.

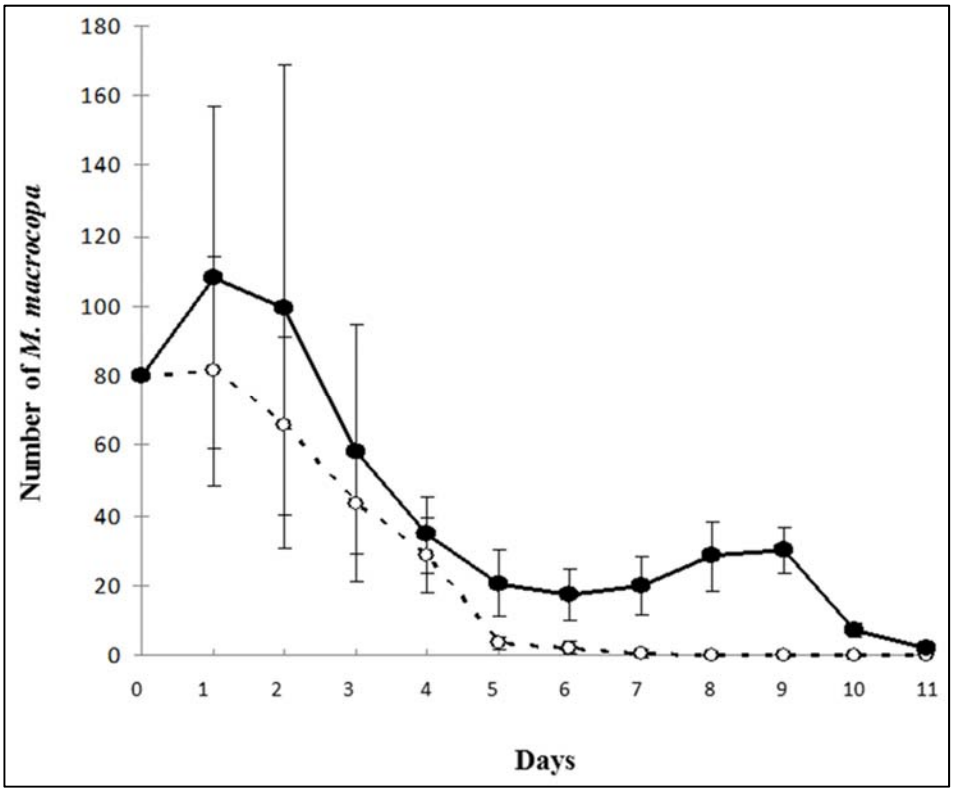


Fig 5: Population growth of *Moina macrocopa* at the initial stocking density of 80 ind. Per 50 mL culture medium. —●— Indicates wastewater effluent (RAS); –○– indicates control (De-chlorinated water). Error bars show mean ± of the standard deviation

Table 2: The *F* and *P*-value from one-way ANOVA analysis used in the comparison of population density between the effluent cultures (T1, T2, T3 and T4) along the culture period.

Number of *Moina* in culture treatments								
Day no.	**T1**		**T2**		**T3**		**T4**	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
1	0.063	0.806	0.356	0.564	0.186	0.068	1.245	0.291
2	0.022	0.886	0.148	0.708	0.209	0.657	1.268	0.286
3	8.368	0.016*	0.481	0.504	0.094	0.765	0.826	0.385
4	18.060	0.002**	4.946	0.050	3.637	0.086	0.910	0.363
5	49.574	0.000***	29.325	0.000***	80.369	0.000***	18.857	0.001**
6	21.076	0.001**	36.019	0.000***	25.490	0.001**	25.900	0.000***
7	28.212	0.000***	18.386	0.002**	24.690	0.001**	33.380	0.000***
8	10.485	0.031*	6.825	0.059	33.707	0.004**	24.103	0.008**
9	48.332	0.000***	55.944	0.000***	52.137	0.000***	128.273	0.000***
10	19.692	0.011*	108.000	0.000***	43.750	0.003**	36.750	0.004**
11	49.000	0.002**	24.143	0.008**	48.000	0.002**	36.577	0.004**

P-values with asterisk denote the significant level of study (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). T1 denotes the density of 4 ind.; T2: 20 ind.; T3: 40 ind. and T4: 80 ind. /culture.

Table 3: The significance values of all population densities in experimental period (in days) (A), and the interaction of density versus days in various population densities in the study (B).

A)			**B)**		
	Population densities		**Interaction**	***F*-value**	***P*-value**
Day no.	**T1, T2, T3 and T4**		**Population density:**		
	F-value	*P*-value	T1		
1	15.790	0.000***	Density × Days	14.464	0.000***
2	6.561	0.003**			
3	5.341	0.007**	T2		
4	4.926	0.010*	Density × Days	7.388	0.000***
5	8.735	0.001**			
6	4.505	0.014*	T3		
7	3.635	0.031*	Density × Days	2.208	0.028*
8	1.640	0.256			
9	9.500	0.000***	T4		
10	12.367	0.002**	Density × Days	7.743	0.000***
11	3.690	0.062			

P-values with asterisk denote the significant level of study (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). T1 denotes the density of 4 ind.; T2: 20 ind.; T3: 40 ind. and T4: 80 ind. /culture.

