ORIGINAL ARTICLE

Some aspects in early life stage of climbing perch, *Anabas testudineus* larvae

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Abstract

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The sexual maturity of female climbing perch, *Anabas testudineus* was studied by determining fecundity and gonadosomatic index (GSI). It was found that the size at sexual maturity of female climbing perch was 15.20±1.24 cm (mean±SD) in total length and 61.10±17.32 g in body weight. The eggs were floating and rounded. The fertilized eggs had a diameter of 830±39 μm. The fecundity was 24,120.5±3,328.24 ova/fish and gonadosomatic index (GSI) was 10.4±2.5%.

Newly hatched larvae of climbing perch were produced by induced spawning using chemical injection (Suprefact and Motilium). The sexually mature fishes were cultured in fiber-glass tank (water volume 300 liters) with the ratio of male and female brooders 2:1. The fertilization rate, hatching out and hatching rate experiments were carried out using a 15-liter glass aquarium (water volume 10 liters) containing 7,000-9,000 eggs. It was found that the eggs were floating and rounded. The fertilized eggs had a diameter of 830±39 μm. The average fertilization rate was 92.67%, hatching out was 20 hr 30 min and average hatching rate was 87.44% at a water temperature of 27.0-30.5°C. Sampling of the newly-hatched larvae was done at 2-hour intervals, when 20 of them were randomly taken and preserved in 10% buffered formalin for later deter-

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mination of yolk absorption time. Observation using a microscope revealed that newly hatched larvae were 2.02±0.20 mm in total length and had yolk sacs of 111.33±46.19 mm^3 in volume. The yolk sacs were completely absorbed within 92 hr after hatching at a water temperature of 27.0-30.5ºC. Up until full mouth development (start of feeding), 2-hourly samplings of twenty newly hatched larvae were taken from an aquarium for observation of the size of mouth opening. All the larvae had open mouths about 28 hr after hatching (2.95±0.59 mm TL), with the mouths measuring 328.42±32.23 mm in height.

The feeding experiments were carried out using a 15-liter glass aquarium (water volume 10 liters) containing 1,000 larvae aged 1 days post-hatching (just before the mouth opened). They were fed with rotifer at a density of 10 ind/ml. Twenty larvae were collected at random from the aquarium at 2-hourly intervals, preserved in 10% buffered formalin, and then dissected to determine the presence of rotifer in the digestive tract. The digestive tracts were fixed at 32 hr of hatching at water temperatures of 27.0-30.5ºC, and measured 477.63±47.80 mm in mouth height. The average number of rotifer in the digestive tract at the start of feeding was 1.50 individual/larva.

A starvation experiment was carried out using a 15-liter glass aquarium (water volume 10 liters) with three replications. Two hundred newly hatched larvae were kept without feeding. Larvae started to die at 216 hr and totally died within 348 hr after hatching at water temperature ranging between 27.0 and 30.5ºC.

The feeding scheme experiments were done in a 15-liter glass aquarium (water volume 10 liters) containing 500 of two-day old larvae (stage at first feeding). It was found that larval climbing perch aged 3-10 days (average total length 3.02-4.97 mm) consumed rotifer. The larvae of age 8-15 days (average total length 3.94-12.60 mm) consumed *Moina*. The larvae of age 8-10 days (average total length 3.94-4.97 mm) consumed both rotifer and *Moina*. The larvae of age 11-day (average total length 5.51 mm) consumed only *Moina*. The larvae of age 14-15 days (average total length 7.34-12.60 mm) consumed both *Moina* and artificial feed. Larvae aged more than 16 days consumed only artificial feed.

Determining the daily food uptake by the larvae and juveniles was done in a 15-liter glass aquarium (water volume 10 liters) containing 100 larvae. The larvae consumed the living food organism i.e. rotifer or *Moina* depending on larval stage, with density of rotifer 100 individual/ml or with density of *Moina* 10 individual/ml. The amount of food intake was calculated based on changes of food density in the aquarium with and without fish larvae at 2-hour intervals. It was found that the larvae of age 3-6 days (average total length 3.02-3.71 mm) consumed only rotifer. The average uptake of rotifer in digestive tract per day of larvae age 3 and 6 days old were 9 and 16 individual/larva, respectively. The 9-days old larvae (average total length 4.43 mm) consumed both rotifer and *Moina*, the average uptake of rotifer in digestive tract in a day was 19 individual/larva and that of *Moina* was 10 individual/larva. The larvae of age 12-15 days (average total length 6.11-12.60 mm) consumed only *Moina*. The average uptake of *Moina* in digestive tract per day of larvae age 12 and 15 days old were 98 and 113 individual/larva, respectively.

Key words : fecundity, yolk absorption, mouth development, start of feeding, starvation, feeding scheme, daily food uptake, larviculture, climbing perch, *Anabas testudineus*

บทคัดย่อ

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ลักษณะบางประการในระยะวัยอ่อนของปลาหมอไทย

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ทำการศึกษาปริมาณความดกของไข่ และความสัมพันธ์ระหว่างน้ำหนักตัวกับอวัยวะสืบพันธุ์ (Gonadosomatic index, GSI) ของปลาหมอไทยที่สมบูรณ์เพศ พบว่าปลาหมอไทยที่สมบูรณ์เพศพร้อมที่จะขยายพันธุ์ มีความยาวลำตัวทั้งสิ้นเฉลี่ย 15.2±1.24 ซม. และน้ำหนักลำตัวเฉลี่ย 61.1±17.32 กรัม ไข่ของปลาหมอไทยเป็นประเภทไข่ลอย ลักษณะกลม มีปริมาณความดกของไข่เฉลี่ย 24,120.5±3,328.24 ฟอง ความสัมพันธ์ระหว่างน้ำหนักตัวกับอวัยวะสืบพันธุ์เท่ากับ 10.4±2.5%

