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การตอบสนองทางชีวประวัติของไรแดงเล็ก (*Moina micrura*) ที่เลี้ยงโดยให้อาหารความเข้มข้นแตกต่างกัน

Life Table Responses of *Moina micrura* Fed with Different Food Concentrations

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การศึกษาผลของความเข้มข้นของคลอเรลลา (*Chlorella vulgaris*) ต่อการตอบสนองทางชีวประวัติของไรแดงเล็ก (*Moina micrura*) โดยเลี้ยงไรแดงเล็กด้วยคลอเรลลาความเข้มข้นแตกต่างกัน 4 ชุดการทดลอง คือ 0, 400, 40,000 และ 4,000,000 เซลล์ต่อมิลลิลิตร ผลการทดลองพบว่า ไรแดงเล็กที่เลี้ยงโดยให้เรลลา 4,000,000 เซลล์ต่อมิลลิลิตรเป็นอาหารมีจำนวนลูกต่อครอก (10.4 ± 0.4 ตัวต่อครอก) และจำนวนลูกต่อแม่ (65.3 ± 1.9 ตัวต่อแม่) สูงกว่าชุดการทดลองอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ไรแดงเล็กที่เลี้ยงโดยไม่ให้อาหารมีจำนวนลูกต่อครอกและจำนวนลูกต่อแม่ต่ำที่สุด ($P < 0.05$) อายุเมื่อมีลูกครอกแรก จำนวนครอกต่อแม่ และอายุขัยของไรแดงเล็กในแต่ละชุดการทดลองไม่แตกต่างกันทางสถิติ ($P > 0.05$) การศึกษาครั้งนี้แสดงให้เห็นว่า ความเข้มข้นของอาหารมีผลต่อจำนวนลูกต่อครอกและจำนวนลูกต่อแม่ของไรแดงเล็ก

คำสำคัญ : ความเข้มข้นของอาหาร ; ชีวประวัติ ; ไรแดงเล็ก ; ไรน้ำ

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Abstract

This study examined the effect of *Chlorella vulgaris* concentrations on life table responses in *Moina micrura*. The four diets tested in the experiment were 0, 400, 40,000 and 4,000,000 cells per mL of *Chlorella vulgaris*. The result showed that the greatest number of offspring per brood (10.4 ± 0.4 offspring per brood) and total number of offspring per female (65.3 ± 1.9 offspring per female) were observed in *M. micrura* fed with 4,000,000 cells per mL of *C. vulgaris* ($P < 0.05$). Culturing of *M. micrura* with no food supply produced the lowest number of offspring per brood and total number of offspring per female ($P < 0.05$). Age at maturity, number of broods per female and life span of *M. micrura* were no significant difference between treatments ($P > 0.05$). The results of this study indicate that food concentrations had effects on the number of offspring per brood and the total number of offspring per female of *M. micrura*.

Keywords : food concentration ; life history ; *Moina micrura* ; water flea

Introduction

Food level is one of the most important factors on life history of cladoceran species by effect on reproduction (Martinez-Jeronimo & Gutierrez-Valdivia, 1991; Burak, 1997; Folt *et al*., 1999; Nandini & Sarma, 2000; Pavon-Meza *et al*., 2005; Xi *et al*., 2005), growth (Ovie & Egborge, 2002; Nandini & Sarma, 2002; Fernandez-Araiza *et al*., 2005; Din & Altaff, 2010; Ertan *et al*., 2011; Azuraidi *et al*., 2013) and survival (Nandini & Sarma, 2000; Xi *et al*., 2005). Early reproduction, high growth rate and enhance the number of offspring were recorded when increased food concentration (Burak, 1997; Dumont *et al*., 1995; Ferrao-Filho *et al*., 2003; Pavon-Meza *et al*., 2005; Xi *et al*., 2005; Azuraidi *et al*., 2013). On the other hand, if food supply is not sufficient, still reproduced, but lower rate compared to those supplied with high food concentration (Azuraidi *et al*., 2013) and higher mortality rates can be expected (Martinez-Jeronimo & Gutierrez-Valdivia, 1991).

M. micrura, a small aquatic microcrustacean, is commonly found in Thailand (Saengphan *et al*., 2013). They can be found in a wide range of different habitats (Petrusek, 2002) include temporary pools, permanent water and fish ponds (Saengphan *et al*., 2013), thus they are considered to be a cosmopolitan species with extensive morphological and ecological plasticity (Petrusek, 2002). Moreover, they have small size and high protein content therefore they are suitable for a starter live food for nursing aquatic animal larvae (Din & Altaff, 2010; Gogoi *et al*., 2016). In other countries, there have research about use of *M. micrura* as live food for nursing aquatic animals larvae such as *Macrobrachium rosenbergii* (Alam *et al*., 1993a, b; Das *et al*., 2007), *Heterobranchus longifilis*

(Kerdchuen & Legendre, 1994), *Channa striatus* (War *et al*., 2011), *Clarias gariepinus* (Okunsebor & Ayuma, 2011; Adewumi, 2015) and other fish species (Wang *et al*., 2008; Anano & Eguia, 2016).

