Cold Preservation of Chironomid Larvulae (*Chironomus fuscipes* Yamamoto, 1990): Nutritional Value and Potential for Climbing Perch (*Anabas testudineaus* Bloch, 1792) Larval Nursing

Nithas Thipkonglars[1*], Wara Taparhudee[2], Methee Kaewnern[2] and Khamchai Lawonyawut[3]

ABSTRACT

The study on the efficacy of chironomid larvulae (*Chironomus fuscipes* Yamamoto, 1990) as live food for climbing perch (*Anabas testudineaus* Bloch, 1792) larvae compared with rotifer was conducted at the Inland Aquaculture Research Institute, Department of Fisheries, Phra Nakorn Si Ayutthaya province from October to December 2008. Three experiments were conducted. The first experiment determined the nutritive value of chironomid larvulae in terms of proximate composition, amino acid profiles, and fatty acid composition. Results showed that protein, fat, fiber, and ash contents in chironomid larvulae were 55.62, 4.57, 6.54 and 14.45%, respectively. Chironomid larvulae contained complete amino acid and fatty acid profiles. The second experiment determined the efficacy of protective media on the preservation of chironomid larvulae at 4°C. It revealed that embryonic solution showed the best result. The third experiment compared the efficacy of the preserved larvulae and rotifer as live food for rearing climbing perch larvae. Results showed that growth and survival of climbing perch larvae were not significantly different between the treatments ($P>0.05$).

Keywords: chironomid, preservation, cooling, nutritional value, climbing perch

INTRODUCTION

Chironomid larvae are normally found in freshwater environment. Chironomids play an important role in the nutrients recycling food chain of inland water ecosystems (Armitage *et al*., 1995). Moreover, they are valuable as live food for cultured fish and other aquatic organisms in ponds (Habib *et al*., 1992; Yusoff *et al*., 1996), and for ornamental fish aquaculture. They are excellent sources of protein (De la Noue and Choubert, 1985), lipid, vitamins and minerals (McLarney *et al*., 1974). The demand for their products is increasing in aquaculture (Ametage *et al*., 1995). Chironomid larvae are mainly collected from natural habitats, thus their quality could be affected by heavy metal contamination, infectious diseases and parasites. Many

[1]Tak Provincial Fisheries Office, Department of Fisheries, Muang, Tak, Thailand 63000
[2]Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok, Thailand 10903
[3]CJ CheilJedang Corporation, Wattana, Bangkok, Thailand 10110
*Corresponding author, email: nithast@hotmail.com

researchers have tried to increase their production using different culture media such as chicken manure, horse manure, fish meal, ground pellets or other media in the laboratory (McLachlan, 1977) and outdoor culture systems (McLarney *et al*., 1974; Shaw and Mark, 1980; Habib *et al*., 1992). However, the main problem is the inability to achieve mass production due to variations in chironomid species composition. Collection of chironomid egg mass from natural sources and adding into the culture system may improve its production. However, environmental variations also affect abundance of chironomid egg mass. Therefore, the culture of chironomids under captivity could be a way to produce their egg masses (Thipkonglars *et al*., 2008). After hatching, chironomid larvulae are free living (Cranston, 2004), and some of their characteristics i.e. body size, continuous movement, and dispersed by drifting in water bodies including bottom, may also be appropriate for the first feeding of some freshwater fish species.

The nursing of fish larvae under controlled hatchery conditions requires not only the development of specific culture techniques, but also the method to produce live food for the developing larvae. The natural diet of most cultured fish species consists of a wide diversity of phytoplankton (diatoms, flagellates) and zooplankton (ciliates, cladocerans, copepods, decapod larvae, rotifers). Normally, freshwater fish hatchery operations have a more limited choice of live food organisms, compared with a seawater fish hatchery. Some species of freshwater fish such as climbing perch, *Anabas testudineaus* (Bloch, 1792) and marble goby, *Oxyeleotris marmorata* (Bleeker, 1852) require very small live food at the first stage of their lives. Currently, rotifers are the only live food organisms for freshwater fish larvae. Dhert *et al*. (2001) noted that the production of rotifers for fish larvae nursing faces frequent problems such as: (1) unpredictability of rotifer mass production, (2) difficulty to manage and harvest large rotifer populations, and (3) difficulty of producing a rotifer which is free of floccules and safe, from a microbial point of view. Thus, the development of alternative live food organisms and simple cold preservation are necessary to provide more choices for freshwater fish larvae rearing.

