ผลของฟอสฟอรัสต่อการรอดตายและการเจริญเติบโตของไรน้ำนางฟ้าสิรินธร (*Streptocephalus sirindhornae*)

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

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บทคัดย่อ

ไรน้ำนางฟ้าสิรินธร (*Streptocephalus sirindhornae*) เป็นสัตว์ไม่มีกระดูกสันหลังจำพวกครัสเตเชียชนิดหนึ่งที่อาศัยในแหล่งน้ำจืด และยังเป็นผู้บริโภคอันดับที่หนึ่งที่มีความสำคัญในระบบนิเวศน้ำจืดที่ถ่ายทอดพลังงานจากผู้ผลิตไปยังผู้บริโภคอันดับสูงขึ้นได้ นอกจากนี้ยังเป็นอาหารของปลาสวยงามและสัตว์ไม่มีกระดูกสันหลังที่สำคัญทางเศรษฐกิจ ในการศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของฟอสฟอรัสต่อการรอดตายและการเจริญเติบโตของไรน้ำนางฟ้าสิรินธร โดยไรน้ำนางฟ้าสิรินธรจะได้รับสาหร่าย *Chlorella vulgaris* ที่เลี้ยงใน COMBO medium ที่มีปริมาณฟอสฟอรัสแตกต่างกัน คือ 25 (low-P) และ 500 (high-P) μM เป็นอาหาร โดยเลี้ยงไรน้ำนางฟ้าที่อุณหภูมิ 28°C ที่สว่าง:มืด=16:8 ชั่วโมง เป็นเวลา 8 วัน จากการศึกษาพบว่าไรน้ำนางฟ้ากลุ่มที่ได้รับอาหารที่มีปริมาณของฟอสฟอรัสสูงมีอัตราการรอดตายสูงกว่ากลุ่มที่ได้รับฟอสฟอรัสต่ำอย่างมีนัยสำคัญ ($p < 0.05$) ทั้งนี้อาจเนื่องมาจากฟอสฟอรัสเป็นธาตุอาหารที่เป็นองค์ประกอบของ exoskeleton รวมถึงเอนไซม์ที่สำคัญและสารพันธุกรรมในไรน้ำนางฟ้าสิรินธร ซึ่งอาจจะมีส่วนช่วยให้ไรน้ำนางฟ้าสิรินธรมีอัตราการรอดตายสูงขึ้น อย่างไรก็ตามในการศึกษานี้ พบว่าการเจริญเติบโตของไรน้ำนางฟ้าในแต่ละกลุ่มทดลองไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ($p > 0.05$)

คำสำคัญ : ไรน้ำนางฟ้า, 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_2HPO_4 in high-P medium, and 25 µM K_2HPO_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 $1x10^6$ 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 ($5.09 \pm 1.48 \times 10^7$ cells/ ml), which were higher than the maximum growth of *Chlorella* in low-P COMBO ($9.72 \pm 0.63 \times 10^6$ 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).

