

การศึกษาชีวประวัติของไรน้ำสามชนิด (Cladocera : Moinidae) ในประเทศไทยในห้องปฏิบัติการ

Laboratory Study on Life History of Three Water Flea species (Cladocera : Moinidae) in Thailand

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Received : 21 June 2019

Revised : 26 August 2019

Accepted : 6 September 2019

บทคัดย่อ

การศึกษานี้เพื่อเปรียบเทียบชีววิทยาของไรน้ำที่มีการเลี้ยงในเชิงพาณิชย์ คือ ไรแดง (*Moina macrocopa*) และ ไรแดงสยาม (*Moina siamensis*) กับไรน้ำที่พบในธรรมชาติ คือ ไรแดงเล็ก (*Moina micrura*) โดยแยกชนิดของไรน้ำและเลี้ยงในห้องปฏิบัติการ ผลการศึกษาพบว่า ไรน้ำทั้ง 3 ชนิดมีลูกครอกแรกเมื่ออายุ 2 วัน ไรแดงเล็กมีจำนวนครอกต่อแม่และจำนวนลูกต่อแม่น้อยกว่าไรแดงสยามอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) แต่ไม่แตกต่างทางสถิติกับไรแดง ($P > 0.05$) จำนวนลูกต่อครอกของไรแดงเล็ก (10.9 ± 2.08 ตัวต่อครอก, $n = 10$) และไรแดงสยาม (8.7 ± 2.45 ตัวต่อครอก, $n = 10$) ไม่แตกต่างกันทางสถิติ ($P > 0.05$) ไรแดงมีจำนวนลูกต่อครอก (16.5 ± 4.45 ตัวต่อครอก, $n = 10$) สูงกว่าไรน้ำชนิดอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ไรแดงสยามมีอายุ (8.7 ± 1.70 วัน, $n = 10$) ยาวนานกว่าไรแดงเล็ก (5.4 ± 1.17 วัน, $n = 10$) และไรแดง (5.3 ± 1.16 วัน, $n = 10$) อย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ไรแดงมีความยาวลำตัวสูงสุด ($500 - 1,500$ ไมโครเมตร) และไรแดงเล็กมีความยาวลำตัวต่ำสุด ($200 - 850$ ไมโครเมตร) ($P < 0.05$) อัตราการเจริญเติบโตจำเพาะของไรแดงสูงกว่าไรแดงสยามและไรแดงเล็กอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) การศึกษานี้แสดงให้เห็นว่า ไรแดงเล็กมีลักษณะทางชีววิทยาไม่แตกต่างกับไรน้ำที่มีการเลี้ยงในเชิงพาณิชย์ และไรแดงเล็กมีขนาดเล็กกว่าไรน้ำชนิดอื่นจึงเหมาะในการใช้เป็นอาหารในการอนุบาลลูกสัตว์น้ำวัยอ่อน

คำสำคัญ : ชีววิทยา, ไรแดงเล็ก, ไรแดง, ไรแดงสยาม, ไรน้ำ

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Abstract

Life histories of commercial water fleas, *Moina macrocopa* and *Moina siamensis*, and natural water flea *Moina micrura* were compared. Specimens were isolated, cultured separately in the laboratory and observed until the animals died. Life history parameters showed that all three species matured to adulthood within 2 days. *M. micrura* had the lowest number of broods and total number of offspring per female, significantly different from *M. siamensis* ($P < 0.05$) but not significantly different from *M. macrocopa* ($P > 0.05$). Number of offspring per brood was not significantly different ($P > 0.05$) between *M. micrura* at 10.9 ± 2.08 , $n = 10$ and *M. siamensis* at 8.7 ± 2.45 , $n = 10$, with greatest number of offspring per brood observed in *M. macrocopa* at 16.5 ± 4.45 , $n = 10$ ($P < 0.05$). *M. siamensis* had a significantly longer lifespan (8.7 ± 1.70 days, $n = 10$) than *M. micrura* (5.4 ± 1.17 days, $n = 10$) and *M. macrocopa* (5.3 ± 1.16 days, $n = 10$) ($P < 0.05$). Largest body length was measured in *M. macrocopa* (500 - 1,500 μm) with the smallest specimen recorded in *M. micrura* (200 - 850 μm) ($P < 0.05$). *M. macrocopa* had higher specific growth rate than other species ($P < 0.05$). Results suggested that *M. micrura*, with smallest body length compared to the other two species, had similar characteristic traits to commercial water fleas as an appropriate feedstuff for new-born aquatic larvae.

