# ผลของความเข้มข้นของอาหารต่อการเติบโตและการรอดชีวิตของไรน้ำนางฟ้าไทย (*Branchinella thailandensis* Sanoamuang, Saengphan and Murugan) Effect of Food Concentrations on Growth and Survival of the Fairy Shrimp *Branchinella thailandensis* Sanoamuang, Saengphan and Murugan

นุกูล แสงพันธุ์<sup>1</sup> และ ละออศรี เสนาะเมือง<sup>2</sup>\* <sup>1</sup>คณะวิชาประมง วิทยาลัยเกษตรและเทคโนโลยีสุพรรณบุรี <sup>2</sup>ศูนย์วิจัยอนุกรมวิธานประยุกต์ ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น Nukul Saengphan<sup>1</sup> and La-orsri Sanoamuang<sup>2</sup>\* <sup>1</sup>Faculty of Fisheries, Suphanburi College of Agriculture and Technology <sup>2</sup>Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University

# บทคัดย่อ

ศึกษาผลของความเข้มข้นของอาหารต่อการเติบโตและการรอดชีวิตของไรน้ำนางฟ้าไทย โดยทำการทดลองเลี้ยงไรน้ำ นางฟ้าไทยที่มีอายุแตกต่างกัน (1-5, 6-10 และ 11-20 วัน) โดยให้อาหารแยกทีละชนิด (สาหร่ายคลอเรลลา รำละเอียด สาหร่าย สไปรู้ไลนาผง) ที่มีความเข้มข้นแตกต่างกัน นอกจากนั้นได้ทดลองให้อาหารผสมของทั้งสามชนิดในอัตราส่วนที่แตกต่างกันด้วย ในแต่ละการทดลองใช้ตัวอ่อนไรน้ำนางฟ้าระยะนอเพลียสที่มีอายุ 1 วันจำนวน 20 ตัว (3-4 ซ้ำ) เลี้ยงในถังพลาสติกที่มีน้ำประปา ปริมาตร 2 ลิตร โดยใช้ระบบน้ำนิ่งที่มีการเปลี่ยนถ่ายน้ำและมีการเดิมอากาศเล็กน้อย ผลการศึกษาพบว่าทั้งคลอเรลลา หรือ รำละเอียดสามารถใช้เป็นอาหารเลี้ยงไรน้ำนางฟ้าได้ดีโดยที่ไม่ต้องผสมกับอาหารชนิดอื่น ปริมาณที่เหมาะสมสำหรับใช้เลี้ยงไรน้ำ นางฟ้าอายุ 1-5 วัน และ 11-20 วัน คือ 1x10<sup>6</sup> เซลล์คลอเรลลาต่อมิลลิลิตร และ 1.6 มิลลิกรัมน้ำหนักแห้งของรำละเอียดต่อตัว และ ปริมาณที่เหมาะสมที่อายุ 6-10 วัน คือ 2x10<sup>6</sup> เซลล์คลอเรลลาต่อมิลลิลิตร และ 2.4 มิลลิกรัมน้ำหนักแห้งของรำละเอียดต่อตัว ส่วน ปริมาณที่เหมาะสมของสไปรูลินาผงสำหรับเลี้ยงไรน้ำนางฟ้าอายุ 1-5 วัน คือ 0.8 มิลลิกรัมน้ำหนักแห้งต่อตัว สำหรับสัดว่นของ อาหารทั้งสามชนิดเมื่อใช้เป็นอาหารผสมที่มีผลทำให้ไรน้ำนางฟ้ามีการรอดชีวิตสูงกว่าลัดส่วนอื่นคือ คลอเรลลา 50-70% ของความ เข้มข้นที่เหมาะสม และอาหารผสมที่ใช้อาหารทั้งสามชนิดร่วมกัน

