

Article

# Effect of different salinities gradient on fatty acid composition, growth, survival and reproductive performance of *Moina macrocopa* (Straus 1820) (Crustacea, Cladocera)

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**Abstract:** Salinity is a known factor which shapes population dynamics and community structure through direct and indirect effects towards aquatic ecosystems. The responses of *Moina macrocopa* (Cladocera) collected from Setiu Wetland lagoon (Terengganu) were evaluated through manipulative laboratory experiments to understand the ability of *M. macrocopa* to tolerate under high salinity stress. Specifically, the fatty acid composition, growth, survival and reproduction of this cladocerans species were examined. Sodium chloride (NaCl) were used in the treatments water with the concentration 0, 4, 6, 8, 12, and 15psu. Fatty acid levels were determined using Gas Chromatography and Mass Spectrophotometry (GC-MS). Results clearly indicate that normal conditions give the highest content of fatty acid, especially the polyunsaturated fatty acid content like EPA (eicosapentaenoic acid), ALA (alpha-linoleic acid), ARA (arachidonic acid) and DHA (docosahexaenoic acid). Furthermore, *M. macrocopa* survival also was best at 0psu, with percentage reached 98%, whereas the opposite occurred at 15psu, with approximately 9% of viable animals survived. On other aspects, *M. macrocopa* also showed the highest reproduction rate at 0psu (e.g. average initial age of reproduction, 4.33±0.58 days) compared with other salinities level. Interestingly, the difference in terms of growth at different salinities was unapparent, an unexpected outcome when adverse effects such as osmoregulation pressure on the organism are considered. Based on the results, we conclude that *M. macrocopa* can only tolerate salinity below 8psu and, is unable to withstand stressful environmental condition engendered with salinities above 8psu.

**Keywords:** freshwater zooplankton; *Moina*; salinity tolerance; NaCl; fatty acid

## 1. Introduction

Industrialization and urbanization often cause a variety of negative impacts on the aquatic ecosystem, especially the freshwater ones (e.g., due to proximity to source and, lower volumes and exchange rates or dilution). Salt concentrations of freshwater habitat have increased because of industrial activities and urbanization, with resultant impacts on freshwater animals (Ghazy et al., 2009). Agricultural activities, urbanization and other coastal development projects are rapidly taking place in Southeast Asia that can drastically change the geomorphological structures (M. N. Lani et al., 2018). Setiu Wetland lagoon is considered as a major

location for brackish water mariculture activity including finfish cage, pond culture and shellfish farming (M. N. Lani et al., 2018). This anthropogenic interference has both directly and indirectly degraded the natural environment of Setiu Wetland lagoon. Major concerns regarding anthropogenic activities at Setiu Wetlands include resulting water contamination, coastal destruction and, productivity impact (Zaleha et al., 2018). The increase of water sea levels and, coastal erosions have contributed tremendously to salinity changes (Ekaterina et al., 2017) and, have damaged aquatic habitat and ecosystems (Reliana, 2012). Aquatic organisms are sensitive towards changes in water condition. Small fishes are more opportunistic in nature that they can adapt and survive in stressful conditions found in shallow lagoons (Zaleha et al., 2018). Zooplankton is an important community in the aquatic ecosystem for energy transfer from the primary producer to fishes (Soni and Thomas, 2014). Factors such as species of zooplankton and ecosystem type heavily influence the feeding of small fishes (Souza et al., 2013). Ecosystem condition affects both foraging efficiency and predation risk and, may beneficially increase the former and decrease the latter in fish (Scharf, 2006).

Zooplankton species distribution and abundance are influenced by various environmental factors such as water transparency, climate change and food nutritional content. The abundance and diversity (El Gamal et al., 2014) of zooplankton and phytoplankton (Ekaterina et al., 2017) are affected by the increase of salinity in the environment. Therefore, water salinity shift also can alter the original taxa composition and ecological process, such as primary productivity, decomposition, nutrient cycles and, food web function (Emanuela et al., 2010). Salinity increase in freshwater has the potential to reduce zooplankton richness especially in the cladoceran community and thus, change adaptation of the species to a more salt tolerant species (Reliana, 2012). Research on cladocerans has been widely conducted, due to their ecological importance, sensitivity towards environmental changes and ease of handling (Sarma & Nandini, 2006; Ana Carolina et al., 2011). Cladocerans are an important group in the zooplankton community with most of the species living in freshwater environments with salinities of <1psu (Ghazy et al., 2009). This is due to osmoregulation adaptations that only allows them to tolerate lower salinity levels (Bruno et al., 2012). A few of cladoceran species have shown an ability to adapt to salinity changes. However, this adaptation can impact the growth and reproduction of cladoceran species in comparison with response to their original habitat. Cladocerans are reported to be abundant in freshwater ecosystems as compared to brackish water environments (Forro et al., 2008; Erondur et al., 2017). However, there are a few cladoceran species that can live in a saline environment; mostly from brackish water species which can tolerate salinity beyond 13psu such as *Daphnia pulex*, *Daphnia thomsoni* and *Daphnia magna* (Bruno et al., 2012).

*Moina* sp., commonly referred to as water fleas, are crustaceans within the family of Moinidae which inhabit freshwater and also thrives in both brackish and marine environment. The reproductive cycle of *Moina* sp. has both a sexual and asexual phase. Normally, the population consists of all females that reproduce asexually. Under optimum conditions, *Moina* sp. reproduce at only 4 to 7 days of age, with a brood size of 4 to 22 per female (Rottman et al., 2014). Broods are produced every 1.5 to 2.0 days (Rottman et al., 2014), with most females producing 2 to 6 broods during their lifetime (Rottman et al., 2014). Under adverse environmental conditions, males are produced, and sexual reproduction occurs resulting in resting egg (ephippia) production. This case is similar to brine shrimp eggs production. The stimuli for the switch from asexual to sexual reproduction in populations of *Moina* sp. is an abrupt reduction in the food supply and significant change in environmental conditions. The density of *Moina* sp. cultures routinely reaches densities of 5,000 individuals per Litre (19,000/gallon) and therefore, they are well adapted to intensive culture (Rottman et al., 2014). *M. macrocopa* are rich in protein and nutrients. They are excellent live foods for fish and prawn larvae when compared to other live feeds such as rotifer and *Artemia* (Loh, 2012). However, the fatty acid content of *Moina* sp. varies when they are cultivated with different culture media and different levels of environmental stress (Loh, 2012).