Keywords : life history, *Moina micrura*, *Moina macrocopa*, *Moina siamensis*, water flea

Introduction

Moina, a small aquatic microcrustacean, is commonly found around the world because their ephippial eggs could be easily transported overland to new habitats (Petrušek *et al.*, 2004). The reproductive cycle of *Moina* has both a sexual and asexual phase (Rottmann *et al.*, 2017). Under optimal conditions, population growth is mainly through parthenogenesis, a type of asexual reproduction whereby embryos develop without fertilization, producing only female offspring, resulting in rapid propagation and population growth (Azuraide *et al.*, 2013). Under adverse environmental conditions, sexual reproduction takes place which produces male offspring and resting eggs with an extra shell layer, also known as ephippia (Kleiven *et al.*, 1992; Rottmann *et al.*, 2017).

M. micrura is a commercially important zooplankton (Azuraide *et al.*, 2013; Vignatti *et al.*, 2013) and a widely accepted live food starter suitable for nursing aquatic animal larvae (Azuraide *et al.*, 2013; Gogoi *et al.*, 2016) such as freshwater prawn *Macrobrachium rosenbergii* (Alam *et al.*, 1993a, b; Das *et al.*, 2007), white shrimp *Litopenaeus schmitti* (Martin *et al.*, 2006), loaches *Misgurnus anguillicaudatus* (Wang *et al.*, 2008), African catfish *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994), *Channa striatus* (War *et al.*, 2011), *Clarias gariepinus* (Okunsebor & Ayuma, 2011; Adewumi, 2015) and *Leiopotherapon plumbeus* (Anano & Eguia, 2016).

In Thailand, *M. macrocopa* is the most popular aquacultural food source followed by *M. siamensis* and *M. micrura*. Intensive research and culture exist on *M. macrocopa* and *M. siamensis* but little information is available

regarding *M. micrura*, which most studies have been done in laboratories in temperate zone where is different in culture condition such as food concentration, food type, culture volume, nutrition conditions, and temperature (Benider *et al.*, 2002; Sipaubá-Tavares & Bachion, 2002; Martínez-Jeronimo *et al.*, 2007; Chen *et al.*, 2015). Advantages of *M. micrura* are smaller body size than both *M. macrocopa* and *M. siamensis*, and therefore suitable as live food for nursing fish larvae, it can survive in both fresh and brackish water (Saint-Jean & Bonou, 1994; Liu, 2008) and high protein content of 49.31 - 75.29 % (Saengphan *et al.*, 2016), which similar to the protein content in *Artemia* of 53.7 - 60.5 % (Peykaran *et al.*, 2014). As *Artemia* spp. has to be imported from abroad as feedstuff for nursing marine aquatic animals. Historically, Thailand has relied on importing at least 200 - 600 tons of *Artemia* cysts per year from other countries to feed shrimps, fish and other aquatic animals at a cost of more than 500 million baht (Department of Industry Promotion, 2008).

Thus, here, the biological characteristics of *M. macrocopa* and *M. siamensis* were defined and compared with *M. micrura* to better understand their life histories as useful knowledge for large-scale *M. micrura* culture to satisfy the fish feed requirements of the Thai aquacultural industry.

Methods

Experimental animals

M. micrura and *M. siamensis* were collected from fish ponds using a 60 µm size plankton net in Suphanburi Province, Thailand (14°49'38.7" N, 99°41'47.7" E). Specimens were kept in 1 L plastic bottles before sorting in the laboratory to identify and isolate *M. micrura* and *M. siamensis* 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. macrocopa* was obtained from the Department of Fisheries, Suphanburi College of Agriculture and Technology. The three water flea species were taken to the laboratory at Mahasarakham University for life history studies. All experiments were conducted in 1 L glass beakers containing 500 mL of dechlorinated tap water as stocking culture and fed approximately 4×10^6 cells per mL of *Chlorella vulgaris* every 2 days (Azuraiddi *et al.*, 2013) in room constant temperatures between 27 - 30 °C and 12:12 (Light : Dark).