คำสำคัญ : ไรน้ำนางฟ้า Branchinella thailandensis คลอเรลลา สไปรูไลนาผง

\*Corresponding author. E-mail: la\_orsri@kku.ac.th

## Abstract

A number of experiments were conducted to assess the suitability of three food sources (*Chlorella* sp., rice bran and *Spirulina* powder) on growth and survival of the fairy shrimp *Branchinella thailandensis* Sanoamuang, Saengphan and Murugan at different ages and various concentrations. Each experiment, 20 individuals of 1-day old nauplii of the fairy shrimp were reared in a circular plastic container containing 2 l of dechlorinated tap water with 3-4 replications. All experiments were conducted in a static/renewal culture system with gentle aeration. The results showed that *Chlorella* sp. and rice bran alone are suitable for feeding the fairy shrimp as a mono-diet. Optimal concentrations were 1x10<sup>6</sup> cells *Chlorella* sp. ml<sup>-1</sup> and 1.6 mg rice bran dry weight individual<sup>-1</sup> at day 1-5 and 11-20, and 2x10<sup>6</sup> cells *Chlorella* sp. ml<sup>-1</sup> and 2.4 mg rice bran dry weight individual<sup>-1</sup> at day 6-10. The optimal food concentration of *Spirulina* powder was 0.8 mg dry weight individual<sup>-1</sup> at day 1-5. In general, the combination of *Chlorella* sp. at 50-70% of optimal concentrations with rice bran or/and *Spirulina* powder and the combination of 3 food sources gave a higher survival rate than any other ratios.

Keywords : fairy shrimps, Branchinella thailandensis, Chlorella sp., Spirulina powder

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## Introduction

Thailand is a tropical country, rich in inexpensive to even free waste products and by-products from agroindustry. Recently, three new species of fairy shrimps have been recorded in Thailand, *viz. Streptocephalus sirindhornae* Sanoamuang, Murugan, Weekers and Dumont, 2000; *Branchinella thailandensis* Sanoamuang, Saengphan and Murugan, 2002 and *Streptocephalus siamensis* Sanoamuang and Saengphan, 2006 (Sanoamuang *et al.*, 2000a; 2002; Sanoamuang & Saengphan, 2006). According to Boonmak *et al.* (2007), *B. thailandensis* is characterized by a high individual biomass, a high fecundity producing >4,000 cysts per female during a life span of ca. 1 month, and it is easily cultured.

Fairy shrimps are non-selective filter feeders. Various food sources, both live and inert, have been used successfully for culturing fairy shrimps from nauplii to adults. Best results have always been achieved with a diet of live algae. However, for high-density mass culture of anostracans, microalgae are relatively expensive and labor-intensive (Lavens & Sorgeloos, 1991; Coutteau *et al.*, 1992; Ali & Brendonck, 1995). For large-scale cultures on a cost-effective basis, an inexpensive algal substitute should be developed. Other foods were then considered, e.g. rice bran, a mixture of corn and pea bran, palm oil effluent, yeast and *Spirulina* powder (Coutteau *et al.*, 1992; Ali & Brendonck, 1995; Maeda-Martinez *et al.*, 1995; Ali & Dumont, 2002; Sriputhorn & Sanoamuang, 2007).

In Thailand, *Chlorella* sp. is widely cultured for feeding *Moina micrura* Kurz, with low cost of fertilizers and waste or by-product from agro-industries. In addition, recent experiments showed that *Chlorella* sp. and rice bran were suitable food for *Branchinella thailandensis* (Saengphan *et al.*, 2006). Alternatively, *Spirulina* powder was reported as a suitable source for supplementing food of fairy shrimps (De Walsche *et al.*, 1991). In this study, a number of experiments were conducted on a small scale to assess the suitability of three food sources (*Chlorella* sp., rice

bran and *Spirulina* powder) on the growth and survival of *B. thailandensis* at different ages at various concentrations.