The objectives of this study were to investigate the nutritional value of the chironomid larvulae, (including amino acid profiles and fatty acid compositions), find the optimal protective medium for chironomid larvulae cold preservation, and compare its efficacy as a live food for rearing of climbing perch larvae with that of rotifers.

MATERIALS AND METHODS

Preparation of Live Feed

Chironomid larvulae *Chironomus fuscipes* (Yamamoto, 1990) used in nutritional value analysis and as live feed were prepared by collecting newly hatched larvae from chironomids reared under captivity. After the adult chironomid females oviposited, their egg masses were collected and hatched in 500 ml beakers with aeration. After hatching, aeration was stopped to allow the newly hatched larvae to separate from the egg shells, then the egg shells were removed. Freshwater rotifer, *Brachionus calyciflorus*

(Pallas) and water flea, *Moina macrocopa* (Straus) used in this study were produced from live feed production units of the Inland Aquaculture Research Institute (IARI), Department of Fisheries, Phra Nakhon Si Ayutthaya, Thailand.

Nutritional Value Analysis

Collected samples were stored at -30°C prior to analysis, which included determination of moisture (AOAC, 2005; method no. 930.15), ash (AOAC, 2000; method no. 942.05), protein (AOAC, 2005; method no. 981.10), fat (AOAC, 2005; method no. 954.02) and fiber (DMSc and ACFS, 2003). The complete amino acid profile of chironomid larvulae on wet weight (mg 100 g^{-1}) were determined according to AOAC (2005). In addition, the fatty acid composition was analyzed following AOAC (2000) method no. 996.06. Analytical data on nutritional values of rotifer and water flea were based on the report of Poompoung (2004) who collected samples from the same production source.

A Comparison of Using Protective Media for Cold Preservation

This experiment was designed using Completely Randomized Design (CRD) with 6 treatments and 3 replications. Variant treatments of protective media were as follows: distilled water (control), 0.9% NaCl, Modified Fish Ringer's solution, embryo solution, 2% glucose solution, and 1% trehalose solution. One hundred chironomid larvulae were stocked in 50 ml volumetric flash containing 10 ml of each of the experimental media. Antibiotics (penicillin and streptomycin) were added in all experimental units at 50 IU and 0.1 mg ml^{-1}, respectively, and cooled down in a refrigerator at 4°C. The survival rate of chironomid larvulae was determined after preservation at 24, 48, 72, 96 and 120 hrs. The chironomid larvulae were considered as surviving if they could repeatedly contract their abdominal muscles. Survival rate data of chironomid larvulae were analyzed by one-way ANOVA. The data was transformed for normalization of variance by arcsine transformation prior to analysis, and Duncan's Multiple Range Test (DMRT) was used to determine which means are different at the significant level of 0.05.

Preparation of Fish Samples

Climbing perch *Anabas testudineaus* larvae (Bloch, 1792) were obtained from a commercial hatchery. Active, newly hatched larvae were collected immediately after hatching and transferred into 40 L plastic tanks with aeration. Temperature was kept constant at 26°C (range ±0.5°C) and under a natural photoperiod. After the majority of the larvae had absorbed their yolk sac at 3 days post-hatch, boiled chicken egg yolk was fed four times a day for the next 2 days. At the end of the preparatory period, the 5-day-old climbing perch reached a total length (TL) and wet body weight (BW) of 4.63 mm and 0.0004735 g, respectively.