Life history experiments

Parthenogenetic females of each species were cultured separately in 100 mL glass beakers containing 40 mL dechlorinated tap water. After maturation (when the first instar is present), new-born *Moina* (< 24 hour old) were cultured separately in 50 mL transparent plastic cups containing 30 mL dechlorinated tap water and fed approximately 4×10^6 cells per mL of *C. vulgaris* every 2 days. Each treatment was 10 replicates. Maturation time and lifespan were observed and recorded until the animals died. Once reproduction started, all neonates from each female were removed and counted under a stereomicroscope every day until the animals died to determine the number of offspring per brood and total number of offspring per female. Body length of females was determined

using a light microscope equipped with a micrometer eyepiece (Vignatti *et al.*, 2013) every day throughout the experiment. A minimum number of 10 specimens were measured each day in all species. Specific growth rate was calculated according to the formula:

$$g = [\ln(l_t) - \ln(l_o)] \times 100 / t \quad (1)$$

where l_o and l_t are mean individual length initially and after t days.

Statistical analysis

Data variances were presented as standard deviation (SD) of the mean of 10 replicates and analyzed using one-way 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

Results showed that all three water flea species matured to adulthood within 2 days. Mean number of broods per female was significantly higher ($P < 0.05$) in *M. siamensis* than *M. micrura* and *M. macrocopa*. *M. macrocopa* had the lowest mean number of broods per female but highest ($P < 0.05$) number of offspring per brood at between 6 and 25. Highest ($P < 0.05$) total number of offspring per female for whole lifespan was observed in *M. siamensis* with lowest found in *M. micrura* but not significantly different ($P > 0.05$) from *M. macrocopa* (Table 1).

There was a significant difference in lifespan amongst treatments ($P < 0.05$). Longest lifespan was found in *M. siamensis* (8.7 ± 1.70 days) followed by *M. micrura* (5.4 ± 1.17 days) and *M. macrocopa* (5.3 ± 1.16 days) (Table 1).

Table 1 Life history parameters (mean \pm SD, minimum and maximum values) of *M. micrura*, *M. macrocopa* and *M. siamensis*. Different letters in the same row indicate significant difference among treatments ($P < 0.05$).

Life history characteristics		<i>M. micrura</i>	<i>M. macrocopa</i>	<i>M. siamensis</i>
Maturation time (days)	Mean	2.0 \pm 0.00 (n = 10)	2.0 \pm 0.00 (n = 10)	2.0 \pm 0.00 (n = 10)
	Min.-max.	3 - 4	3 - 4	5 - 10
Number of broods per female	Mean	3.9 \pm 0.32 ^b (n = 10)	3.6 \pm 0.52 ^b (n = 10)	7.2 \pm 1.75 ^a (n = 10)
	Min.-max.	3 - 4	3 - 4	5 - 10
Number of offspring per brood	Mean	10.9 \pm 2.08 ^b (n = 10)	16.5 \pm 4.45 ^a (n = 10)	8.7 \pm 2.45 ^b (n = 10)
	Min.-max.	5 - 15	6 - 25	5 - 12
Total number of offspring per female for whole life span	Mean	52.3 \pm 13.36 ^b (n = 10)	62.1 \pm 23.47 ^b (n = 10)	81.6 \pm 21.97 ^a (n = 10)
	Min.-max.	26 - 70	24 - 98	48 - 110
Life span (days)	Mean	5.4 \pm 1.17 ^b (n = 10)	5.3 \pm 1.16 ^b (n = 10)	8.7 \pm 1.70 ^a (n = 10)
	Min.-max.	4 - 7	4 - 7	6 - 11

Body length of the three water fleas showed significant differences ($P < 0.05$) between species. Largest body length was measured in *M. macrocopa* (500 - 1,500 μ m), followed by *M. siamensis* (500 - 1,290 μ m). The smallest specimen was recorded in *M. micrura* (200 - 850 μ m) ($P < 0.05$). Body lengths of *M. micrura*, *M. macrocopa* and *M. siamensis* are presented in *Figure 1*. Growth data indicated that *M. macrocopa* grew faster than the other species, especially during day 2.