## Materials and Methods

#### **Culture of Fairy Shrimps**

Cysts of fairy shrimp B. thailandensis were obtained from continuous laboratory cultures of the Applied Taxonomic Research Center, Khon Kaen University, Thailand. They were incubated in 3-I plastic containers with dechlorinated tap water for 24-hr intervals. Nauplii were initially fed when they were 12 hours old. Each experiment, 20 individuals of 1-day old nauplii of the fairy shrimp were reared in a circular plastic container containing 2 I of dechlorinated tap water with 3-4 replications. All experiments were conducted in a static/renewal culture system. Gentle aeration was provided through 0.5 mm diameter holes drilled at 5 cm intervals in an air tube (4 mm diameter) placed on a circular bottom of containers. The medium was renewed 20% on alternative days, except during the experiment on water renewal. Food was supplied twice a day. Cultures of Chlorella sp. were performed using the procedure of Saengphan et al. (2005).

#### **Data Analysis**

After the fairy shrimps were reared until the dates set according to the experiment design, body length of each fairy shrimp was measured using a microscope fitted with camera lucida calibrated against a stage micrometer. Duncan's New Multiple Range Test was used to compare means of the treatments after the analysis found significance.

# Experiment 1: Determination of *Chlorella* concentrations on growth and survival of *B. thailandensis* at day 1-5.

Three algal concentrations of  $2.5 \times 10^5$ ,  $5 \times 10^5$  and  $1 \times 10^6$  cells ml<sup>-1</sup> were designed in quadruplicates to feed the shrimp nauplii from day 1 to 5 twice a day. Survival and body length were measured at day 5 of culture.

# Experiment 2: Determination of *Chlorella* concentrations on growth and survival of *B. thailandensis* at day 6-15.

Nauplii at the age of 1-5 days were fed with *Chlorella* sp. 1 x  $10^{6}$  cells ml<sup>-1</sup> twice a day. Shrimps then were randomly chosen, moved to twelve containers and fed with 3 food concentrations:  $0.5 \times 10^{6}$ , 1 x  $10^{6}$  and 2 x  $10^{6}$  cells ml<sup>-1</sup> twice a day between day 6 and 15. Numbers and body length were measured at day 10 and 15.

# Experiment 3: Determination of rice bran concentrations on growth and survival of *B. thailandensis* at day 1-5.

Rice bran was fed to shrimp nauplii twice a day at day 1-5 with 3 food concentrations: 0.8, 1.6 and 2.4 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup>. Experiments were run in quadruplicates. Survival and length were measured at day 5 of culture.

# Experiment 4: Determination of rice bran concentrations on growth and survival of *B. thailandensis* at day 6-15.

Nauplii at first 1-5 days were fed with rice bran 0.8 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> twice a day. Shrimps then were randomized to twelve containers and fed with 3 food concentrations: 0.8, 1.6 and 2.4 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup>, between 6 and 15 days of culture. Experiments were run in quadruplicate. Number and length were measured at day 10 and 15.

# Experiment 5: Determination of *Spirulina* powder concentrations on growth and survival of *B. thailandensis* at day 1-5.

*Spirulina* powder was fed to nauplii twice a day at day 1-5 with 4 food concentrations: 0.2, 0.4, 0.6 and 0.8 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup>. Experiments were run in triplicate. Survival and length were measured at day 5 of culture.

# Experiment 6: Determination of *Spirulina* powder concentrations on growth and survival of *B. thailandensis* at day 6-15.

Nauplii at first 1-5 days were fed with *Spirulina* powder 0.8 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> twice a day. Shrimps then were randomized to twelve containers and fed with 3

food concentrations: 0.6, 0.8 and 1.6 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup>, twice a day between day 6 and 15 days of culture. Experiments were run in quadruplicate. Number and length were measured at day 10 and 15.

# Experiment 7: Determination of the food ratio of mixed feed: 2 food sources

Preliminary study on the optimal ratio of 3 food types indicated that the animal growth was better in mixed feed with a high ration of *Chlorella* sp. Therefore, the ratio of *Chlorella* sp. should be at least 50% of their optimal concentration. Experiment 7 was designed to use rice bran (R) or *Spirulina* powder (S) mixed with *Chlorella* sp. (C) at various ratios for feeding to fairy shrimp from day 1-15 as followed:

The first 5 days, food concentrations used were at  $1 \times 10^{6}$  cells ml<sup>-1</sup>, 0.8 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> and 0.6 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> of *Chlorella* sp., rice bran and *Spirulina* powder, respectively. Then between day 6 and 15, food concentrations used were at 1 x--0 10<sup>6</sup> cells ml<sup>-1</sup>, 1.6 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> and 0.8 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> of *Chlorella* sp., rice bran and *Spirulina* powder, respectively. On day 5, 10 and 15, all animals were counted and two shrimps of each replication were randomly chosen for measuring growth (not returned to the containers). Experiments were run in triplicates.