Experiment on Climbing Perch Larvae Rearing

The experiment was designed in a CRD with 2 treatments and 6 replications. The treatments were: (1) feeding climbing perch larvae with rotifers from 5 to 12 days of age, and (2) feeding climbing perch larvae with chironomid larvulae from 5 to 12 days of age. Both treatments were fed *ad libitum*,

Table 1. Composition of various protective media used in the experiment on chironomid larvulae (*Chironomus fuscipes*) cold preservation

protective media	Composition (g l^{-1})								
	NaCl	KCl	$NaHCO_3$	$CaCl_2$	$MgCl_2$	1 g l^{-1} $MgSO_4$	glucose	trehalose	pH
0.9% NaCl	9.000	-	-	-	-	-	-	-	7.0
Modified Fish Ringer's solution	6.500	3.000	0.200	0.300	-	-	-	-	7.5-7.8
Embryo solution	0.584	0.010	-	0.118	0.204	0.4 ml	-	-	7.0
Glucose solution	-	-	-	-	-	-	20.000	-	7.0-7.5
Trehalose solution	-	-	-	-	-	-	-	20.000	7.0-7.5

Source: Pewnane (n.d.)

and afterwards water flea was given continuously to all treatments from 10-29 days of age. Experimental units were set up in rectangular glass aquaria (20x25x30 cm) containing 10 liters of water with aeration under natural light conditions. Each aquarium contained 200 fish larvae (20 fish $liter^{-1}$). The water exchange rate was 30% of total volume day^{-1} on days 1-7, and 50% of total volume day^{-1} on days 8-24. Sampling days were fixed at 6, 12, 18 and 24 days after rearing. During sampling, 40 larvae were removed from the aquaria and total length (TL) and weight (WT) were measured to estimate growth rate. Growth was assessed by calculating specific growth rate (SGR). Dead larvae were removed and counted daily to determine rate of survival. During the experiment, water quality parameters such as temperature, dissolved oxygen, pH, alkalinity, hardness, and total ammonia were determined using the methods of American Public Health Association (1980) and The Chemical Analysis of Freshwater (Stainton *et al*., 1977) on days 0, 12, and 24. Data comparisons between groups were performed using Student's T-test with an overall significance level of 0.05. Percentage data was arcsine transformation before statistical analysis.

RESULTS

Nutritional Value of Chironomid Larvulae

The chironomid larvulae *Chironomus fuscipe* has a nutrient proximate composition as follows: moisture 88.34% (wet weight), protein 55.62%, fat 4.57%, fiber 6.54% and ash 14.45% by dry weight (Table 2). The amino acid profiles of chironomid larvulae showed they have both essential and non-essential amino acids (Table 3). The fatty acid profile includes both unsaturated and saturated fats (Table 4). Results showed that the level of protein and fiber contents in chironomid larvulae were lower than those of rotifer and water flea. Furthermore,

chironomid larvulae had fat higher than water flea but lower than rotifer, and also had higher ash content than rotifer and water flea. Chironomid larvulae had methionine content higher than rotifer and water flea but lower in threonine. In addition, chironomid larvulae had linoleic acid content higher than rotifer and water flea but lower in linolenic acid.

Table 2. Proximate composition (g $100g^{-1}$) of chironomid larvulae (*Chironomus fuscipes*) compared with rotifer (*Branchionus calyciflorus*) and water flea (*Moina macrocopa*)

Sample	Proximate composition					Sample condition
	Moisture	Protein	Fat	Fiber	Ash	
Chironomid	88.34					Wet sample
larvulae	11.31	55.62	4.57	6.54	14.45	Dry sample
Rotifer[a]	92.04					Wet sample
	3.65	68.08	4.94	13.63	6.45	Dry sample
Water flea[a]	89.96					Wet sample
	8.51	64.68	2.97	6.83	9.03	Dry sample