Growth curves of the three water fleas showed a parabolic shape with two successive phases: the first, from birth to 2 days presented fast increase in size at maximal growth rate, with the second, from the second day to death showing slow growth rate (*Figure 1*).

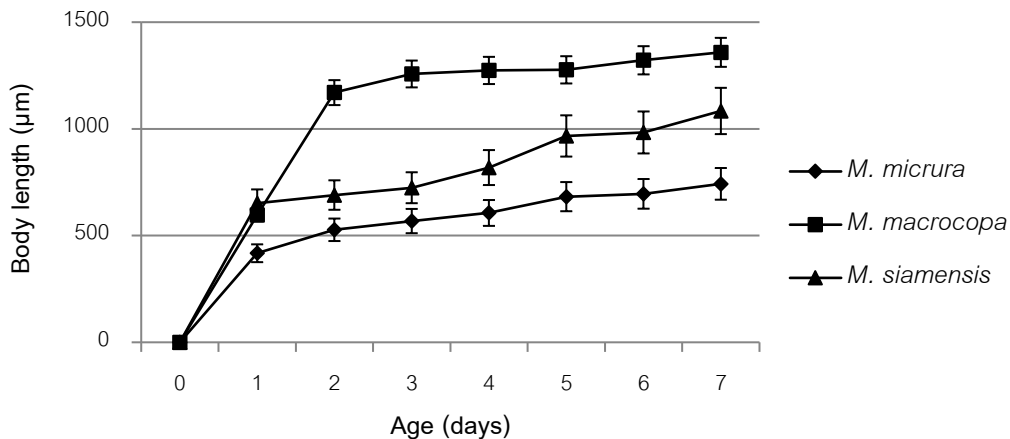


Figure 1 Mean body length (µm) of *M. micrura*, *M. macrocopa* and *M. siamensis* based on laboratory cultures for 7 days.

Specific growth rates of *M. micrura*, *M. macrocopa* and *M. siamensis* were $9.6 \pm 1.05\%$ per day (n = 10), $13.7 \pm 1.05\%$ per day (n = 10) and $8.5 \pm 0.77\%$ per day (n = 10), respectively. Comparative specific growth rate showed that *M. macrocopa* had higher specific growth rate than other species ($P < 0.05$) (Figure 2).

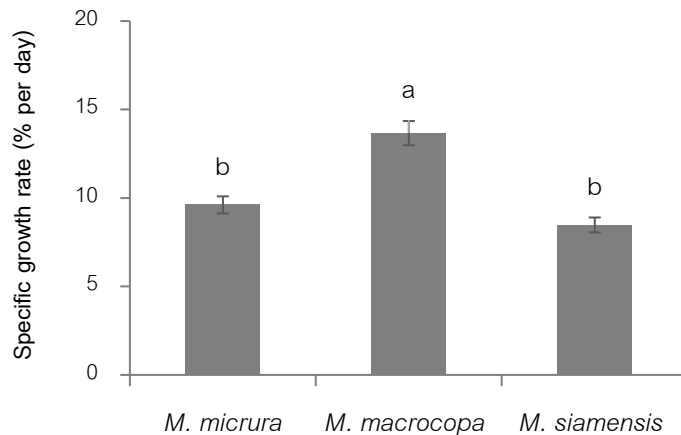


Figure 2 Mean specific growth rate (% per day) of *M. micrura*, *M. macrocopa* and *M. siamensis* based on laboratory cultures for 7 days. Different letters indicate significant difference among treatments ($P < 0.05$).