Studies have shown that lowering ambient temperature tends to increase the production of C20-22 polyunsaturated fatty acid production in planktonic crustacean (Zaleha et al., 2014). However, the study on the impact other environmental parameters on freshwater cladocerans, such as salinity change, is still limited and some quantitative data are only available on the fatty acid profiles of zooplankton at different temperatures but, salinity-induced effects on the fatty acid composition of *M. macrocopa* have not been evaluated (Gama-Flores et al., 2015). Lipids and fatty acids are structural components of cell membranes that play an important role in growth, survival and reproduction of aquatic organisms, especially in the early life stages of larvae (Gama-Flores

et al., 2015). Salinity changes in the freshwater habitat can exert an impact on the distribution or, community structure of water fleas thus, affecting feeding behavior and indirectly, also having a slightly impact on nutritional composition. The current study investigated the tolerance of *M. macrocopa* in response to salinity changes. The objectives of this study were to evaluate the ability of *M. macrocopa* in adapting to a saline environment (Inger et al., 2010) and, to investigate the potential of *M. macrocopa* in saline tolerance (Bruno et al., 2012). Specifically, the study aimed to assess the impact of salinity on fatty acid composition, growth, survival and, reproduction of *M. macrocopa* when exposed to different salinity levels. *M. macrocopa* are also considered as a useful indicating animal to evaluate impacts of increasing salinity on the aquatic environment.

## 2. Materials and Methods

### 2.1. Cultivation of *Moina macrocopa*

Live *M. macrocopa* were collected from the lagoon of Setiu Wetland, located in the state of Terengganu (N 5° 68' E 102° 70'), and cultured in the Hatchery of University Malaysia Terengganu prior to use in experiments. Zooplankton net (sizes from 50 until 200  $\mu\text{m}$ ) was used to sample zooplankton in the sampling area. Salinity, dissolved oxygen and temperature of water at the sampling site was measured using a YSI Multi-probe instrument (YSI Model 33).

*Moina* sp. were cultured in a 100 mL flask filled with freshwater and, were periodically upscaled to 2000 L tanks for mass culture once the density reached 10 individuals per milliliter (ind/mL). Water parameters were maintained at 26°C to 30°C with a photoperiod of 12 h light: 12 h dark (Loh et al., 2012). In the current study, individuals in the neonates' stage >24 hours old, were used (Ghazy et al., 2009) and, isolation of neonates was carried out using nets with different mesh sizes (50  $\mu\text{m}$  until 200  $\mu\text{m}$ ). During the experiment, *M. macrocopa* were fed with fresh food of microalgae at a concentration of  $14 \times 10^7$  cells/mL (Gani et al., 2016). Microalgae *Nannochloropsis* sp. were used as feed to maintain similarity with the original habitat food resource. Pure microalgae stock was cultured at room temperature (25°C until 28°C) with 24 hours of light. The algal strain *Nannochloropsis* sp. was obtained from the UMT hatchery algal laboratory and, the algae were cultured as feed for *M. macrocopa*. Microalgae were harvested when the culture reached the exponential phase (Hasnun et al., 2011) or before the decline phase (Rihab et al., 2013). To maintain the salinity of *M. macrocopa* cultivation, the *Nannochloropsis* sp. was cultured at a lower salinity by gradually decreasing the salinity until it reached the same range of *M. macrocopa* cultivation.

Changes in growth, survival and reproduction of *M. macrocopa* were examined at six different salinities. The salinity level used was within the range of those able to occur within the original habitat of Setiu Lagoon. According to M. N. Lani *et al.*, (2018) and Suratman *et al.*, (2014), the water salinity in Setiu Wetland ranges from 0psu until 35.0psu. In the laboratory, *M. macrocopa* was tested at 0, 4, 6, 8, 12, and 15psu (El Gamal *et al.*, 2014), for the similarity of the range of salinity in Setiu wetland. The effect of salinity treatments was conducted by using sodium chloride (NaCl) solution as test waters. Every treatment ran in parallel with control in three replicates, each replicate contained 10 neonates in 100 mL test water in 250 mL glass beakers. The artificial test water was diluted with the synthetic freshwater media to the respective test salinity. Test media were prepared by diluting saline water with synthetic freshwater media until the required salinities were recorded with a salinity conductivity- temperature Meter (YSI Model 33). The culture medium (0psu) was used as a control to mimic the original habitat of freshwater *M. macrocopa* in nature with no salinity level.

The average percentage survival of *M. macrocopa* was determined every alternate day by using a zooplankton counting chamber. The initial density is known, the final density of *M. macrocopa* was then recorded to generate a survival estimate (final density divided by initial counted density). The growth of *M. macrocopa* was determined daily by measuring body length (from the base of the caudal spine to the anterior edge of the head) with a projection microscope. The experiment was carried out for 21 days until all the cohort dies. To measure the reproductive capacity of *M. macrocopa*, life table parameter was used to record and analyze reproduction data. Life table variables included the initial age at maturation, longevity (days), survival/survivorship, average

longevity, gross reproduction rate, net reproduction rate, generation time and, life expectancy. These variables were estimated as follows:

Age of first maturation (day) = age at which the first brood appears from a female

Longevity (day) = the average number of days of survival of the female

Survival/Survivorship =  $\sum l_x$

Average longevity =  $\sum \frac{n_x}{n}$

Gross reproduction rate =  $\sum m_x$

Net reproduction rate ( $R_0$ ) =  $\sum l_x m_x$

Generation time (T) =  $\sum \frac{l_x m_x x}{R_0}$

Life expectancy ( $e_x$ ) =  $\frac{T_x}{n_x}$

Where,

$l_x$  = Proportion individual surviving to age x

$n_x$  = Number of individual alive for each age class

n = Number of animals

$m_x$  = Age-specific fecundity (number of neonates produced per surviving female at age x)

$T_x$  = Generation time at age x

The intrinsic rate of population increases ( $r$ ) was calculated at the end of the experiment, and all the data were recorded at 15 until 16 days which was equivalent to the average lifespan (cycle) of *M. macrocopa*.

## 2.2. Analysis of Fatty acid

The zooplankton samples from each treatment were collected and freeze-dried prior to fatty acid analysis. Fatty acids (FAs) were extracted from freeze-dried *M. macrocopa* samples using a technique of Püttman *et al.*, (1993) for both qualitative and quantitative examination. The extracted FAs were transesterified into fatty acid methyl esters (FAME) using strong acid at 80°C to 85°C for about an hour. After this treatment, purified water and hexane were added and, the upper organic layer transferred to a vial (Ichihara and Fukubayashi, 2010). This step was performed several times to achieve complete extraction of FAME. Samples were then dried and dissolved again in 20 µl hexane to get 50 times concentration which removes all solvent peaks (i.e., toluen). The concentrated samples were then injected into gas chromatography–mass spectrometry (GC-MS) to read the spectra using caprylic acid (CH<sub>3</sub> (CH<sub>2</sub>)<sub>6</sub>COOH) as an internal standard.