4. Discussion

The present study evaluated the growth performance of *Moina macrocopa* at different stocking densities using effluent water as a culture medium from a Nile Tilapia RAS system. There are several factors that could affect the life history of *M. macrocopa* in a culture environment, such as population density, trophic conditions, water quality, and water-borne chemical cues [23]. Among these, population density is presumably the major limitation to the overall growth performance of *M. macrocopa*, this is mainly because of the competition of space, food and bioaccumulation of excretion materials.

The population density of *M. macrocopa* at T1 (4 ind./ 50 mL, or equivalent to 100 ind./ L) showed a better growth performance compared to T3 and T4. *M. macrocopa* grew at T2 shared the similar growth trend as in the density of T1. In terms of time and efficiency, *M. macrocopa* at T1 reached its peak population on day-5, which was 3 days earlier than the density of T2 (highest production at day-8). Furthermore, *M. macrocopa* cultivated at T2 required higher starting density as compared to T1. Therefore, instead of using a higher culture density (≥ T2), the density T1 is preferable with regarding on the cost and time consuming. This also means that *M. macrocopa* could be harvested within a shorter period of time, thus allowing more number of cultivation batches per cycle. A short production time is also important for commercial livefeed producers as it could greatly reduce operational time and cost in the hatchery [7].

Generally, *M. macrocopa* has a higher density adaptation in a captive culture environment compared to other cladoceran species e.g. *M. micrura*. According to Jana and Pal [15], the growth performance of *M. micrura* was limited at the population density of 75 ind./ L. Nevertheless, *M. macrocopa* showed a better adaptation at the density of 100 to 500 ind./ L, which is equivalent to 4 ind. and 20 ind./ 50 mL shown in the study. Our study suggested that *M. macrocopa* should not be exceeded 500 ind./ L in an enclosed culture environment, because high stocking density may possibly lead to a population collapse. This could be caused by insufficient of space, food availability, sexual transformation, and/or allelopathic effects [4, 8, 10, 12, 13, 14, 16, 22, 25, 26]. The food supply of *Moina* embryos are closely related to the energy reserves of the females where the embryos are fed

by the placentas [3, 10]. The survival of neonates is dependent upon the level of reserves which remain after the completion of the embryonic development in the female's brood pouch. Therefore, high population densities cause the zooplankton to experience limitation in food supplies, hence reducing the population growth rate [23].

Population density has a direct association with sexual reproduction [22]. When environmental conditions are unfavourable, high population density would shift the reproduction mode of *Moina* from asexual to sexual (gamogenesis). Unfavourable conditions would then induce the production of males and sexual females, which leads to the formation of ephippia (resistant eggs). When the environmental conditions become favourable again, the resistant eggs are then hatch and produce only parthenogenetic females [8, 14]. At high population densities such as T3 and T4, the reproduction mode could possibly had already shifted from parthenogenesis to gamogenesis causing the number of newborns to decrease, and thus reducing the overall population number [23].

Studies also showed that high population densities might be affecting the organisms to modify their feeding behaviour by releasing and accumulating chemical substances [28], nonetheless, these chemical substances are not the products of food metabolism. They can inhibit the population growth and produce smaller sized animals as reported in the *Daphnia* population study [4]. These effects are termed as 'negative interference' or 'allelopathy'.

Our study demonstrated that *M. macrocopa* can be cultured using organic farm wastes. This is also in agreement with Nandini *et al*., [21] and Golder *et al*., [9]. The authors showed that *Moina* spp. can be cultivated using crude wastewater from treatment plants and terrestrial animal waste products. However, *Moina* cultivation using domestic wastes as a food source posing a high risk of pathogenic contamination or toxicant pollution [24]. Bio-magnification of accumulated toxicants may eventually affect their prey, for example fish fry after feeding with contaminated livefeed. This problem could be mitigated through using aquaculture effluents from a fish culture system. Under a constantly monitored environment, the effluents discharged in the RAS system are considerably safer than using untreated wastewater or animal wastes as the culture medium for *M. macrocopa*.

5. Conclusion

Our study showed that *M. macrocopa* could be cultivated using RAS effluent, however, the population density of *M. macrocopa* for a start-up culture is recommended lower than 20 ind./ 50 mL of RAS effluent. From the view of commercial perspective, population density with 4 ind./ 50 mL, or equivalent to 100 *Moina* per liter is suggested for an optimal growth of *M. macrocopa* within a shorter cultivation time.

6. Acknowledgment

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