Source: [a]Poompoung (2004)

Table 3. Amino acid profiles (mg $100g^{-1}$) in chironomid larvulae (*Chironomus fuscipes*), rotifer (*Branchionus calyciflorus*) and water flea (*Moina macrocopa*)

Amino acid	Chironomid Larvulae	Rotifer[a]	Water flea[a]
Essential amino acid			
Arginine	1,905.89	2,684.36	3,125.47
Histidine	1,503.48	1,846.43	1,623.22
Isoleucine	1,188.89	1,255.95	1,446.51
Leucine	2,683.38	3,387.99	3,508.02
Lysine	3,053.72	3,515.10	3,166.77
Methionine	1,201.26	60.46	561.68
Phenylalanine	2,122.71	2,193.65	2,616.22
Threonine	817.97	2,210.68	2,411.62
Tryptophan	490.24	418.62	609.01
Valine	1,310.53	1,807.28	2,200.52
Non-essential amino acid			
Alanine	3,099.81	2,435.10	3,615.20
Aspartic acid	3,510.53	5,287.46	4,833.76
Cystine	824.54	426.24	37.87
Glutamic acid	5,183.67	6,767.47	6,171.57
Glycine	1,891.88	1,977.47	2,461.06
Proline	1,621.35	2,376.50	2,232.45
Serine	766.57	2,681.70	2,758.42
Tyrosine	1,106.57	2,585.90	2,324.09

Source: [a]Poompoung (2004)

Table 4. Fatty acid composition (g $100g^{-1}$) in chironomid larvulae (*Chironomus fuscipes*), rotifer (*Branchionus calyciflorus*) and Water flea (*Moina macrocopa*)

Fatty acid	Chironomid Larvulae	Rotifer[a]	Water flea[a]
Lauric acid (C12:0)	0.10	0.07	0.05
Myristic acid (C14:0)	0.19	0.42	-
Myristoleic acid (C14:1)	-	0.17	0.08
Pentadocanoic acid (C15:0)	-	0.07	0.04
Palmitic acid (C16:0)	0.67	1.74	1.08
Palmitoleic acid (C16:1 n-7)	0.19	0.61	0.22
Heptadecanoic (C17:0)	0.10	0.04	0.06
Cis-10 Heptadocanoic acid (C17:1)	-	0.03	0.04
Stearic acid (C18:0)	0.48	0.52	0.33
Oleic acid (C18:1 n-9)	-	1.65	1.29
Cis-Vacconic acid (C18:1 n-7)	-	0.23	0.30
cis-9,12-Linoleic acid (C18:2 n-6)	1.16	0.68	0.86
a-Linolenic acid (C18:3 n-3)	0.10	0.65	1.16
Arachidic acid (C20:0)	0.10	0.05	0.03
Arachidonic acid (C20:4 n-6)	0.10	0.04	0.05
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5 n-3)	0.29	0.11	0.04
Docosahexaenoic acid (DHA)	-	0.14	-

Source: [a]Poompoung (2004)

Cold Preservation of Chironomid Larvulae Using Protective Media

Results showed that survival rates of chironomid larvulae which were cold preserved at 4°C in different protective media were significantly different ($p<0.01$). Using embryonic solution gave the greatest result allowing the larvae to be preserved for 96 hrs with 10.33% survival rate, followed by trehalose at 1.67%. Cooling could keep them for 72 hrs while NaCl, Modified Fish Ringer Solution and glucose could not be used to preserve the larvulae (Table 5).