## 2.3. Data analysis

The data in this study were expressed as Mean ± SD and were analyzed using one-way analysis of variance (ANOVA) to test the interaction between different ranges of salinity. Wherever applicable, post hoc Tukey's and Duncan multiple comparison tests were used to determine the significant differences of means between treatments. The level of significant difference was set at  $P < 0.05$ .

## 3. Results

### 3.1. Survival and growth of *M. macrocopa* in different salinities

The survival and growth of *M. macrocopa* at different salinity levels are shown in Table 1 and 2 below. The highest survival of *M. macrocopa* occurred at 0psu (98.00±4.47 %) ( $P < 0.05$ , refer to Table 1) while the least survival (approximately 9%) occurred at the salinity of 15psu. The survival rate at 4psu (92.67±7.23 %) and 6psu (83.33±12.69 %) were lower than in the control at 0psu salinity ( $P > 0.05$ , refer to Table 1). The growth of *M.*

*macrocopa* showed a positive relationship with the lowering of salinities at 0psu ( $1.35\pm 0.28$  mm), 4psu ( $1.32\pm 0.25$  mm), and 6psu ( $1.30\pm 0.25$  mm) with growth not being significantly affected. When the salinity reached 8psu ( $0.72\pm 0.61$  mm), 12psu ( $0.39\pm 0.54$  mm) and 15psu ( $0.23\pm 0.43$  mm), the growth of *M. macrocopa* became slower. There was no significant difference between salinity ranges on the growth of *M. macrocopa*.

### 3.2. Age of first maturation and average longevity

The life table parameters of *M. macrocopa* are shown in Table 3. Salinity significantly influenced the initial age of reproduction ( $P < 0.05$ , refer to Figure 2). Females began to reproduce after  $4.33\pm 0.58$  days at 0psu, compared longer delay for 4psu ( $5.33\pm 0.58$  days), 6psu ( $6.00\pm 0.58$  days) and 8psu ( $12.54\pm 0.58$  days). However, there was no difference in reproduction between salinity 0psu and 4psu ( $P > 0.05$ , refer to Figure 2). The average longevity of *M. macrocopa* was negatively correlated with salinity ( $P > 0.05$ , refer to Figure 3), average longevity being reduced with increase in salinity. Female of *M. macrocopa* survived longer at 0psu ( $12.33\pm 0.58$  days), compared with survivals at 4psu ( $9.67\pm 0.58$  days), 6psu ( $9.67\pm 0.58$  days) and 8psu ( $3.35\pm 0.62$  days). Therefore, there was no significant difference in longevity between 4psu and 6psu ( $P < 0.05$ , refer to Figure 3). Apart from that, there was no life table data can be recorded at 12psu and 15psu, since all the *M. macrocopa* dead before being able to reproduce.

### 3.3. Net reproduction rate and gross reproduction rate

A net reproduction rate is the average number of females in a population produced during the lifetime of a female. The net reproduction rates of *M. macrocopa* ranged with rates for 0psu ( $12.35\pm 0.77$  offspring/female), 4psu ( $9.45\pm 0.77$  offspring/female), 6psu ( $4.92\pm 1.91$  offspring/female) and 8psu ( $2.34\pm 0.77$  offspring/female) that revealed significant difference between salinity and reproduction rate ( $P > 0.05$ , refer to Figure 4). The increase of salinity showed a statistical difference in gross reproductions rates of *M. macrocopa* ( $P < 0.05$ , refer to Figure 5). The highest gross reproduction rate is at 0psu ( $13.33\pm 0.58$  offspring/female) followed by 4psu ( $12.00\pm 1.00$  offspring/female), 6psu ( $11.00\pm 0.7$  offspring/female) and 8psu ( $5.34\pm 0.58$  offspring/female). The net reproduction rates and gross reproduction rate of *M. macrocopa* decreased with salinity increases. However, there was no significant difference between the 0psu as control and 4psu ( $P > 0.05$ , refer to Table 3), for net reproduction or gross reproduction rate.

### 3.4. Generation time and intrinsic rate of population increase

The time from laying of eggs to when an individual reaches sexual maturity is termed as generation time. The generation time for *M. macrocopa* was shorter at 6psu ( $6.98\pm 0.96$  days) and longer at 8psu ( $11.23\pm 0.77$  days). There was no significant difference in generation time between all the treatments ( $P > 0.05$ , refer to Figure 6). Based on the results, salinity showed an inverse relationship with the intrinsic rate ( $P < 0.05$ , refer to Figure 7). The *M. macrocopa* showed a high intrinsic rate at 0psu ( $0.16\pm 0.007$ ) compared with the rate at 4psu ( $0.052\pm 0.028$ ), 6psu ( $0.061\pm 0.004$ ) and 8psu ( $0.078\pm 0.004$ ).

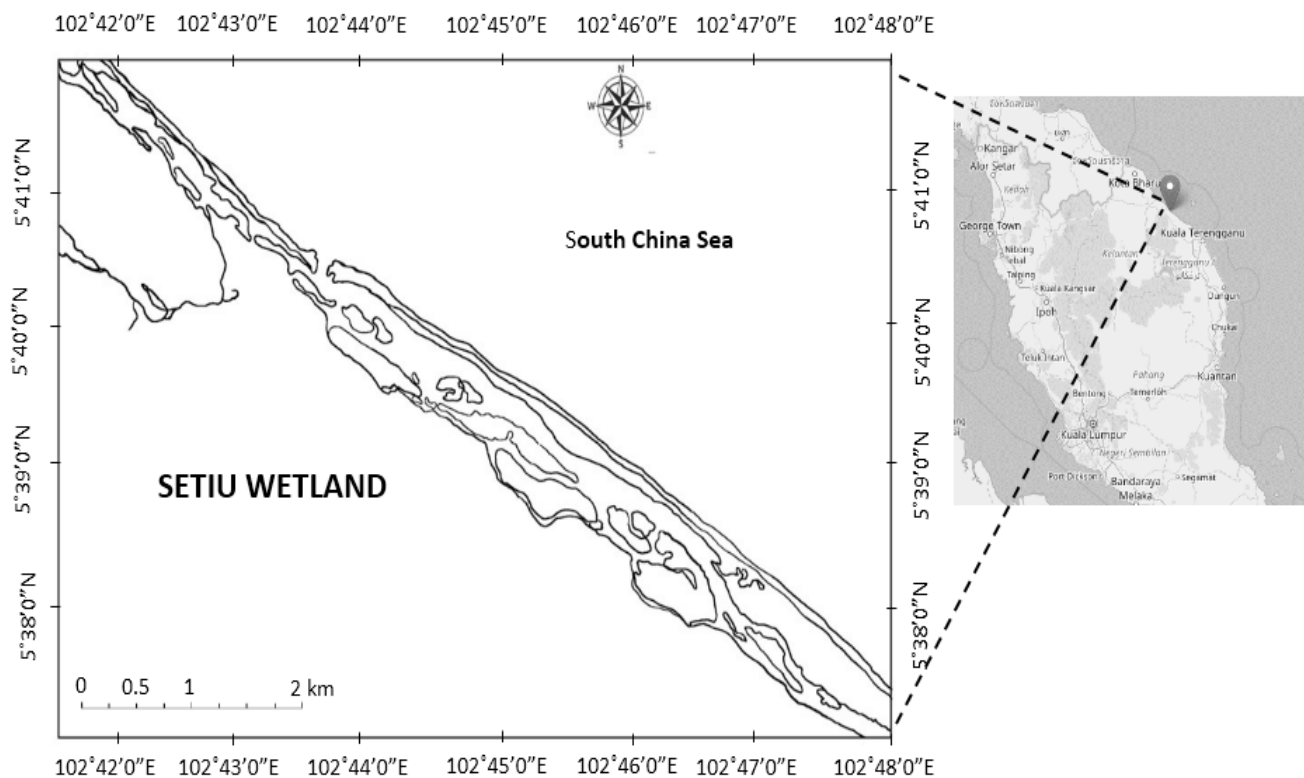
### 3.5. Fatty acid compositions of *M. macrocopa*