Discussion

Life history of the three Moinidae species from Thailand shows rapid maturation time (2 days), which is faster than *M. mongolica* from China, which takes 3.4 days at 30 °C (He *et al.*, 2001). The age of first reproduction is very important to the population of the ecosystem (Roff, 2001). It may vary for up to 10 days (Dumont & Negrea, 2002) which the environment affects the development period of the reproductive age. When limited natural resources, the slow reproduction occurs to limit the population and the population will increase again when the environment comes back properly (Sarma *et al.*, 2005). Chen *et al.* (2015) reported that *M. micrura* grow faster when temperature rise. In addition, they will reproduce faster when in an environment is more predators (Pietrzak *et al.*, 2015).

Offspring number of Thai Moinidae were higher than shown in previous studies. Total number of offspring per female of *M. micrura*, *M. macrocopa* and *M. siamensis* were 26 - 70, 24 - 98 and 48 - 110, respectively covering the entire lifespan. Martinez-Jeronimo *et al.* (2007) reported that *M. micrura* produced 23 - 43 offspring per female when fed with 3.6×10^4 cells per mL of *Chlorella* sp. at culture volumes 30 - 120 mL (1 individual per 20 mL), while 11 - 26 offspring per female in *M. macrocopa* fed with 0.5×10^6 - 4.5×10^6 cells per mL of *C. vulgaris* (Nandini & Sarma, 2000). These results indicated that *Moina* from Thailand were suitable for commercial production because they produced a high number of progenies, resulting in rapid population growth. Offspring number of Thai Moinidae in this study is different from previous studies, maybe due to differentiation in culture condition like food density, food type, culture volume and temperature, that may affect number of offspring per female (Nandini & Sarma, 2000; Sipauba-Tavares & Bachion, 2002; Martinez-Jeronimo *et al.*, 2007; Chen *et al.*, 2015), while increase in food level resulted in increased offspring production (Rose *et al.*, 2000).

Lifespan of *M. micrura*, *M. macrocopa* and *M. siamensis* were 4 - 7, 4 - 7 and 6 - 11 days, respectively. *M. macrocopa* in our study had a shorter lifespan than the maximal 17.4 days reported by Benider *et al.* (2002), which found when fed with *Chlorella sorokiniana* at 6.25×10^6 cells per mL, but concurred with Nandini & Sarma (2000) at 5 ± 1 days, fed with 4.5×10^6 cells per mL of *C. vulgaris*. Lifespan of *M. micrura* was close to the study of Sipauba-Tavares & Bachion (2002) at 5 - 6 days, fed 4×10^6 cells per mL of *Ankistrodesmus gracillius*. Differentiation in lifespan between these studies probably due to environmental condition difference such as food concentration, population density, temperature and water quality. Short lifespans result from environmental risks of predation (Pietrzak *et al.*, 2015) and increasing temperature (Benider *et al.*, 2002). Conversely, longevity was recorded with low food concentration and decrease in temperature (Nandini & Sarma, 2000; Benider *et al.*, 2002). Nutrition conditions, population density and crowding influenced both longevity and survival (Benider *et al.*, 2002).

Differentiation in life history strategies between species might be due to the diversity of natural habitats (Vanschoenwinkel *et al.*, 2010; Dararat *et al.*, 2011). In Thailand, *M. macrocopa* was found only in rearing ponds or ponds flooded by water drained from rearing ponds of *M. macrocopa* (Saengphan *et al.*, 2013) but in other countries

like Slovakia, the Czech Republic, India and Argentina, *M. macrocopa* are commonly found in permanent water bodies as small village ponds or sewage ponds, temporary pools (such as rain pools or ditches) and fishponds (Petrušek, 2002; Vignatti *et al.*, 2013). Generally, rapid maturation and short life span are found in temporary pools (Vanschoenwinkel *et al.*, 2010; Dararat *et al.*, 2011). Conversely, late reproduction and long lifespan are observed in permanent water bodies or long-lasting pond (Vanschoenwinkel *et al.*, 2010; Dararat *et al.*, 2011). However, a short lifespan and rapid maturation of *M. micrura* in Thailand is recorded in temporary pools, permanent water and fishponds (Saengphan *et al.*, 2013). They are also considered to be a cosmopolitan species with extensive morphological and ecological plasticity, occurring in a wide range of different habitats (Petrušek, 2002). *M. siamensis*, which recorded a long lifespan, inhabits ephemeral pools in contrast to the general trend. Populations in temporary ponds are expected to show rapid growth, early start of reproduction and have shorter lifespans (Vanschoenwinkel *et al.*, 2010; Dararat *et al.*, 2011).