# Experiment 8: Determination of the food ratio of mixed feed: 3 food sources

Experiment 7 indicated that the animal growth was better in mixed feed with the ration of *Chlorella* sp. 50-70%. Therefore the rations of rice bran and *Spirulina* powder in mixed feed were further tested. The experimental designs were as followed: C: R = 4: 4 C: R: S = 4: 3: 1 C: R: S = 4: 2: 2

The experiments were run in quadruplicate. Food concentrations and data collections followed in experiment 7.

#### Results

The survival and growth of *B. thailandensis* fed with three *Chlorella* sp. densities at day 5, 10 and 15 were shown in Table 1 and 2. The fairy shrimps at day 5 increased both their survival and length from the preliminary study

(concentrations of 5 x 10<sup>3</sup>, 2.5 x 10<sup>4</sup>, 5 x 10<sup>4</sup> and 2.5 x 10<sup>5</sup> cells ml<sup>-1</sup>). There were no significant differences (p>0.05) on survival between these food concentrations. The body length of shrimps fed at the highest food concentration, however, was significantly (p<0.05) greater than those were fed at the lower concentration (Table 1). The survival at day 10 and 15 did not differ significantly (p<0.05) greater in length than at the lower levels except that at day 15, there was no significant difference between the animals fed at 1 x 10<sup>6</sup> and 2 x 10<sup>6</sup> cells ml<sup>-1</sup> (Table 2).

*Table 1* Survival and growth (mean ± SD) of 1-day old *Branchinella thailandensis* nauplii (20 individuals) fed with different levels of *Chlorella* sp. at day 5.

<i>Chlorella</i> sp. (cells ml <sup>-1</sup> )	Survival (individuals)	Body Length (mm)	
2.5 x 10 <sup>5</sup>	$19.0 \pm 1.41^{a}$	$13.81 \pm 0.23^{a}$	
5 x 10⁵	$19.0\pm0.82^{a}$	$14.72\pm0.42^{ m b}$	
1 x 10 <sup>6</sup>	$18.0 \pm 1.41^{a}$	$17.11\pm0.19^{\circ}$	

Means, within a column, sharing a common superscript are not significantly different (p > 0.05).

Table 2Survival and growth (mean ± SD) of 1-day old Branchinella thailandensis nauplii (20 individuals) fed with<br/>different levels of Chlorella sp. at day 10 and 15.

Chlorella sp.	Day 10		Day 15		
(cells ml⁻¹)	Survival (individuals)	Body Length (mm)	Survival (individuals)	Body Length (mm)	
5 x 10⁵	$19.5\pm0.58^{a}$	$16.64 \pm 0.44^{a}$	$16.25 \pm 1.26^{a}$	$17.85 \pm 0.75^{a}$	
1 x 10 <sup>6</sup>	$19.5\pm0.58^{\text{a}}$	$18.48\pm0.50^{\text{b}}$	$17.25 \pm 0.96^{a}$	$19.35\pm0.62^{\scriptscriptstyle b}$	
2 x 10 <sup>6</sup>	$19.0 \pm 1.41^{a}$	$19.33\pm0.63^{\circ}$	$16.25 \pm 1.26^{a}$	$20.25\pm0.53^{\text{b}}$	

Means, within a column, sharing a common superscript are not significantly different (p > 0.05).

With reference to the rice bran experiments, the survival of the shrimps fed with the highest food concentration of 2.4 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> at day 5 was significantly (p<0.05) lower than that of any other levels. In contrast with the survival, the body length of the shrimps fed with the 2.4 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> was significantly (p<0.05) greater than that of the other levels (Table 3).