Fatty acid compositions which trigger the growth and survival rate of fish and crustaceans' larvae when fed on *M. macrocopa* are bold and shown in Table 4. The fatty acid contents were significantly affected by salinity levels that cohorts were exposed to ( $P < 0.05$ ). The highest level of fatty acids content considering saturated, and polyunsaturated fatty acids in *M. macrocopa* was recorded for the salinity of 0psu ( $P < 0.05$ ). The highest level of fatty acid as the sum of saturated, monosaturated and polyunsaturated, as a percentage of total fatty acids, was recorded in the control treatment at the lowest salinity level (98.95%, salinity 0psu, refer to Table 4). The amount of EPA (C20:5) in *M. macrocopa* were higher at the lowest salinity levels 0psu ( $35.21\pm 0.67$  %) than at higher salinity levels ( $P < 0.05$ ). The increase of salinity from 0psu ( $17.96\pm 0.39$  %) to 15psu ( $6.54\pm 0.81$  %) significantly abated the C18:1 monosaturated fatty acids in *M. macrocopa* ( $P < 0.05$ , refer to Table 4). The content of polyunsaturated fatty

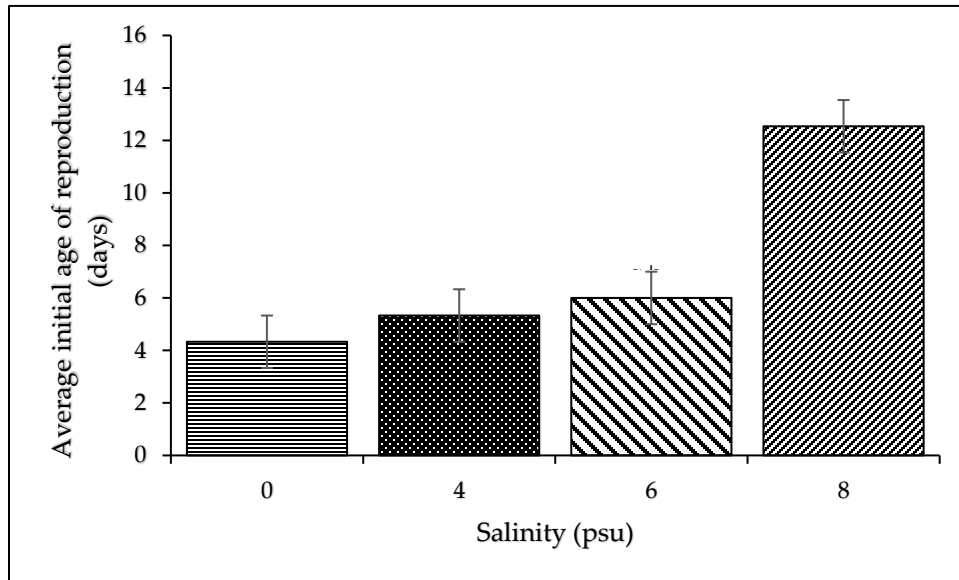


acids (PUFA) was significantly higher for salinity level treatment of 0psu (98.95%) than at higher salinity levels in *M. macrocopa* ( $P < 0.05$ ). The highest polyunsaturated fatty acids were recorded for eicosapentaenoic acid (EPA) ( $35.21 \pm 0.67\%$ ) at the salinity of 0psu ( $P < 0.05$ ). The highest linoleic acid (C18:2) content in *M. macrocopa* was found for the salinity levels of 0psu and 4psu ( $31.66 \pm 0.64\%$ ,  $23.69 \pm 1.6\%$ , respectively,  $P < 0.05$ , refer to Table 4). The highest alpha-linoleic acid (ALA) content in *M. macrocopa* was also found at 0psu and 4psu ( $16.45 \pm 3.7\%$ ,  $13.08 \pm 0.04\%$ , respectively,  $P < 0.05$ , refer to Table 4). The amount of C18:4 in *M. macrocopa* decreased from  $14.64 \pm 2.5$  to  $4.68 \pm 0.01\%$  as salinity levels increased from 0psu to 15psu, respectively ( $P < 0.05$ , refer to Table 4). Arachidonic acid (ARA) and Docosahexaenoic acid (DHA) are deficient, almost at non-detected (ND) levels, in *M. macrocopa*. DHA was the lowest FA analytical results among all the fatty acids found in *M. macrocopa* ( $P < 0.05$ , refer to Table 4).

