ผลของฟอสฟอรัสต่อการรอดตายและการเจริญเติบโตของไร้นางฟ้าสิรินธร (Streptocephalus sirindhornae) 

Effects of Phosphorus on Survival and Growth of Fairy Shrimp, Streptocephalus sirindhornae

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บทคัดย่อ

ไร้นางฟ้าสิรินธร (Streptocephalus sirindhornae) เป็นสัตว์ไม่มีกระดูกสันหลังจำพวกครัสเตเชียชนิดหนึ่งที่อาศัยในแหล่งน้ำจืด และยังเป็นผู้บริโภคอันดับที่หนึ่งที่มีความสำคัญในระบบนิเวศน์น้ำจืดที่กำáficoพิจารณาได้จากการค้นพบในป่าที่มีพืชและสัตว์ที่มีความสัมพันธ์กับไร้นางฟ้าสิรินธรอยู่มาก การศึกษาล่าสุดพบว่าฟอสฟอรัสเป็นธาตุอาหารที่จำเป็นต่อการรอดตายและการเจริญเติบโตของไร้นางฟ้าสิรินธร โดยไร้นางฟ้าสิรินธรจะได้รับสาหร่าย Chlorella vulgaris ที่เลี้ยงใน COMBO medium ที่มีปริมาณฟอสฟอรัสแตกต่างกัน คือ 25 (low-P) และ 500 (high-P) μM เป็นอาหาร โดยเลี้ยงไร้นางฟ้าที่อุณหภูมิ 28˚C ที่สว่าง:มืด = 16:8 ชั่วโมง เป็นเวลา 8 วัน จากการศึกษาพบว่าไร้นางฟ้ากลุ่มที่ได้รับอาหารที่มีปริมาณของฟอสฟอรัสสูงจะมีอัตราการรอดตายสูงกว่ากลุ่มที่ได้รับฟอสฟอรัสต่ำมีนัยสำคัญ (p < 0.05) ทั้งนี้อาจเกิดมาจากฟอสฟอรัสเป็นธาตุอาหารที่มีส่วนในการสังเคราะห์ซึ่งมีการสังเคราะห์ผ่านกระบวนการของ exoskeleton รวมถึงอื่นๆที่สำคัญและส่งผลต่อการรอดตายของไร้นางฟ้าสิรินธร ซึ่งอาจจะมีส่วนช่วยให้ไร้นางฟ้าสิรินธรมีอัตราการรอดตายสูงขึ้น อย่างไรก็ตามในการศึกษานี้พบว่าการเจริญเติบโตของไร้นางฟ้าในแต่ละกลุ่มทดลองไม่มีความแตกต่างกันอย่างมีนัยสำคัญ (p > 0.05)

คำสำคัญ : ไร้นางฟ้า, ฟอสฟอรัส, Streptocephalus sirindhornae, การรอดตาย, การเจริญเติบโต

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Abstract

Fairy shrimp, Streptocephalus sirindhornae is a freshwater crustacean and acts as a primary consumer that transfers energy and nutrients from producers to higher trophic levels. It has been used as a live food for ornamental fish and the other economical crustaceans. In this study, we aimed to clarify the effects of phosphorus on survival and growth of S. sirindhornae. The fairy shrimps were fed with Chlorella vulgaris cultured in COMBO medium containing two different concentrations of phosphorus (25 (low-P) and 500 (high-P) μM) and cultured under the same conditions: 28˚C at 16-h light and 8-h dark photoperiod. We observed their growth and survival until they became adults (8 days). The results showed that the survival of S. sirindhornae fed with high-phosphorus Chlorella significantly higher than those fed with low-phosphorus group (p < 0.05) due to the important of phosphorus on component of the exoskeleton, enzymes, and nucleic acids of fairy shrimps. However, the growth of fairy shrimps was not significantly different between the treatments (p > 0.05).