There were no significant (*p*>0.05) differences between food concentrations on the survival of the shrimps at day 10 and 15. The shrimps fed rice bran at the concentration of 2.4 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> were significantly (*p*<0.05) larger than those fed at the lower levels at day 10, but did not differ significantly (*p*>0.05) with the 1.6 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> concentration at day 15 (Table 4).

*Table 3* Survival and growth (mean ± SD) of 1-day old *Branchinella thailandensis* nauplii (20 individuals) fed with different levels of rice bran at day 5.

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	Rice bran (mg ind. <sup>-1</sup> d <sup>-1</sup> )	Survival (individuals)	Body Length (mm)	
	0.8	$15.25 \pm 1.71^{ m b}$	$10.60 \pm 0.39^{a}$	
	1.6	$15.00\pm0.82^{ m b}$	$11.57\pm0.73^{\text{a}}$	
	2.4	$11.75 \pm 1.26^{a}$	$12.72\pm0.80^{\text{b}}$	

Means, within a column, sharing a common superscript are not significantly different (p > 0.05).

*Table 4* Survival and growth (mean ± SD) of 1-day old *Branchinella thailandensis* nauplii (20 individuals) fed with different levels of rice bran at day 10 and 15.

Rice Bran	Day	10	Day 15		
(mg ind. <sup>-1</sup> d <sup>-1</sup> )	Survival (individuals)	Body Length (mm)	Survival (individuals)	Body Length (mm)	
0.8	16.0 <u>+</u> 1.63 <sup>ª</sup>	11.93 <u>+</u> 0.61 <sup>a</sup>	9.25 <u>+</u> 2.22 <sup>a</sup>	13.36 <u>+</u> 0.09 <sup>ª</sup>	
1.6	16.0 ± 1.41 <sup>ª</sup>	12.51 ± 0.43ª	10.50 <u>+</u> 1.00 <sup>a</sup>	15.23 <u>+</u> 0.99 <sup>b</sup>	
2.4	15.0 <u>+</u> 1.41 <sup>a</sup>	14.11 ± 0.44 <sup>b</sup>	9.00 <u>+</u> 1.63 <sup>a</sup>	15.20 <u>+</u> 0.88 <sup>b</sup>	

At day 5, the shrimps fed with *Spirulina* powder at the concentrations of 0.6 and 0.8 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> at day 5 had significantly (p<0.05) higher survival than those fed at the lower concentrations (Table 5). The animals fed at the concentration of 0.2 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup>

were significantly (p<0.05) smaller than those fed at the higher concentrations. However, the body length at every food level was low; between 4.60 and 5.54 mm (Table 5). The shrimps fed with *Spirulina* powder at all levels died before day 10.

Spirulina powder (mg ind. <sup>-1</sup> d <sup>-1</sup> )	Survival (individuals)	Body Length (mm)
0.2	$3.33\pm1.53^{\text{a}}$	$4.60\pm0.06^{a}$
0.4	$6.00\pm2.00^{a}$	$5.48\pm0.29^{\text{b}}$
0.6	$17.00 \pm 1.73^{\circ}$	$5.54\pm0.05^{\text{b}}$
0.8	$18.00\pm1.00^{ m b}$	$5.39\pm0.42^{\text{b}}$

*Table 5* Survival and growth (mean ± SD) of 1-day old *Branchinella thailandensis* nauplii (20 individuals) fed with different levels of *Spirulina* powder at day 5.

Means, within a column, sharing a common superscript are not significantly different (p > 0.05).

The survival and growth of the shrimps fed with various ratios of three food sources at day 5, 10 and 15 are shown in Table 6. The suitability of *Chlorella* sp. rations on growth and survival of the shrimps at day 15 were

between 50 and 70%. Most of the animals died before measuring at day 15. Only the shrimps fed with *Chlorella* sp. survived at day 15 at ~50% of the initial number with the same size of animals at day 10 (Table 6).