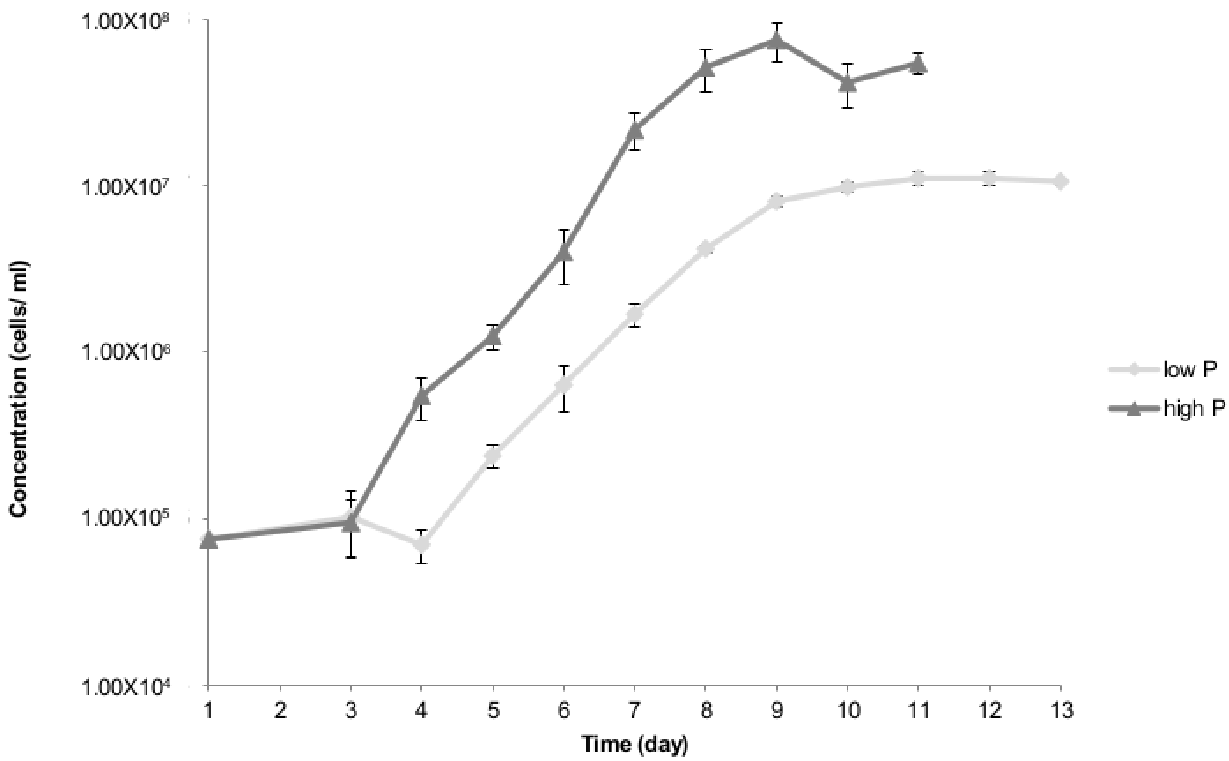

Figure 1 Growth of *Chlorella* cultured in two different phosphorus concentrations. The concentration of *Chlorella* cultured in low and high P COMBO (25 and 500 μM of phosphorus, respectively) was estimated and plotted against culturing time. There was no significant difference between groups (Welch's t-test, $p > 0.05$).

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

Treatment	P (μM)	N:P	Growth rate of *C. vulgaris*
low-P	25	40	1.05 ± 0.15
high-P	500	2	0.98 ± 0.34

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, $p < 0.05$).

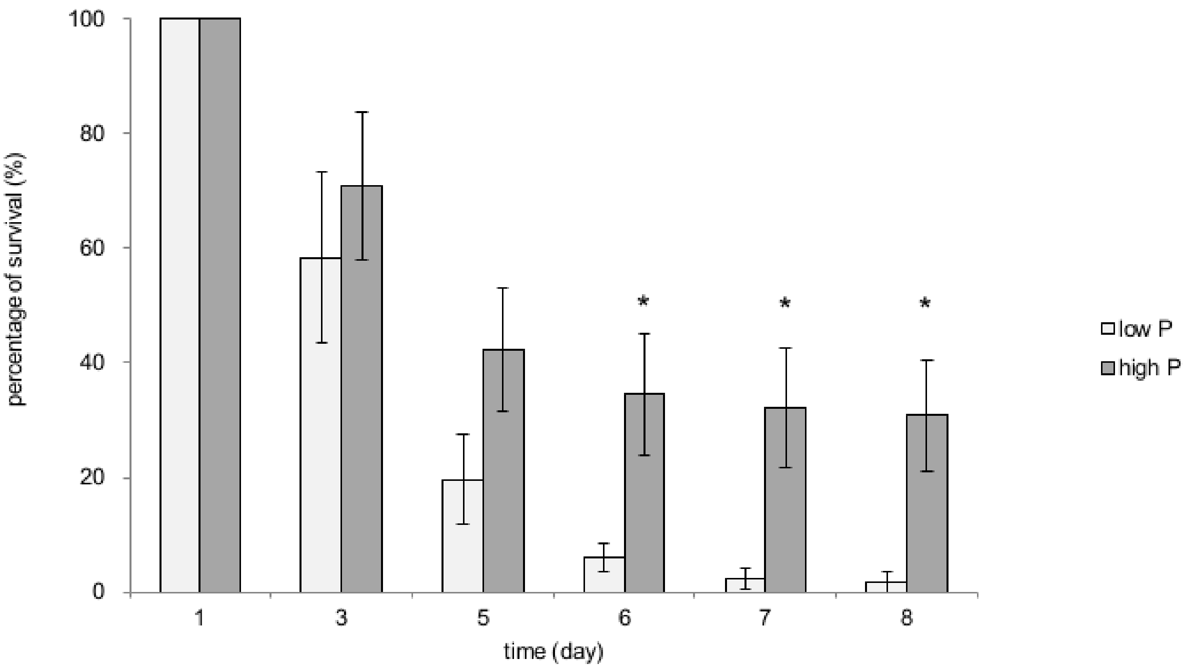

Figure 2 Survival *S. sirindhornae* fed with *Chlorella* cultured in low and high P COMBO (25 and 500 μM of phosphorus, respectively). Asterisks above the bars indicate significant differences between treatments (Welch's t-test, $p < 0.05$).