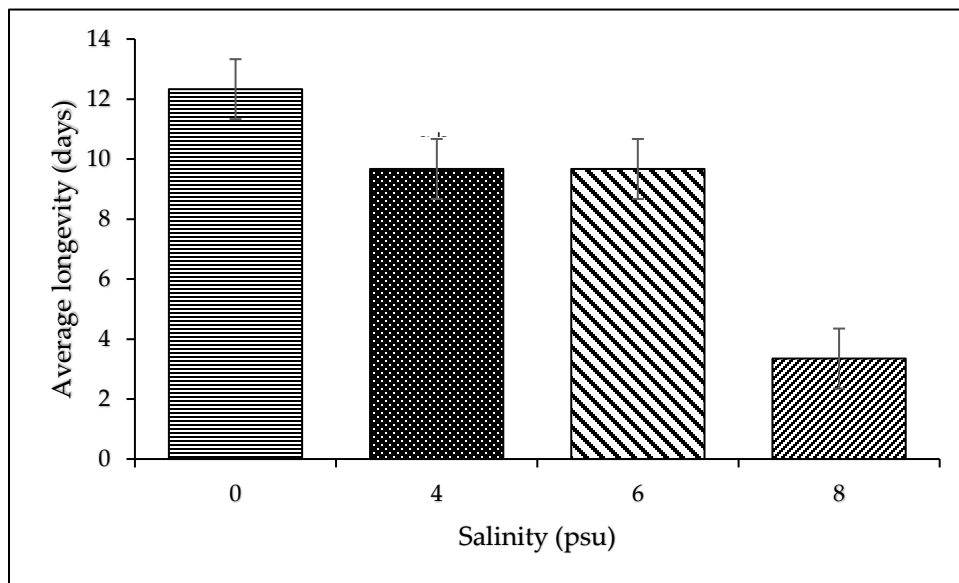
### 3.6 Figures, Tables



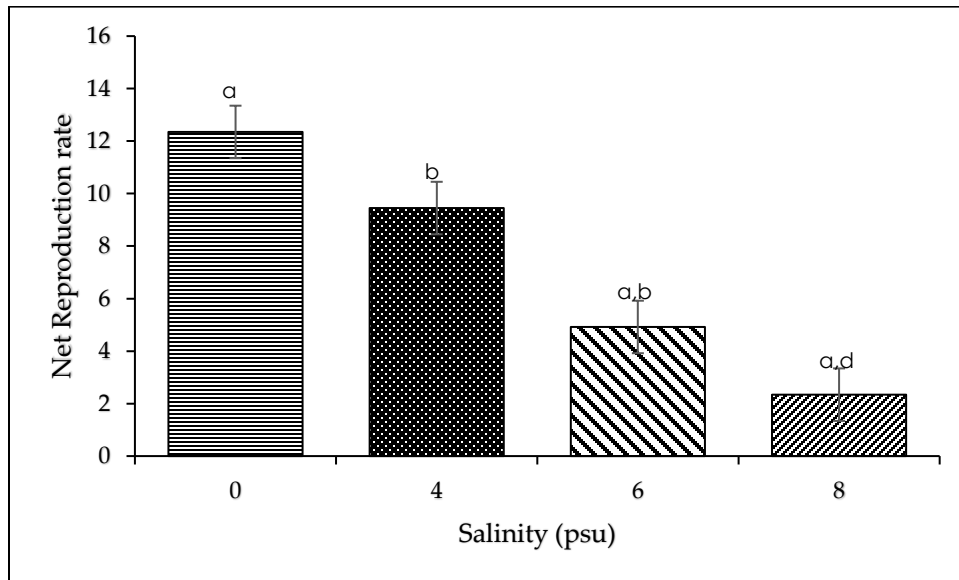
**Figure 1.** The location of sampling area at Setiu Wetland, Terengganu.



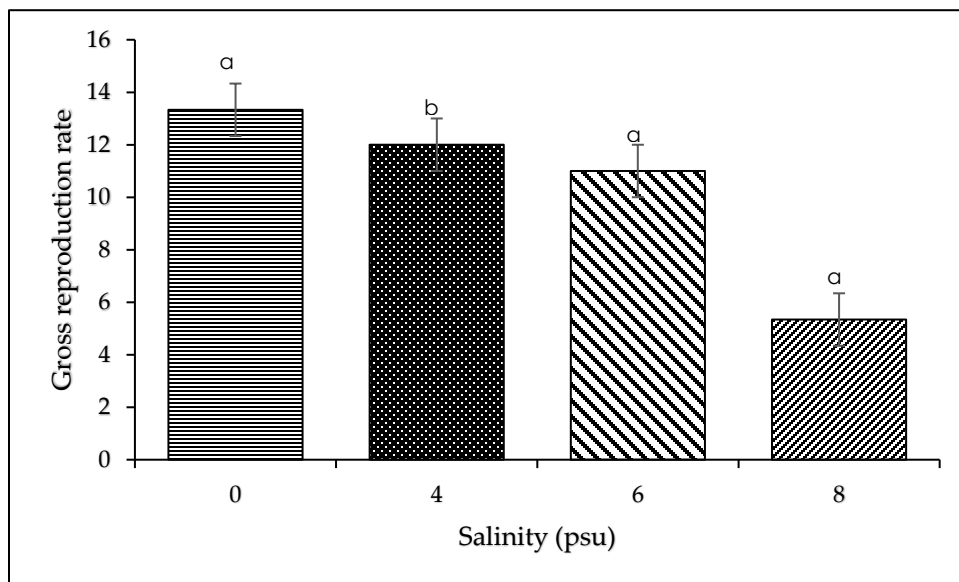
**Figure 2.** The average initial age of reproduction of *M. macrocopa* on different range of salinity. The different small letters indicate significant different between different salinity ( $P<0.05$ ).



**Figure 3.** The average longevity of *M. macrocopa* on different range of salinity. The different small letters indicate significant different between different salinity ( $P<0.05$ ).

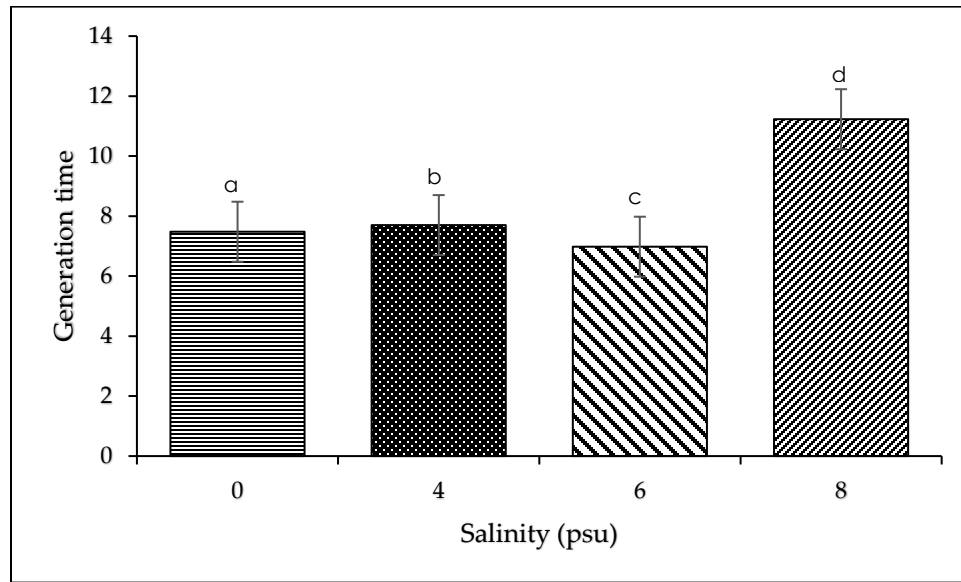


**Figure 4.** The net reproduction rate of *M. macrocopa* on different range of salinity. The different small letters indicate significant different between different salinity ( $P<0.05$ ).