Table 5. Average survival rates of chironomid larvulae at various retention times (hr) after cold preservation method with different protective media

Protective media	Retention time (hr)					
	0	24	48	72	96	120
Control	100	99.33±1.15[a]	81.67±7.51[a]	0[c]	0[c]	0
0.9% Nacl	100	74.33±6.51[b]	0[b]	0[c]	0[c]	0
Modified Fish Ringer Solution	100	0.33±0.58[c]	0[b]	0[c]	0[c]	0
Embryonic Solution	100	98.67±1.53[a]	81.67±7.64[a]	32.67±11.02[a]	10.33±4.51[a]	0
2% Glucose	100	00.67±0.58[c]	0[b]	0[c]	0[c]	0
1% Trehalose	100	99.33±1.15[a]	87.33±4.16[a]	21.67±7.64[b]	1.67±0.58[b]	0

Remark: Average values with different letters in the same column are statistically significantly different ($P<0.01$)

Comparison between Chironomid Larvulae and Rotifer in Climbing Perch Larval Rearing

Results showed that after rearing climbing perch larvae with 2 live food species (T1= fed with rotifers from 5-12 days of age, T2= fed with chironomid larvulae from 5-12 days of age) for 24 days, the total length (TL), weight (WT), and specific growth rate (SGR) of climbing perch larvae were not significantly different between treatments (P>0.05) (Fig. 1-3).

The average survival rates on the final stage (days 24) were 47.53±5.59% for T1 and 49.28±3.55% for T2, respectively (Fig. 4), which were not significantly different (P>0.05).

Average water quality parameters in each treatment during the rearing period are shown in Table 6. The data show that all parameters were within the suitable range for aquaculture.

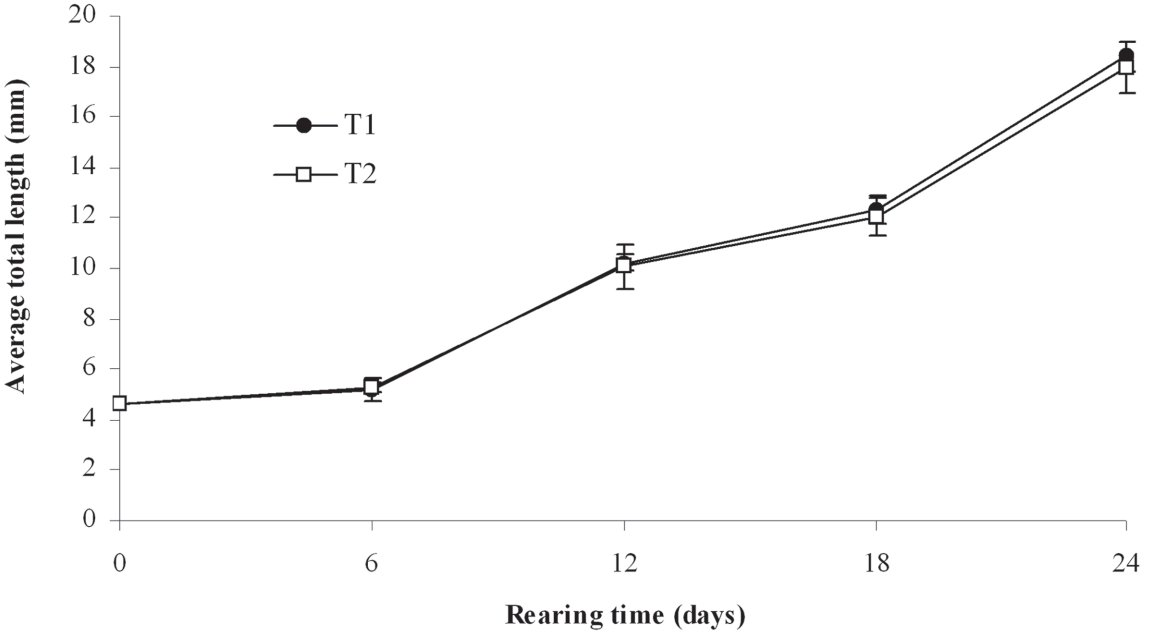

Figure 1. Average total length of climbing perch larvae reared with 2 live food types (T1= fed with rotifers from 5-12 days of age, T2= fed with chironomid larvulae from 5-12 days of age) for 24 days