Mass production of *M. micrura* can be produced by culture with microalga (Alam *et al*., 1991; Ovie & Egborge, 2002; Azuraidi *et al*., 2013). In Thailand, *Chlorella* sp. is one of the most frequent and commonly used for zooplankton culture. Hence, the objective of the present study was to define the effect of *C. vulgaris* concentration on biological characteristics of the optimum and sustained culture of *M. micrura* which is a useful knowledge for *M. micrura* culture at large - scale production for aquaculture business in Thailand.

Methods

Experimental animals

M. micrura were collected by using a 60 micrometers plankton net from fish pond in Suphanburi province, Thailand (14°49′38.7″ N, 99°41′47.7″ E). The specimens were kept in 1 L plastic bottles. In the laboratory, the samples were sorted and identified to isolate *M. micrura* under stereomicroscope (Nikon model SMZ 745) and light microscope (Carl Zeiss model Primo Star), respectively following the procedures of Goulden (1968) and Pascual *et al*. (2014). *M. micrura* was taken to the laboratory at Mahasarakham University for the experiments study. *M. micrura* was maintained in 1 L glass beaker containing 500 mL of dechlorinated tap water for the stocking culture and fed approximately 4,000,000 cells per mL of *C. vulgaris* every two days (Azuraidi *et al*., 2013) in room constant temperatures between 27 - 30 °C.

The experiments

Parthenogenetic female was cultured separately in 50 mL glass beakers containing 30 mL dechlorinated tap water. After maturation (when the first instar is present), neonate *M. micrura* females age less than 24 hours old were use in the experiments. Each neonate was transferred and cultured separately in 50 mL plastic transparent cups at a certain food concentration during its entire lifetime.

Each of the algal concentrations was serially diluted from a concentrated stock of *C. vulgaris*, which cultured and harvested by Algaeba Company Limited in Nonthaburi province. *C. vulgaris* concentrations in the experiment were 0, 400, 40,000 and 4,000,000 cells per mL. Each treatment was four replicated. The food concentrations were measured using a haemacytometer according to the formula:

$$\text{Concentration (cells per mL)} = \frac{\text{Mean number of cells counted per squares}}{4 \times 10^{-6}}$$

where, 4×10^{-6} = the volume of sample over the small square area which is equivalent to 0.000004 cm^3 (mL) (0.02 × 0.02 × 0.01 cm). Algal feed concentrations in all treatments were checked before and after the food supply, which was given every two days (Azuraidi *et al*., 2013).

Maturation time and life span were observed and recorded until the animals died. Upon reproduction, all neonate from each individual female were separated and counted under a stereomicroscope every 3 h until the animals died to determine the number of offspring per brood and the total number of offspring per female.

Statistical analysis

The variances of the data were presented as standard deviation (SD) of the mean of four replicates. The collected data were analyzed using one-way ANOVA (analysis of variance). Significant differences among treatments were determined by Duncan's multiple range tests at a 0.05 level of probability using SPSS version 14.0.

Results

The result shows that the first reproduction occurred at 2 - 2.8 days. *M. micrura* fed with high food concentration had shorter age at maturity than cultures fed with low food concentration, but not a significant difference between treatments ($P > 0.05$) (*Table 1*).

The highest number of offspring per brood (10.4 ± 0.4 offspring per brood) and total number of offspring per female (65.3 ± 1.9 offspring per female) were recorded when *M. micrura* fed with the highest food concentration of 4,000,000 cells per mL *C. vulgaris* ($P < 0.05$). Cultures *M. micrura* with 40,000 cells per mL of *C. vulgaris* produced the second highest number of offspring per brood (6.41 ± 0.43 offspring per brood) and total number of offspring per female (39.3 ± 4.8 offspring per female). On the other hand, cultures with no food supply produced the lowest number of offspring per brood and total number of offspring per female ($P < 0.05$). The total number of broods per female ranged from 5.5 to 6.3. High number of broods per female was observed when fed *M. micrura* with high *C. vulgaris* density and low number of broods per female was founded when fed with low *C. vulgaris* density but not significant differences ($P > 0.05$) between food concentrations (*Table 1*).