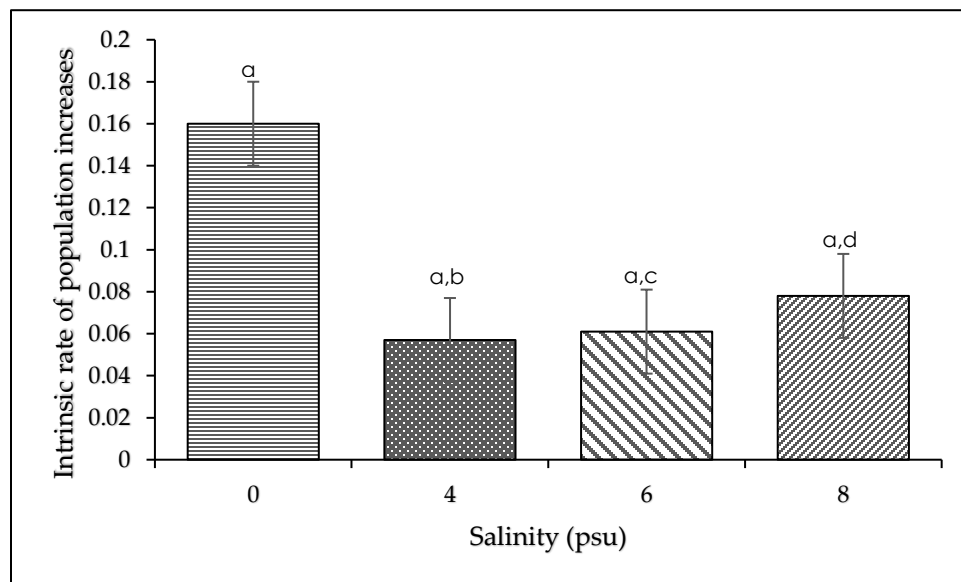


**Figure 5.** The gross reproduction rate of *M. macrocopa* on different range of salinity. The different small letters indicate significant different between different salinity ( $P<0.05$ ).





**Figure 6.** The generation time of *M. macrocopa* on different range of salinity. The different small letters indicate significant different between different salinity ( $P<0.05$ ).



**Figure 7.** The intrinsic rate of population increase of *M. macrocopa* on different range of salinity. The different small letters indicate significant different between different salinity ( $P<0.05$ ).

**Table 1.** The survival rate of *M. macrocopa* on different ranges of salinity. All values are mean  $\pm$  standard deviation (n=3). The different small letters indicate significant different between different salinity ( $P<0.05$ ).

Salinity (psu)	Survival rate (%) (mean $\pm$ SD)
0	98.00 $\pm$ 4.47 <sup>a</sup>
4	92.67 $\pm$ 7.23 <sup>b</sup>
6	83.33 $\pm$ 12.69 <sup>b,c</sup>
8	29.38 $\pm$ 36.42 <sup>d</sup>
12	16.67 $\pm$ 28.88 <sup>d,e</sup>
15	8.96 $\pm$ 25.44 <sup>d,f</sup>

**Table 2.** Growth rate of *M. macrocopa* on different ranges of salinity. All values are mean  $\pm$  standard deviation (n=3). The different small letters indicate significant different between different salinity ( $P<0.05$ ).

Salinity (ppt)	Growth rate (mm) (mean $\pm$ SD)
0	1.35 $\pm$ 0.28 <sup>a</sup>
4	1.32 $\pm$ 0.25 <sup>a,b</sup>
6	1.30 $\pm$ 0.25 <sup>a,c</sup>
8	0.72 $\pm$ 0.61 <sup>b,c</sup>
12	0.39 $\pm$ 0.54 <sup>c,d</sup>
15	0.23 $\pm$ 0.43 <sup>c,d</sup>

**Table 3** Life table parameters of *M. macrocopa*. All values are mean  $\pm$  standard deviation (n=3). The different small letters indicate significant different between different salinity ( $P<0.05$ ).

Salinity (ppt)	Average initial age of reproduction (days)	Average longevity (days)	Net Reproduction rate	Gross reproduction rate	Generation time	Intrinsic rate of population increases
0	4.33 $\pm$ 0.58 <sup>a</sup>	12.33 $\pm$ 0.58 <sup>a</sup>	12.35 $\pm$ 0.77 <sup>a</sup>	13.33 $\pm$ 0.58 <sup>a</sup>	7.48 $\pm$ 0.46 <sup>a</sup>	0.16 $\pm$ 0.007 <sup>a</sup>
4	5.33 $\pm$ 0.58 <sup>b</sup>	9.67 $\pm$ 0.58 <sup>a,b</sup>	9.45 $\pm$ 0.77 <sup>b</sup>	12.00 $\pm$ 1.00 <sup>b</sup>	7.70 $\pm$ 0.19 <sup>b</sup>	0.057 $\pm$ 0.028 <sup>a,b</sup>
6	6.00 $\pm$ 0.58 <sup>a,b</sup>	9.67 $\pm$ 0.58 <sup>a,c</sup>	4.92 $\pm$ 1.91 <sup>a,b</sup>	11.00 $\pm$ 7x10 <sup>5</sup> <sup>a</sup>	6.98 $\pm$ 0.96 <sup>c</sup>	0.061 $\pm$ 0.004 <sup>a,c</sup>
8	12.54 $\pm$ 0.58 <sup>a</sup>	3.35 $\pm$ 0.62 <sup>a,d</sup>	2.34 $\pm$ 0.77 <sup>a,d</sup>	5.34 $\pm$ 0.58 <sup>a</sup>	11.23 $\pm$ 0.77 <sup>d</sup>	0.078 $\pm$ 0.004 <sup>a,d</sup>
12	-	-	-	-	-	-
15	-	-	-	-	-	-

\*Life table parameters for 12psu and 15psu cannot be measured and recorded because all the treatments dead before being able to reproduce.

**Table 4** Fatty acid composition (% total fatty acids) of *Moina macrocopa* grown at different salinities. All values are mean  $\pm$  standard deviation (n=3). The different small letters indicate significant different between; different salinity (P<0.05). The bold fatty acid species are described in the results.