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*.

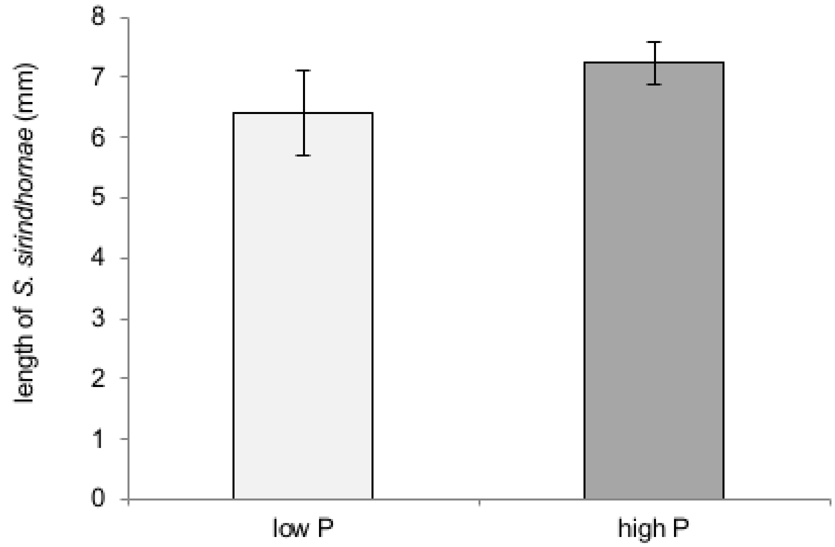

Figure 3 Body length of adult *S. sirindhornae* fed with *Chlorella* cultured in low and high P COMBO (25 and 500 μM of phosphorus, respectively).

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;

Frost *et al*., 2008). 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

Acharya, K., Kyle, M., and Elser, J.J. (2004) Effects of stoichiometric dietary mixing on Daphnia growth and reproduction. *Oecologia*, *138*, 333–40.

Becker, C. and Boersma, M. (2005) Differential effects of phosphorus and fatty acids on Daphnia magna growth and reproduction. *Limnol. Oceanogr, 50*, 388–397.

Biro, P.A., Abrahams, M. V., Post, J.R., and Parkinson, E.A. (2006) Behavioural trade-offs between growth and mortality explain evolution of submaximal growth rates. *J. Anim. Ecol.*, *75*, 1165–1171.

Boersma, M. and Kreutzer, C. (2002) Life at the edge: Is food quality really of minor importance at low quantities? *Ecology*, *83*, 2552–2561.

Boonmak, P., Saengphan, N., and Sanoamuang, L. (2007) Biology and fecundity of two fairy shrimps, Streptocephalus sirindhornae Saoamuang, Murugan, Weekers and Dumont and Branchinella thailandensis Sanoamuang, Saengphan and Murugen. *KKU Res J*, *12*, 125–131.

Calder, P.C. and Jackson, A.A. (2000) Undernutrition, infection and immune function. *Nutr. Res. Rev.*, *13*, 3–29.

Dararat, W., Starkweather, P.L., and Sanoamuang, L. (2011) Life History of Three Fairy Shrimps (Branchiopoda: Anostraca) from Thailand. *J. Crustac. Biol.*, *31*, 623–629.

van Donk, E. and Hessen, D.O. (1993) Grazing resistance in nutrient-stressed phytoplankton. *Oecologia*, *93*, 508–511.

Frost, Paul C, Ebert, D., and Smith, V.H. (2008) Bacterial infection changes the elemental composition of Daphnia magna. *J. Anim. Ecol.*, *77*, 1265–1272.

Frost, P. C., Ebert, D., and Smith, V.H. (2008) Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. *Ecology*, *89*, 313–318.

Frost, P.C., Evans-White, M.A., Finkel, Z. V., Jensen, T.C., and Matzek, V. (2005) Are you what you eat ? Physiological in an elementally world imbalanced stoichiometry. *Oikos*, *109*, 18–28.

Kegley, E.B., Spears, J.W., and Auman, S.K. (2001) Dietary phosphorus and an inflammatory challenge affect performance and immune function of weanling pigs. *J. Anim. Sci.*, *79*, 413–419.

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E., and Herrera, L. (1998) COMBO: A defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*, *377*, 147–159.

Lurling, M. and van Donk, E. (1997) Life history consequences for Daphnia pulex feeding on nutrient-limited phytoplankton. *Freshw. Biol.*, *38*, 693–709.

Muangsan, N., Niamsanit, S., and Siripornadulsil, S. (2006) Black disease of fairy shrimps and associated bacteria. *KKU Res J*, *11*, 39–45.