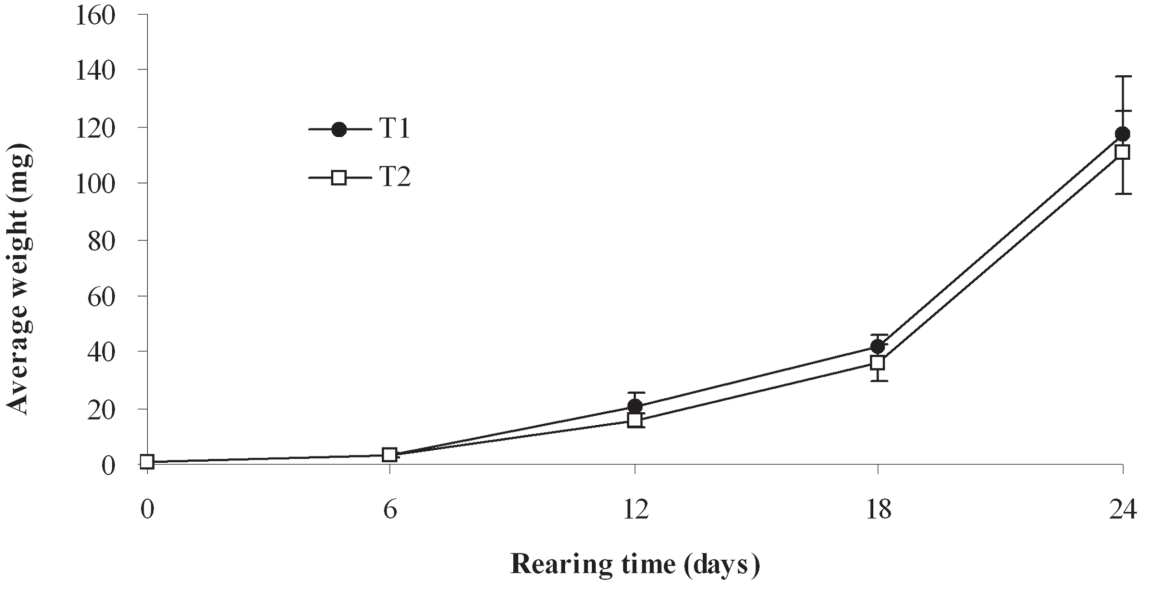

Figure 2. Average weight of climbing perch larvae reared with 2 live food types (T1= fed with rotifers from 5-12 days of age, T2= fed with chironomid larvulae from 5-12 days of age) for 24 days