Keywords: fairy shrimp, phosphorus, Streptocephalus sirindhornae, survival, growth

Introduction

Fairy shrimp Streptocephalus sirindhornae is a freshwater crustacean widely found in temporary pool, pond, flooding plain and other freshwater ecosystem (Sanoamuang et al. 2000). Fairy shrimp is an important primary consumer in an aquatic ecosystem, and it is typically highly abundant. Therefore, S. sirindhornae is a keystone species to maintain the equilibrium of freshwater ecosystem (Boonmak et al. 2007). The changes of their survival and growth may affect to higher consumers in a food chain. In Thailand, S. sirindhornae has been cultured and widely used as a live feed for ornamental fish to increase the levels of carotenoid, canthaxanthin, astaxanthin and β-carotene in skin and muscle of flowerhorn cichlid. The hatching percentage of S. sirindhornae is 64%, however, the survival rate of this fairy shrimp has not been studied yet. Phallocryptus spinosa and Streptocephalus simplex are also used for feeding of ornamental fish (Seidgar 2015; Simhachalam et al., 2015; Sornsupharp et al. 2015). Therefore, the affecting factors to improve production of fairy shrimp should be investigated.

The factors that affect to survival and growth of animal are divided into biotic factors and abiotic factors. Biotic factors affecting survival and growth of fairy shrimps are widely studied, especially, pathogen infection causing black disease in fairy shrimps (Thie 1998; Muangsan et al. 2006; Saejung et al. 2011, 2012, 2014). Temperature, photoperiod, and desiccation also impact to the hatching of fairy shrimp’s cysts (Saengphan et al. 2005; Vanschoenwinkel et al., 2010; Dararat et al., 2011). Previously, some studies revealed that type of food and nutrient composition are important factors to control survival and growth of freshwater crustacean (Saengphan & Sanoamuang 2009; Sornsupharp et al., 2013). In addition, diet stoichiometry significantly affects to the growth and reproduction of the freshwater crustacean Daphnia. High quality of food (low C:P ratio) increase growth rate and
clutch size of *Daphnia*. However, the mechanism for this improvement is still unclear (Acharya *et al.*, 2004). Therefore, phosphorus is one of interesting elements, which is an essential component in nucleotides and as important biomolecules required in aquatic invertebrates to change their life history (Becker & Boersma 2005; Frost *et al.*, 2008). However, the effects of phosphorus in diet are still unknown in fairy shrimp. Therefore, we aimed to clarify the effects of phosphorus in the diet on survival and growth of *S. sirindhornae*.

**Methods**

*Chlorella vulgaris* strain and growth experiment

*Chlorella vulgaris* Beijerinck was obtained from the National Institute for Environmental Studies (NIES, Tsukuba, Japan). To clarify the importance of dietary phosphorus in *S. sirindhornae*, this study was divided into two treatments by culturing *Chlorella vulgaris* in two different concentrations of phosphorus; 25 and 500 μM (low-P and high-P, respectively) by modifying the composition of COMBO medium (Kilham *et al.* 1998). We used 500 μM K$_2$HPO$_4$ in high-P medium, and 25 μM K$_2$HPO$_4$ and 975 μM KCl to supplement K concentration in low-P medium. The 3.75x10$^6$ cells of *Chlorella* were inoculated in 50 ml of sterilized COMBO medium with two different concentrations of phosphorus; low-P and high-P COMBO. Subsequently, three replicates of *Chlorella* were incubated under 16-h light and 8-h dark photoperiod at 23°C with continuous aeration. Numbers of *Chlorella* cells were daily counted until reaching maximum growth by using hemocytometer (Bright-Line, NY, USA), and the concentrations of *Chlorella* were calculated and plotted versus time.