Paes, C.R.P.S., Faria, G.R., Tinoco, N.A.B., Castro, D.J.F.A., Barbarino, E., and Lourenco, S.O. (2016) Growth, nutrient uptake and chemical composition of Chlorella sp. and Nannochloropsis oculata under nitrogen starvation. *Lat. Am. J. Aquat. Res.*, *44*, 275–292.

Saejung, C., Hatai, K., and Sanoamuang, L. (2012) Bath efficacy of sodium hypochlorite, oxytetracycline dihydrate and chloramphenicol against bacterial black disease in fairy shrimp Branchinella thailandensis. *Aquac. Res.*, *45*, 1697–1705.

Saejung, C., Hatai, K., and Sanoamuang, L. (2014) The in-vitro antibacterial effects of organic salts, chemical disinfectants and antibiotics against pathogens of black disease in fairy shrimp of Thailand. *J. Fish Dis.*, *37,* 33–41.

Saejung, C., Hatai, K., Wada, S., Kurata, O., and Sanoamuang, L. (2011) Clinical observations of black disease in fairy shrimps, Streptocephalus sirindhornae and Branchinella thailandensis, from Thailand and pathogen verification. *J. Fish Dis.*, *34*, 911–20.

Saengphan, N. and Sanoamuang, L. (2009) Effect of Food Concentrations on Growth and Survival of the Fairy Shrimp Branchinella thailandensis Sanoamuang , Saengphan and Murugan. *Burapha Sci. Spec.*, *2009*, 19–28.

Saengphan, N., Shiel, R.J., and Sanoamuang, L. (2005) The cyst hatching pattern of the Thai fairy shrimp, Branchinella thailandensis Sanoamuang, Saengphan & Muragen, 2002 (Anostraca). *Crustaceana, 78,* 513–523.

Saengphan, N., Shiel, R.J., and Sanoamuang, L. (2003) The cyst hatching pattern of the Thai fairy shrimp, Branchinella thailandensis Sanoamuang&Murugan, 2002 (Anostraca: Crustacea) in laboratory cultures. *KKU Res J*.

Sanoamuang, L., Murugan, G., Weekers, P.H.H., and Dumont, H.J. (2000) Streptocephalus sirindhonae, new species of freshwater fairy shrimp (Anostraca) from Thailand. *J. Crustac. Biol.*, *20*, 559–565.

Seidgar, M. (2015) The effects of Fairy Shrimp Phallocryptus spinosa (Branchiopoda : Anostraca) as live food on reproduction performances and color of freshwater ornamental fish prawns. *Int. J. Aquat. Sci.*, *6,* 37–44.

Simhachalam, G., Sampath Kumar, N.S., and Govinda Rao, K. (2015) Biochemical composition and nutritional value of Streptocephalus simplex as live feed in ornamental fish culture. *J. Basic Appl. Zool.*, *72*, 66–72.

Sornsupharp, B., Lomthaisong, K., Dahms, H.U., and Sanoamuang, L. (2015) Effects of dried fairy shrimp Streptocephalus sirindhornae meal on pigmentation and carotenoid deposition in flowerhorn cichlid; Amphilophus citrinellus (Gunther, 1864) x Cichlasoma trimaculatum (Gunther, 1867). *Aquac. Res.*, *46,* 173–184.

Sornsupharp, S., Dahms, H.U., and Sanoamuang, L. (2013) Nutrient composition of fairy shrimp Streptocephalus sirindhornae nauplii as live food and growth performance of giant freshwater prawn postlarvae. *Aquac. Nutr.*, *19*, 349–359.

The R Core Team (2013) R : a language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria.

Thie, A. (1998) Microflora associated with the digestive tract of the fairy shrimp Branchinella spinosa. *FEMS Microbiol. Lett.*, *158,* 201–205.

Urabe, J. and Watanabe, Y. (1992) Possibility of N or P limitation for planktonic cladocerans : An experimental test. *Limnol. Oceanogr.*, *37*, 244–251.

Vanschoenwinkel, B., Seaman, M., and Brendonck, L. (2010) Hatching phenology, life history and egg bank size of fairy shrimp Branchipodopsis spp. (Branchiopoda, Crustacea) in relation to the ephemerality of their rock pool habitat. *Aquat. Ecol.*, *44*, 771–780.

Vrede, T., Andersen, T., and Hessen, D.O. (1999) Phosphorus distribution in three crustacean zooplankton species. *Limnol. Oceanogr.*, *44*, 225–229.

Zera, A.J. and Harshman, L.G. (2001) The Physiology of Life History Trade-Offs in Animals. *Annu. Rev. Ecol. Syst.*, *32*, 95–126.