Table 6 Survival and growth (mean ± SD) of 1-day old Branchinella thailandensis nauplii (20 individuals) fed with different ratios of mixed feed with Chlorella sp. at least 50% at day 5, 10 and 15 (C = Chlorella sp., R = rice bran, S = Spirulina powder).

Food Ratio Day		5 Day 1		10 Da		y 15
(mg ind.⁻¹ d⁻¹)	Survival	Body Length	Survival	Body Length	Survival	Body Length
	(ind.)	(mm)	(ind.)	(mm)	(ind.)	(mm)
control	$14.75 \pm 1.67^{a}$	$16.43\pm0.71^{a}$	$8.50\pm2.38^{a}$	$18.91 \pm 0.50^{a}$	-	-
C:R = 85:15	$16.25\pm2.06^{ab}$	$15.81\pm0.32^{a}$	$11.00\pm2.94^{\text{a}}$	$18.19\pm0.99^{a}$	-	-
C:R = 70:30	$18.50\pm1.91^{\rm bc}$	$16.00\pm0.53^{\text{a}}$	$16.50 \pm 1.73^{ m b}$	$18.85\pm0.67^{a}$	-	-
C:R = 50:50	$18.25\pm1.71^{\rm bc}$	$16.15\pm0.29^{\text{a}}$	$16.50 \pm 1.73^{ m b}$	$18.80\pm0.18^{\text{a}}$	$10.25\pm2.63$	$18.75 \pm 0.35$
C:S = 85:15	$16.25 \pm 1.56^{ab}$	$16.75\pm0.39^{a}$	$10.50\pm1.29^{a}$	$18.28\pm0.23^{a}$	-	-
C:S = 70:30	$19.24\pm0.50^{\circ}$	$16.61\pm0.42^{a}$	$15.25 \pm 1.26^{\circ}$	$18.03\pm0.75^a$	-	-

Means, within a column, sharing a common superscript are not significantly different (p > 0.05).

When fed the shrimps with various ratios of these food sources, the survival at an equal ratio of rice bran and *Spirulina* powder (C: R: S = 4: 2: 2) was better than that at any other ratios except at day 5 where there was no significant (p>0.05) difference between the ratio of C: R: S = 4: 3: 1 (Table 7). At day 5, there was no significant (p>0.05) difference in the body length of the shrimps among the food ratios. While at day 10, the body length at the

ratio of C: R = 4: 4 was significantly (p<0.05) greater than at the ratio of C: R: S = 4: 3: 1 but no significant difference was noticed when compared with the ratio of C: R: S = 4: 2: 2. Most of the animals died before measuring at day 15. Only the shrimps fed with rice bran and *Spirulina* powder at an equal proportion survived at day 15 at ~30% of initial number (Table 7).

**Table 7** Survival and growth (mean ± SD) of 1-day old *Branchinella thailandensis* nauplii (20 individuals) fed with different ratios of rice bran and *Spirulina* powder in mixed feed at day 5, 10 and 15 (C = *Chlorella* sp., R = rice bran, S = *Spirulina* powder).

Food Ratio	Day 5		Day 10		Day 15	
	Survival	Body Length	Survival	Body Length	Survival	Body Length
	(110.)	()	(ma.)	()	(110.)	()
C:R = 4:4	$17.00\pm0.82^{a}$	$14.55\pm0.83^{\text{a}}$	$10.75\pm1.71^{a}$	$21.06\pm0.53^{ m b}$	0	-
C:R:S =4:3:1	$19.25 \pm 0.96^{\text{b}}$	$14.38\pm0.55^{a}$	$11.75\pm2.50^{\text{a}}$	$19.51\pm0.80^{a}$	0	-
C:R:S =4:2:2	$19.50\pm1.00^{\text{b}}$	$13.97\pm0.52^{\text{a}}$	$16.00\pm1.41^{\text{b}}$	$20.39\pm0.66^{ab}$	6.75 ± 2.50	$22.21 \pm 0.71$

Means, within a column, sharing a common superscript are not significantly different (p > 0.05).