*Chlorella* culture for feeding to *S. sirindhornae*

The 7.50x10$^7$ cells of *Chlorella* were inoculated in 1 L of sterilized COMBO medium containing 25 and 500 μM of phosphorus and incubated under 16-h light and 8-h dark photoperiod at 23°C with continuous aeration until the concentration of *Chlorella* reached to 4x10$^6$ cells/ ml. This concentration was selected for harvesting because *Chlorella* growth of both treatments was in the same growth phase (exponential phase). Previous study revealed that quantities of nutrients in *Chlorella* in exponential and stationary phases were different (Paes *et al.* 2016). After *Chlorella* concentration reached to 4x10$^6$ cells/ ml, the *Chlorella* cells were centrifuged at 3,000 rpm for 5 minutes, washed the cell pellet with filtered reverse osmosis water (RO water) 2 times, and resuspended in filtered RO water and stored at 4°C until use for feeding.

Hatching of *S. sirindhornae*'s cysts

*Streptocephalus sirindhornae* was originally collected from a temporary pond, which was approximately 2 km far from the north of Khon Kaen University in Ban Non Muang subdistrict, Khon Kaen province, Thailand. Subsequently, they were cultured in a laboratory at Applied Taxonomic Research Center, Khon Kaen University, Thailand (Sanoamuang *et al*., 2000). Their resting eggs were hatched according to the procedure described previously (Saengphan *et al*., 2005). The 1-day-old nauplii *S. sirindhornae* were collected to use in the experiments.
Experimental design and phenotypic analyses of S. sirindhornae

We conducted the experiments to observe survival and growth of S. sirindhornae. This study was divided into 2 treatments: S. sirindhornae fed with Chlorella in low-P and high-P COMBO. Each treatment, twenty-four individuals of S. sirindhornae were used then one juvenile was transferred into each well of a 24-well plate containing 2 ml of RO water. Seven replications were performed in each treatment. Therefore, total number of S. sirindhornae used in each treatment was 168 individuals. All juveniles were cultured at 28 ± 1°C under 16-h light and 8-h dark photoperiod and daily fed with Chlorella cultured in COMBO containing 25 or 500 μM of phosphorus. The 1x10⁶ cells of Chlorella were fed to S. sirindhornae and thereafter fed with double amounts of Chlorella on day 7. Then, the survival of fairy shrimps was daily counted, and their body lengths of survival fairy shrimp were measured from head to caudal furca on day 8 using Image J. Number of survival fairy shrimps of low-P and high-P treatments used to measure the body length were 3 and 42 individuals, respectively.

Statistical analyses

All data are presented as means ± standard error (SE). Data were evaluated with Welch’s t-test at α=0.05 with the R statistical program, version 3.5.1 (The R Core Team 2013).

Results

Phosphorus level affects the growth of Chlorella

Chlorella was cultured in two different levels of phosphorus in the algal culturing medium. The results showed that Chlorella high-P COMBO started to increase after three days and reached to maximum concentration on day 8 (5.09 ± 1.48 x 10⁷ cells/ ml), which were higher than the maximum growth of Chlorella in low-P COMBO (9.72 ± 0.63 x 10⁶ cells/ ml). The lag phase of Chlorella in low-P COMBO was one day longer than that of high-P COMBO (Figure 1). However, the difference in Chlorella growth rates in log phase between both treatments was not observed (Table 1).
Figure 1 Growth of Chlorella cultured in two different phosphorus concentrations. The concentration of Chlorella cultured in low and high P COMBO (25 and 500 μM of phosphorus, respectively) was estimated and plotted against culturing time. There was no significant difference between groups (Welch’s t-test, \( p > 0.05 \)).

Table 1 Phosphorus concentration, molar N:P and growth rate of C. vulgaris in low and high-P COMBO.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P (μM)</th>
<th>N:P</th>
<th>Growth rate of C. vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>low-P</td>
<td>25</td>
<td>40</td>
<td>1.05 ± 0.15</td>
</tr>
<tr>
<td>high-P</td>
<td>500</td>
<td>2</td>
<td>0.98 ± 0.34</td>
</tr>
</tbody>
</table>

There was no significant difference in growth rate among three treatments (Welch’s t-test, \( p > 0.05 \)).