Fatty acid species	0psu	4psu	6psu	8psu	12psu	15psu
<b>Saturated</b>						
C14:0	4.68 $\pm$ 0.01	4.34 $\pm$ 0.06	2.68 $\pm$ 0.28	0.61 $\pm$ 0.03	0.24 $\pm$ 0.03	0.11 $\pm$ <0.01
<b>C16:0</b>	<b>14.97<math>\pm</math>0.24<sup>a</sup></b>	<b>10.22<math>\pm</math>0.69<sup>b</sup></b>	<b>11.09<math>\pm</math>0.83<sup>c</sup></b>	<b>7.78<math>\pm</math>0.57<sup>d</sup></b>	<b>5.58<math>\pm</math>0.05<sup>e</sup></b>	<b>0.90<math>\pm</math>0.30<sup>f</sup></b>
<b>Sum</b>	19.65	14.56	13.77	8.39	5.82	1.01
<b>Monosaturated</b>						
C16:1	7.41 $\pm$ 1.45	6.78 $\pm$ 0.15	5.58 $\pm$ 0.05	4.68 $\pm$ 0.01	3.37 $\pm$ 0.03	1.19 $\pm$ 0.04
<b>C18:1<sup>c</sup></b>	<b>17.96<math>\pm</math>0.39<sup>a</sup></b>	<b>16.45<math>\pm</math>3.7<sup>b</sup></b>	<b>14.62<math>\pm</math>2.5<sup>c</sup></b>	<b>11.78<math>\pm</math>0.04<sup>d</sup></b>	<b>9.6<math>\pm</math>1.11<sup>e</sup></b>	<b>6.54<math>\pm</math>0.81<sup>f</sup></b>
C22:1 <sup>c</sup>	0.79 $\pm$ 0.08	0.77 $\pm$ 0.16	0.66 $\pm$ 0.01	0.38 $\pm$ 0.02	0.19 $\pm$ 0.04	0.09 $\pm$ 0.07
<b>Sum</b>	26.16	24	20.86	16.84	13.16	7.82
<b>Polyunsaturated</b>						
<b>C18:2<sup>b</sup></b>	<b>31.66<math>\pm</math>0.64<sup>a</sup></b>	<b>23.69<math>\pm</math>1.6<sup>b</sup></b>	<b>12.23<math>\pm</math>0.18<sup>c</sup></b>	<b>10.22<math>\pm</math>0.69<sup>d</sup></b>	<b>7.78<math>\pm</math>0.57<sup>e</sup></b>	<b>3.23<math>\pm</math>0.52<sup>f</sup></b>
<b>C18:3<sup>a</sup>(ALA)</b>	<b>16.45<math>\pm</math>3.7<sup>a</sup></b>	<b>13.08<math>\pm</math>0.04<sup>b</sup></b>	<b>12.38<math>\pm</math>0.02<sup>c</sup></b>	<b>11.78<math>\pm</math>0.04<sup>d</sup></b>	<b>6.54<math>\pm</math>0.81<sup>e</sup></b>	<b>0.59<math>\pm</math>&lt;0.01<sup>f</sup></b>
<b>C18:4<sup>a</sup></b>	<b>14.64<math>\pm</math>2.5<sup>a</sup></b>	<b>13.09<math>\pm</math>0.04<sup>b</sup></b>	<b>11.78<math>\pm</math>0.04<sup>c</sup></b>	<b>10.91<math>\pm</math>0.03<sup>d</sup></b>	<b>9.00<math>\pm</math>1.41<sup>e</sup></b>	<b>4.58<math>\pm</math>&lt;0.01<sup>f</sup></b>
<b>C20:4<sup>b</sup>(ARA)</b>	<b>0.57<math>\pm</math>0.19</b>	<b>0.38<math>\pm</math>0.02</b>	<b>0.014<math>\pm</math>0.06</b>	<b>0.12<math>\pm</math>0.01</b>	<b>0.06<math>\pm</math>&lt;0.01</b>	<b>0.04<math>\pm</math>&lt;0.01</b>
<b>C20:5<sup>a</sup>(EPA)</b>	<b>35.21<math>\pm</math>0.67<sup>a</sup></b>	<b>31.68<math>\pm</math>0.64<sup>b</sup></b>	<b>20.15<math>\pm</math>1.6<sup>c</sup></b>	<b>19.99<math>\pm</math>1.2<sup>d</sup></b>	<b>14.96<math>\pm</math>1.10<sup>e</sup></b>	<b>4.68<math>\pm</math>0.01<sup>f</sup></b>
<b>C22:6<sup>a</sup>(DHA)</b>	<b>0.42<math>\pm</math>0.06</b>	<b>0.35<math>\pm</math>0.19</b>	<b>0.19<math>\pm</math>0.04</b>	<b>0.09<math>\pm</math>&lt;0.01</b>	<b>0.07<math>\pm</math>&lt;0.01</b>	<b>0.04<math>\pm</math>&lt;0.01</b>
<b>Sum</b>	98.95	82.27	56.87	53.11	38.41	13.16

<sup>a</sup> $\omega$ -3 fatty acids, <sup>b</sup> $\omega$ -6 fatty acids, and <sup>c</sup> $\omega$ -9 fatty acids.

\*ALA: Alpha-linolenic acid; ARA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

#### 4. Discussion

Freshwater ecosystems are now becoming increasingly threatened, partly due to the rise in salinity of groundwater (i.e., from seawater intrusion) and, water regime modifications which reduce the frequency of high-flow, flushing events (Iannacone & Alvarino, 2002; Cañedo-Argüelles *et al.*, 2016; Kayla *et al.*, 2017) and, these will significantly impact both the survival rate (Rahma *et al.*, 2017) and, reproduction (Grezesiuk and Mikulski, 2006; Bezirci *et al.*, 2012; Rahma *et al.*, 2017) of *M. macrocopa*. This study demonstrated how the increase of salinity especially the content of sodium chloride (NaCl) affects survival and life history traits of a freshwater cladoceran species through an examination of effects on *M. macrocopa* which would be representative of impact to an ecological area. The results of the present study indicated that the increase in salinity influenced survival, growth and, reproduction of *M. macrocopa*.

*M. macrocopa* achieved the highest recorded survival rate at treatment salinity of 0psu, compared to other salinity range treatments. As indicated by Haridevan *et al.*, (2015) for another species of cladoceran, *Latonopsis australi*, *L. australis* had the highest survival rate recorded for environmental salinity of 0psu which also decreased with the increase of the salinity level in the natural environment. Moreover, according to the study of Cabrera *et al.*, (2014), the survival rate of *Moina eugenie* decreased with increase in salinity and, Ghazy *et al.*, (2009) further supported this where survival rate of *Daphnia magna* was shown to decrease with increasing salt concentration. It is concluded that the survival rate of *M. macrocopa* is undoubtedly, significantly affected by the increase of salinity levels.