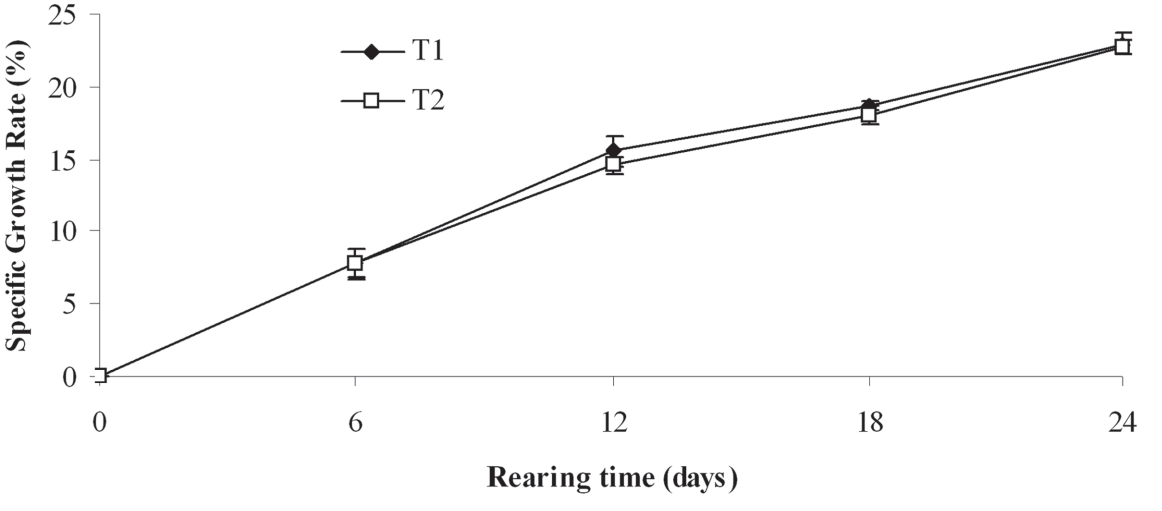

Figure 3. Average specific growth rate of climbing perch larvae reared with 2 live food types (T1= fed with rotifers from 5-12 days of age, T2= fed with chironomid larvulae from 5-12 days of age) for 24 days

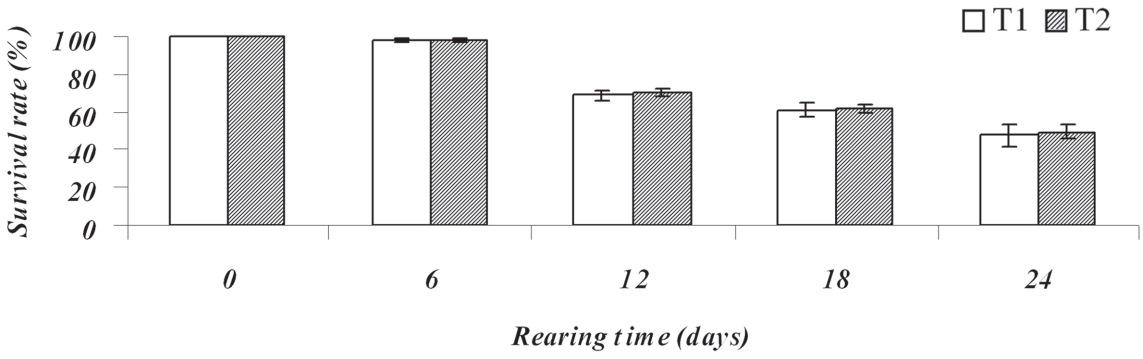

Figure 4. Survival rate of climbing perch larvae reared with 2 live food types (T1= fed with rotifers from 5-12 days of age, T2= fed with chironomid larvulae from 5-12 days of age) for 24 days

Table 6. Water quality conditions during climbing perch larval rearing with 2 treatments (T1= fed with rotifers from 5-12 days of age, T2= fed with chironomid larvulae from 5-12 days of age) at various rearing days

Rearing time	Treatment	Parameter					
		Temperature (^{0}C)	DO (mg l^{-1})	pH	Alkalinity (mg l^{-1})	Hardness (mg l^{-1})	NH_3 (mg l^{-1})
Day 0	T1	27.8±0.1	6.4±0.2	7.2±0.2	114.3±3.4	362.1±8.4	0.22±0.07
	T2	27.7±0.1	6.7±0.6	7.3±0.3	116.5±2.6	361.4±3.2	0.18±0.04
Day 12	T1	23.0±0.1	5.6±0.5	7.1±0.3	106.8±4.7	350.7±10.3	0.34±0.12
	T2	23.2±0.2	5.9±0.3	7.0±0.2	110.4±8.2	358.9±7.6	0.42±0.16
Day 24	T1	25.9±0.1	5.4±0.8	6.9±0.2	108.3±5.3	346.7±8.4	0.53±0.17
	T2	26.0±0.1	5.1±0.8	7.1±0.3	103.2±3.5	352.7±4.8	0.43±0.24

DISCUSSION

The proximate analysis of chironomid larvulae *Chironomus fuscipes* in this study were compared to a report by Habib *et al*. (1997) who studied the nutritional values of fourteen species of chironomids in Malaysia. This present study found higher protein and ash contents, but fat was lower. In addition, chironomid protein was higher than those reported by Chaiyachet (2003), who cultured pool chironomids in Khon Kaen province, but fat and ash contents were lower. This was probably due to different chironomid species, quality and quantity of food consumed (Armitage *et al*. 1995). However, different fatty acid amounts were found in chironomid larvulae, rotifers and water flea. Kiyashko *et al*. (2004) noted that the amount and composition of fatty acids in aquatic animals depended on dietary sources and internal enzymes.