Dietary phosphorus influents the survival of S. sirindhornae

To characterize the effects of dietary phosphorus on the survival of S. sirindhornae, the juveniles were reared and fed with Chlorella cultured in low and high concentrations of phosphorus. Subsequently, percentages of survival were observed until S. sirindhornae became an adult (day 8). The results revealed that the survival of
S. sirindhornae fed with Chlorella cultured in high-P COMBO showed the higher survival on day 6-8, which was significantly different from those fed with Chlorella cultured in low-P COMBO (Figure 2, $p < 0.05$).

**Figure 2** Survival S. sirindhornae fed with Chlorella cultured in low and high P COMBO (25 and 500 μM of phosphorus, respectively). Asterisks above the bars indicate significant differences between treatments (Welch’s t-test, $p < 0.05$).

Phosphorus level does not affect to the growth of S. sirindhornae

This experiment was conducted to estimate the growth of S. sirindhornae by measuring of the body lengths from head to caudal furca. The lengths of adult S. sirindhornae in both treatments were not significantly different (Figure 3, $p > 0.05$) suggesting that phosphorus may not influence the growth of S. sirindhornae.
**Discussion**

In this study, we found that the survival of *S. sirindhornae* fed with *Chlorella* in high-P COMBO was significantly higher than *Chlorella* cultured in low-P COMBO. It seems that phosphorus is accumulated in *Chlorella* and transferred to *S. sirindhornae*, which is consistent with the previous study in *Daphnia* that *Chlorella* cultured in high quality of algal medium (P-rich COMBO medium) increased growth rate and reproduction of *D. pulicaria*, *D. galeata* and *D. magna* (Acharya et al., 2004). Immunity of *S. sirindhornae* might be increased when fed with *Chlorella* in high-P COMBO leading to increase survival of adult *S. sirindhornae*. Previous studies also showed the similar results, which dietary phosphorus is involved in immunity against bacterial pathogens and increase survival in aquatic invertebrates and other animals (Calder & Jackson 2000; Frost et al., 2005; Frost et al., 2008). Furthermore, phosphorus is also related to the contents of free nucleotides, protein and phospholipids that enhance immune function in aquatic invertebrates, which is related to organismal activities (e.g. survival, growth and reproduction) (Vrede et al., 1999; Becker & Boersma 2005; Frost et al., 2005).

A study showed that P-saturated *Scenedesmus subspicatus* and *Selenastrum capricornutum* were efficiently digested and assimilated in *Daphnia*, while P-limited algal cells showed structural and morphological changes, which is leading to reduce their digestibility (van Donk & Hessen 1993). P-limited algae also cause weight decreasing in *Daphnia* and *Bosmina*, on the other hand, P-saturated algal cells are able to alter *Daphnia* stoichiometry and improve immunity to reduce pathogen infection leading to maintaining growth and reproduction of crustaceans (Urabe & Watanabe 1992; Lurling & van Donk 1997; Boersma & Kreutzer 2002; Acharya et al., 2004;
However, we could not observe any difference in *S. sirindhornae* growth among the treatments in this study. This finding suggests that responses of phosphorus limitation are different among the species. The nutritional imbalance causes *S. sirindhornae* to spend more energy to improve immunity and maintain survival (Zera and Harshman 2001; Biro et al., 2006). Therefore, mechanisms controlling the responses from phosphorus limitation are needed to study in further experiments.

Our study suggests that phosphorus is required for survival of *S. sirindhornae*. This finding might be useful for improvement of survival rate in commercial aquaculture of fairy shrimps. However, the importance of phosphorus on fecundity, embryonic development and immunity of *S. sirindhornae* should be investigated to improve the culture of *S. sirindhornae*. The mechanisms of phosphorus enhancing survival of *S. sirindhornae* also should be studied to understand this biological phenomenon.

**Conclusions**

This study suggests that phosphorus is an essential element for increasing the survival of fairy shrimp *S. sirindhornae*. Our finding will be useful for applying in aquaculture to improve production of fairy shrimps. Furthermore, the other abiotic factors and nutrients should be investigated to improve quality and quantity of fairy shrimps for animal food production.

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**References**