There was no significant difference in life span amongst treatments ($P > 0.05$). Long life span was founded when cultures *M. micrura* fed with low *C. vulgaris* density and a short life span was recorded when cultures fed with high *C. vulgaris* density (*Table 1*).

Table 1 Life table responses (mean ± SD, minimum and maximum values) of *M. micrura* fed with difference food concentrations. Different letters in the same row indicate significant difference among treatments ($P < 0.05$).

Life history characteristics		Food concentrations (cells per mL)				P-value
		0	400	40,000	4,000,000	
Maturation time (days)	Mean	2.8 ± 0.5 (n = 4)	2.5 ± 0.6 (n = 4)	2.3 ± 0.5 (n = 4)	2.0 ± 0.0 (n = 4)	0.168
	Min. - max.	2 - 3	2 - 3	2 - 3	2	
Number of offspring per brood	Mean	3.4 ± 0.2^{d} (n = 4)	4.7 ± 0.6^{c} (n = 4)	6.4 ± 0.4^{b} (n = 4)	10.4 ± 0.4^{a} (n = 4)	0.000
	Min. - max.	1 - 6	2 – 8	2 - 11	5 - 16	
Total number of offspring per female	Mean	18.8 ± 1.5^{d} (n = 4)	28.5 ± 3.7^{c} (n = 4)	39.3 ± 4.6^{b} (n = 4)	65.3 ± 1.9^{a} (n = 4)	0.000
	Min. - max.	17 - 20	25 – 33	34 - 44	64 - 68	
Number of broods per female	Mean	5.5 ± 0.6 (n = 4)	6.0 ± 1.2 (n = 4)	6.0 ± 1.2 (n = 4)	6.3 ± 0.5 (n = 4)	0.698
	Min. - max.	5 - 6	5 - 7	5 - 7	6 - 7	
Life span (days)	Mean	9.5 ± 1.0 (n = 4)	9.0 ± 1.2 (n = 4)	8.8 ± 1.5 (n = 4)	7.8 ± 1.0 (n = 4)	0.247
	Min. - max.	8 - 10	8 - 10	7 - 10	7 - 9	

Number of offspring peaking in the early of the life span, during the second to the fifth reproductive event, regardless of treatment. The highest number of offspring (14.3 ± 1.5 neonates) was recorded when fed *M. micrura* with 4,000,000 cells per mL of *C. vulgaris* ($P < 0.05$). Conversely, the lowest clutch size (1.5 ± 0.6 neonates) was founded when cultures *M. micrura* with no food supply ($P < 0.05$) (*Figure 1*).

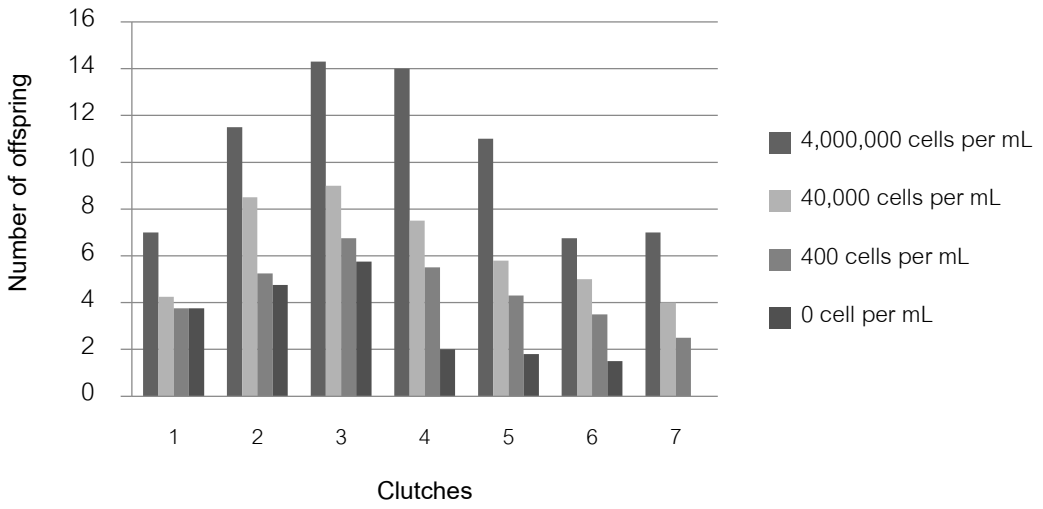

Figure 1 Mean number of offspring per clutch when fed *M. micrura* with different density of *C. vulgaris*.