The current study showed that growth of *M. macrocopa* does not experience a significant reduction in environments with high salinities, up to a point. Results showed positive growth of *M. macrocopa* in salinity levels from 0psu until 6psu. However, when salinity was increased to 8, 12 and 15psu, the growth of *M. macrocopa* became slower. Slowest growth was observed to occur at 15psu, which might have been a result of some regulatory stress that occur in the culture (Zhao *et al.*, 2006). This conclusion is also aligned with the findings of Inger *et al.*, (2010) investigating the growth rate of exotic cladoceran, *Daphnia exilis* which also did not show any changes under salinity regime of 6psu but, started to decline in growth when salinity reached 8psu and above.

Female reproduction was affected by salinity and, the maturation of *M. macrocopa* was delayed at the highest salinity. These results are comparable to those obtained by Albert *et al.*, (2011), who noted that sexual maturation of *M. macrocopa* was delayed by 1 day at a salinity level of 5.5psu when compared to 0psu. Additionally, Ghazy *et al.*, (2009), noted that the reproduction age of *D. magna* was delayed from 7 days until 9 days at salinity levels of 0psu and 2.66psu, respectively.

The results from the current study showed that the average lifespan of *M. macrocopa* was 12.3 days of 0psu and, 9.6 days at 6psu. These are similar results to study by Fernando and Laura (2007), where average lifespan of *D. magna* was also significantly affected by salt concentration and where results showed that maximum average lifespan (57.7 days) occurred at the salinity of 0psu, whereas shortest lifespan (25.2 days) occurred at 7psu. Furthermore, in studies of *Simocephalus vetulus*, results also showed that the lifespan of this cladoceran decreased drastically when the salinity level was increased (Didem and Duygu, 2014).

Offspring net and gross reproduction rates of *M. macrocopa* were also affected when salinity increased. The previous study also showed that the number of offspring produce was the most affected parameters on the life table as caused by an increase of salinity for *D. magna* and *D. longispina*

(Goncalves *et al.*, 2007). Furthermore, in an observation-based study by Haridevan *et al.*, (2015), the findings also showed that the offspring of *Latonopsis australis* were significantly influenced by high salinity. It is concluded that salinity clearly impacts reproduction of offspring of this cladoceran.

Salinity levels impact the intrinsic rate (births minus deaths) for *M. macrocopa* population. This claim was supported in similar research done by Haridevan *et al.* (2015) where the intrinsic rate of *Latonopsis australis* decreased when the salinity levels of treatments increased. In the current study, life table parameters at salinity more than 8psu also cannot be reliably acquired because salinity higher than 8psu is beyond the toleration of *M. macrocopa*. El Gamal *et al.*, (2014) also noted that *D. longispina* reproduced and lived well in salinity below 5psu but then, started to decline when salinity levels increased. Thus, the present study concludes that *M. macrocopa* can achieve the optimum growth population when salinity is below 6psu. Zooplankton is sensitive to change in the aquatic environment that can cause a significant change in ambient conditions within the aquatic ecosystem (Harish and Kiran, 2016). The increase of salt concentration can cause the disappearance of species that cannot tolerate highly saline conditions and, salinity is known to be a strong mechanism that can change aquatic communities (Maria *et al.*, 2012).

Furthermore, no life table data can be recorded for 12psu and 15psu since the *M. macrocopa* are dead before being able to reproduce. Therefore, various freshwater invertebrate species (including cladocerans) are more sensitive to NaCl salinity than to the effect produced by the array of chemical compounds present in sea salt (Kefford *et al.*, 2004). Higher content of salinity level might be fatal to freshwater cladocerans especially *M. macrocopa*. The highest data can be recorded for life table parameter only at 8psu. It is strongly supported by Ghazy *et al.*, 2009, that the highest salinity can provoke stressful condition for cladocerans and cause mortality. Apart from that, *M. macrocopa* that spending most of their life stages under good conditions (food and environment) may adapt their life only with considerable time-lags are more vulnerable to starvation when there is decreasing food concentrations, and stressful environment occur and indirectly cause mortality among this species.

Observations in the current study showed that the expression of fatty acid is higher at the optimum culture conditions (0psu and 4psu) compared to other salinity level treatments. This effect is probably due to the minimal stress undergone by the cultured *M. macrocopa* at these optimum conditions which is finally reflected in the obtainment of a rich fatty acid profile. Similar observations were also reported (Zaleha and Busra, 2012; Zaleha *et al.*, 2014) where live feed underwent minimal stress during the culture period produced superior survival, growth and reproduction. High polyunsaturated fatty acids achieved by *M. macrocopa* might be due to the use of *Nannochloropsis* sp. as food sources, which was conducted to imitate feed in the natural environment. The amount of EPA and DHA in algae varies greatly among different algal species and environmental conditions. EPA content in *Nannochloropsis* sp. is 23.4 mg g<sup>-1</sup> (Rasdi and Qin, 2018). Microalgae such as *Nannochloropsis* sp. have received increasing interest as a target live feed for aquatic animals because of the high contents of EPA (Patil *et al.*, 2007).

## 5. Conclusions

In conclusion, survival, growth, reproduction rate and fatty acid profile of *M. macrocopa* were affected by different treatment ranges of salinity. *M. macrocopa* produced better growth rate, survival rate and, productivity at the salinity of 0psu which is a normal freshwater environment. Salinity at 4psu also provided relatively better growth for *M. macrocopa* and, most of the results from the present



study showed that there is no significant difference between salinity treatments of 0psu and 4psu. Salinity above 8psu, was found to be intolerable for *M. macrocopa* and to be above the critical salinity maximum (CSMax) or acute phase for cladoceran. Apart from that, fatty acid content also significantly abated with increased salinity levels. The highest content of fatty acid produced was with *M. macrocopa* being cultured in optimal salinity levels of 0psu and 4psu, which are considered to be normal in their natural habitat.

Comparing results in the whole NaCl concentration range tested, we can conclude that the impairment effects on fatty acid content, growth, reproduction and survival under the current treatments were provoked by salinity stress. Although the result of the present study shows that the survival rate, life history and fatty acids of *M. macrocopa* were significantly affected by the increase in salinity, future detailed studies are required to understand how freshwater species, including other species of zooplankton, can adapt to a higher saline stressed environment. Thus, these findings can be used by future researchers for further study and research on the development of species characterization and, on the factors affecting the abundance and zooplankton composition within the freshwater community, thus providing more ecologically relevant information on primary productivity in the ecosystem.

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