Growth curves of specimens studied here were similar to Benider *et al.* (2002) who mentioned that growth curves of *Moina* included two phases. The first phase, before the first egg, is characterized by fast growth and the second phase, after sexual maturity of the females, corresponded to a slow growth phase.

Our results showed significant size differences ($P < 0.05$) between the three water flea species. Largest body length was recorded in *M. macrocopa* (500 - 1,550 μm). Our largest species was smaller than *M. macrocopa* from Argentina (600 and 1,800 μm) (Vignatti *et al.*, 2013) but larger than *M. macrocopa* from South America (1,190 μm) (Paggi, 1997) and Rio de Janeiro (1,080 μm) (Elmoor-Loureiro *et al.*, 2010). The body size of *M. macrocopa* and *M. siamensis* (500 - 1,290 μm) is suitable for nursing *Katsuwonus pelamis*, *Girella punctata*, *Clupea pallasii* and *Cyprinus capio* which have mouth sizes between 570 - 930 μm (Shirota, 1970).

The smallest species from our study was *M. micrura* (200 - 920 μm), smaller than *M. micrura* from Lake Kasumigaura in Japan (450 - 1,200 μm) (Hanazato & Yasuno, 1985) and Australia (1,200 μm) (Goulden, 1968) but larger than *M. micrura* from Lake Kivu in Africa (499 μm) (Descy *et al.*, 2012). *M. micrura* is suitable for nursing *Gadus macrocephalus*, *Carassius auratus*, *Konosirus punctata* and *Engraulis japonica* which have small mouths ranging from 250 to 424 μm (Shirota, 1970).

Size of water fleas differed between regions (Vignatti *et al.*, 2013). Body size depends on food concentration (Goulden, 1968; Lampert & Trubetskova, 1996; Sipauba-Tavares & Bachion, 2002; Azuraidi *et al.*, 2013) diet content (Lampert & Trubetskova, 1996; Sipauba-Tavares & Bachion, 2002), population density (Jana & Pal, 1985) and temperature (Lampert & Trubetskova, 1996; Sipauba-Tavares & Bachion, 2002).

The results from this study showed the number of broods and total number of offspring per female of *M. micrura* was not significantly different from *M. macrocopa* ($P > 0.05$) and number of offspring per brood not significantly different ($P > 0.05$) from *M. siamensis*. In addition, they were short lifespan similar to *M. macrocopa* and same rapid maturation as *M. macrocopa* and *M. siamensis*. Thus, they have similar characteristic traits to

commercial water fleas, which possible for large-scale aquaculture production. Moreover, they can survive in both fresh and brackish water (Saint-Jean & Bonou, 1994; Liu, 2008) and contain 49.31 - 75.29 % protein (Saengphan *et al.*, 2016), which close to 53.7 - 60.5 % protein of *Artemia* (Peykaran *et al.*, 2014), making them suitable as live food for nursing marine aquatic animals and can replace *Artemia*, that need to be imported from abroad.

Conclusions

Our results indicated that *M. micrura* had similar characteristic traits to commercial water fleas. They have a short lifespan, small size and show rapid maturation, making them suitable for large-scale aquaculture production. Moreover, they recorded the smallest body length and are, therefore, appropriate as new-born aquatic larval feed.

Acknowledgements

This research was supported by Mahasarakham University Fund. The authors thank the aquaculture team at the Department of Fisheries, Suphanburi College of Agriculture and Technology and the Division of Fisheries, Mahasarakham University for supporting laboratory operation. We also wish to thank Apaporn Jampa, Thanawoot Phengchai, Siriwan Yotapol and Poomarin Kaewwongsa for their assistance in data collection.

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