Discussion

This study examined the life table responses of *M. micrura* fed with different concentrations of *C. vulgaris* (0, 400, 40,000 and 4,000,000 cells per mL), which the range have used for *Miona* culture and research followed by Azuraidi *et al.* (2013) and Saengphan *et al.* (2016). The result shows that maturation time was not a significant difference between treatments ($P > 0.05$), which similarly studied of Martinez-Jeronimo & Gutierrez-Valdivia (1991) who reported first reproduction of *Moina macrocopa* fed with different density of *C. vulgaris* (380,000 and 760,000 cells per mL) was no significant difference between treatments ($P > 0.05$). Maturation time of *M. micrura* from this study (2.0 - 2.8 days) is faster than Martinez-Jeronimo & Gutierrez-Valdivia (1991) studied which showed the first reproduction of *M. macrocopa* occurred on the fourth day. This difference may be due to a difference in the size between species, which *M. macrocopa* are larger in size than *M. micrura* (Rodmongkoldee *et al.*, 2020). The size differences may affect the time needed to attain maturity (Sarma *et al.*, 2005). As a general rule, large species need more time to reach maturity than small ones (Babu & Nayar, 1997; Nandini & Sarma, 2000). Moreover, maturation time tends to decrease with increasing food concentration, which similar to previously studied of Burak (1997) who showed decreases in the food concentration to 10,000 cells per mL of *Scenedesmus* sp. resulted in the increasing age at maturity of *M. macrocopa* to seven days, whereas an increase in the food concentration of 10,000,000 cells per mL of *Scenedesmus* sp. led to a decrease age at maturity to two days at 20 °C. Maturation time is delayed

when food decreases, possibly due to this condition having enough energy for living but not enough for reproduction.

According to our results, the number of offspring per brood and total number of offspring per female increased with increasing food concentration, similar to the finding of Martinez-Jeronimo & Gutierrez-Valdivia, (1991) who showed increasing in the food concentration of 380,000 cells per mL of *C. vulgaris* resulted in the total offspring of *M. macrocopa* to 108 offspring, whereas increased to 760,000 cells per mL of *C. vulgaris* led to increase total offspring to 157 offspring. Moreover, Pavon-Meza *et al*. (2005) has reported gross and net reproductive rates of *Brachionus havanaensis* increased with increasing algal food levels. However, if the amount of food is lower or upper than demand, it may cause a decrease in reproduction. This is supported by the Xi *et al*. (2005), who reported gross reproductive rate of *M. macrocopa* at 33 °C was 56.88 offspring per female when fed with 1,000,000 cells per mL of *C. vulgaris*, while fed with 500,000 and 2,000,000 cells per mL of *C. vulgaris*, the gross reproduction were 27.67 and 33.25 offspring per female, respectively. High food densities affected temporary reduction in dissolved oxygen cause by algal respiration at high densities (Herzig, 1979), excessively high pH from heavy alga bloom resulting in an increase in toxic forms of ammonia (Rottmann *et al*., 2017), decomposition of toxic products and secretions by the algae (Sarma & Rao, 1990), fouling effect of accumulated feces and uneaten food (Hirata, 1980) and obstruction of filtration apparatus and suffocation of zooplankton (Ivleva, 1973; Peters, 1987). Conversely, low food concentration caused insufficient energy for reproduction (Azuraidi *et al*., 2013).

From the results presented, life span trend to increase with decreasing food concentration, which similar to previously studied of Xi *et al*. (2005) who reported excessively high food concentrations (4,000,000 cells per mL at 18 °C and 23 °C and 2×10^6 cells per mL at 28 °C) caused decreased survival in *M. Macrocopa*. In the case of no food supply condition, *M. micrura* was able to living probably due to the presence of some bacteria in the water (Azuraidi *et al*., 2013). This condition also found the longest lifespan may be caused by an increased effort in food gathering and reduced net energy assimilation (Nandini & Sarma, 2000; Xi *et al*., 2005). Furthermore, temperature, salinity and humic substance concentration affected longevity (Folt *et al*., 1999; Benider *et al*., 2002; Pavon-Meza *et al*., 2005; Xi *et al*., 2005; Martinez-Jeronimo and Martinez-Jeronimo, 2007; Santangelo *et al*. 2008; Suhett *et al*., 2011; Engert *et al*., 2013).

Although the maturation time and life span were no significant difference between treatments ($P > 0.05$) unlike the number of offspring per brood and total number of offspring per female, but maturation time and life span tend to decrease with increasing food concentration. These indicate that food concentration tends to affect the maturation time and life span of *M. micrura*.

Conclusions

The results of this study indicated that food concentrations had effect on the life history of *M. micrura*. The high number of offspring per female and number of broods per female observed in these studies suggests that *M. micrura* could be intensively cultured and high concentration of *C. vulgaris* is a suitable as food for the culture. This is important in regard to its suitability for aquaculture in large-scale production.

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