## **Discussion**

The algal food concentrations affected the growth of B. thailandensis, but did not affect the survival. The best growth was obtained at the highest algal concentration except for that at day 15 where the highest food level  $(2 \times 10^{6} \text{ cells m}^{-1})$  did not differ from the growth at  $1 \times 10^{6}$ cells ml<sup>-1</sup>. However, these three levels of Chlorella sp. in the experiments seem to be adequate for the basic needs of the animals; only those fed at lower food densities showed slower growths. These concentrations were similar to those reported by De Walsche et al. (1991) who used Chlorella vulgaris at a concentration of 1 x  $10^5$  cells m<sup>-1</sup> fed semi-continuously to Streptocephalus proboscideus. They found that this diet was adequate for survival, growth and maturation, but not for high cyst production. At day 11-15, B. thailandensis required a lower or the same food concentration as at earlier days. Because their growth rate of this period was lower, which is in agreement with the findings of Hildrew (1985) and Mura et al. (2003) who reported the highest growth rate of fairy shrimps evident before sexual maturity.

The concentrations of rice bran affected both the survival and growth of *B. thailandensis*. The highest food concentration (2.4 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> mg dry weight ind.<sup>-1</sup> d<sup>-1</sup>) was too high for the shrimps at 1-5 days of age,

but suitable for those at day 6-10. The food level of 1.6 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> was found suitable for the shrimps at day 1-5 and 11-15. The animals fed with rice bran were normal in development, but they have pale bodies, lower survival and growth than those fed with the algal food. The reduced food requirement of the older animals confirms the previous experiments.

According to the results of the *Spirulina* powder experiment, to feed this powder as a mono-diet to the shrimps was not suitable for their survival and growth. At day 5, although the survival at the food concentration of 0.6 and 0.8 mg dry weight ind.<sup>-1</sup> d<sup>-1</sup> were as high as the shrimps fed with algae, their growth was only a half of that of the animals fed with rice bran. All of the animals died before day 10, as was reported by De Walsche *et al.* (1991) who found that *S. proboscideus* fed with *Spirulina* all died before the end of experiments. They also suggested that the deaths might be due to the toxicants of this food.

The preliminary study on mixed feed using the rice bran as a main source in the mixed feed showed that it was not suitable for growth of *B. thailandensis* when compared to the animals solely-fed with *Chlorella* sp. However 50% of the rice bran and the algae produced the best growth at day 5 and the second best growth at day 10 and 15. This is in contrast to the finding of Ali & Dumont (2002) who reported that *S. proboscideus* fed with rice bran performed similar results to animals fed with algae, a mixture of corn and pea bran (YM20) or palm oil mill effluent (POME).

The optimal ratio of the algae for the survival and growth of *B. thailandensis* in the mixed feed was at 50-70% of their optimal concentrations. A combination of the three food sources resulted in a better survival than on the two food sources (algae and rice bran). However the results of each experiment were quite variable from time to time, possibly they were caused by extraneous factors e.g. the quantity control of food in the culture container, cyst origin, and culture environment.

## Conclusions

At 6-10 days of age, *B. thailandensis* required a higher food quantity than those of at 1-5 and 11-15 days. These quantities were 2 x  $10^6$  cells *Chlorella* sp. ml<sup>-1</sup> and 2.4 mg rice bran dry weight ind.<sup>-1</sup> d<sup>-1</sup>. While at 1-5 and 6-10 days, the optimal food concentrations were 1 x  $10^6$  cells *Chlorella* sp. ml<sup>-1</sup>, 1.6 mg rice bran dry weight ind.<sup>-1</sup> d<sup>-1</sup> and 0.8 mg *Spirulina* powder dry weight ind.<sup>-1</sup> d<sup>-1</sup> (1-5 day). The results indicated that *Chlorella* sp. and rice bran alone are suitable for feeding the fairy shrimps as a monodiet.

The combination of *Chlorella* sp. at 50-70% of optimal concentrations with rice bran or/and *Spirulina* powder gave a higher survival than those any other ratios. In addition, a combination of the three food-source diets mostly showed a higher survival than those of the two sources.

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