This study showed that the highest survival rate was obtained when embryonic solution was used as a protective media in the chironomid larvulae preservation by cooling, followed by trehalose. They were better than using NaCl, Modified fish ringer solution and glucose. Mongkolpunya (1993) noted that internal protective media could perform better than external protective media. Embryonic solution is an internal protective medium, while the others act as external protective media. The protective media's ability to pass through membranes depended on the molecular weight, shape, and electrochemistry of the molecules (Muir, 2007). Protective media has been proposed to function as a water replacement molecule to stabilize the structure of macromolecules, and depress the freezing point of cells (Crowe *et al.*, 1998). Trehalose exerts its protective effect by stabilizing membrane, probably by replacing water molecules among the phospholipids. From the preservation of *Artemia*, Leger *et al.* (1983) reported that no mortality occurred when cooling newly hatched larvae at 4°C for 24 hours. Anbala Almalul (2000) also reported that mortality was less than 20% when cooling newly hatched *Artemia* larvae for 48 hours. These reports showed similar results to chironomid larvulae preservation. Low temperature can slow metabolism and prevent weight and energy losses.

The study on the potential of chironomid larvulae as live food for rearing of climbing perch larvae show that there is no significant difference in growth (length, weight and SGR) of climbing perch larvae reared in either chironomid and rotifer. The main reason could be that the nutritional values of chironomid larvulae, rotifers and water flea in terms of essential amino acids were similar. Even though rotifers had higher protein content than chironomid larvulae, it exceeds the protein requirement that fish needs for energy (Chantararothai, 1993). In comparison to Doolgindachbaporn (1994) results, there was no significant difference in survival and growth after rearing climbing perch larvae with feed containing protein ranging from 26.74-45.34% for 45 days. Therefore, the protein concentration in chironomid larvulae (55.62%) may be enough for climbing perch larvae requirements. In this study, survival rates of climbing perch larvae were not significantly different between treatments ($P>0.05$). From the results, it showed that fish larvae could also eat chironomid larvulae as they would eat rotifers. Even if chironomid larvulae had bigger body sizes (273.3-614.8 µm) than rotifers (59-200 µm), no ingestion problems were observed. Based on feed adjustment in Doolgindachbaporn (1994), fish larvae could accept all live food in the experiments. Even the survival rates from each treatment showed no significant difference. Water quality parameters in the experiment changed overtime. Since the experiment was conducted in the cool season (November-December, 2008), water temperatures decreased to 23.0-23.2°C in a few days. However, this temperature range did not affect growth of fish larvae. Froese and Pauly (2009) suggested that optimal water temperature for climbing perch should be 22.0-30.0°C. Total ammonia during climbing perch larval rearing increased gradually; nevertheless the total ammonia did not exceed 0.5 mg l^{-1} which was within the range for freshwater fish (Department of Environment Quality Promotion, 2009). However, increased total ammonia might

happen from excretion and decomposition of food scraps. Other water quality parameters (i.e. dissolved oxygen, pH, alkalinity and hardness) in both treatments were not different, and were also within the safe range for aquaculture.

CONCLUSIONS

The results indicate that chironomid larvulae has potential as an appropriate live food for climbing perch larvae older than 5 days. Survival and growth rates of climbing perch larvae were not significantly different from those fed with rotifers. In addition, chironomid larvulae could be cold preserved for future use by using embryonic solution as a preservative media.

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