ทำการเพาะขยายพันธุ์ปลาด้วยวิธีการผสมเทียมโดยการฉีดสารเคมี ได้แก่ Suprefact ร่วมกับ Motilium เป็นการกระตุ้นให้ไข่พัฒนาเร็วขึ้นและมีการตกไข่ ปล่อยพ่อแม่พันธุ์ปลาในถังไฟเบอร์กลาส (ปริมาตรน้ำ 300 ลิตร) อัตราส่วนระหว่างตัวผู้กับตัวเมีย 2:1 พบว่าไข่ที่ได้รับการผสมกับน้ำเชื้อ มีเส้นผ่าศูนย์กลางเฉลี่ยเท่ากับ 830±39 μm อัตราการปฏิสนธิของไข่เฉลี่ย 92.67% ระยะเวลาในการฟักไข่ปลา ประมาณ 20 ชั่วโมง 30 นาที และมีอัตราการฟักเฉลี่ย 87.44% ที่อุณหภูมิของน้ำ 27.0-30.5ºC สุ่มลูกปลาที่ฟักออกมาใหม่ จำนวน 20 ตัว ทุก ๆ 2 ชั่วโมง เก็บดองในฟอร์มาลีนบัฟเฟอร์ 10% เพื่อใช้ทำการศึกษาการยุบตัวของไข่แดงโดยใช้กล้องจุลทรรศน์ พบว่าลูกปลาที่ฟักออกมาใหม่มีความยาวลำตัวทั้งสิ้น 2.02±0.20 มม. ปริมาตรของไข่แดงประมาณ 111.33±46.19 μm^3 ไข่แดงยุบตัวอย่างสมบูรณ์ประมาณ 92 ชั่วโมงหลังจากฟักออกเป็นตัวที่อุณหภูมิของน้ำ 27.0-30.5ºC ศึกษาการพัฒนาของปาก โดยสุ่มลูกปลาจำนวน 20 ตัว จากตู้กระจกที่ใช้สำหรับฟักไข่ทุก ๆ 2 ชั่วโมง พบว่าที่ 28 ชั่วโมงหลังจากฟักออกเป็นตัว (2.95±0.59 มม, TL) ปากของลูกปลาเริ่มเปิด วัดความสูงของปากได้ 328.42±32.23 μm

ศึกษาการเริ่มกินอาหารของลูกปลาหมอไทยโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) ใส่ลูกปลาอายุ 1 วันหลังจากฟักออกเป็นตัว (ระยะก่อนที่ปากจะเปิด) จำนวนตู้ละ 1,000 ตัว โดยให้ลูกปลากินโรติเฟอร์เป็นอาหาร ในอัตราความหนาแน่น 10 ตัว/มล สุ่มลูกปลาจำนวน 20 ตัว จากตู้ปลาที่ใช้ทำการศึกษาทุก ๆ 2 ชั่วโมง เก็บดองในฟอร์มาลีนบัฟเฟอร์ 10% พบว่าที่ 32 ชั่วโมงหลังจากฟักออกเป็นตัวที่อุณหภูมิ 27.0-30.5ºC ความสูงของปาก 477.63±47.80 μm ในระบบทางเดินอาหารปรากฏโรติเฟอร์ ประมาณเฉลี่ย 1.50 ตัว/ลูกปลา ซึ่งหมายความถึงการเริ่มกินอาหารของลูกปลา

ศึกษาการอดอาหารจนตายในลูกปลาหมอไทยโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) จำนวน 3 ซ้ำ ใส่ลูกปลาที่ฟักใหม่ จำนวนตู้ละ 200 ตัว เลี้ยงโดยไม่ให้อาหาร พบว่าลูกปลาหมอไทยเริ่มตายที่ 216 ชั่วโมงหลังจากฟักออกเป็นตัว และตายหมดที่ 348 ชั่วโมง ที่อุณหภูมิ 27.0-30.5ºC

ศึกษาประเภทของอาหารในแต่ละระยะของการเจริญเติบโตโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) ใส่ลูกปลาอายุ 2 วันหลังจากที่ฟักออกเป็นตัว (เริ่มกินอาหาร) จำนวนตู้ละ 500 ตัว พบว่าลูกปลาหมอไทยอายุ 3-10 วัน (ความยาวลำตัวเฉลี่ย 3.02-4.97 มม.) กินโรติเฟอร์เป็นอาหาร ลูกปลาอายุ 8-15 วัน (ความยาวลำตัวเฉลี่ย 3.94-12.60 มม.) กินไรแดงเป็นอาหาร ขณะที่ลูกปลาอายุ 8-10 วัน (ความยาวลำตัวเฉลี่ย 3.94-4.97 มม.) กินทั้งโรติเฟอร์และไรแดง ลูกปลาอายุ 11 วัน (ความยาวลำตัวเฉลี่ย 5.51 มม.) กินไรแดงเพียงอย่างเดียว ขณะที่ลูกปลาอายุ 14-15 วัน (ความยาวลำตัวเฉลี่ย 7.34-12.60 มม.) กินทั้งไรแดงและอาหารสำเร็จรูป และเมื่อลูกปลาอายุ 16 วันขึ้นไปจะกินอาหารสำเร็จรูปเพียงอย่างเดียว

ศึกษาปริมาณอาหารที่ลูกปลาต้องการทั้งวันในแต่ละระยะของการเจริญเติบโตโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) ใส่ลูกปลา จำนวนตู้ละ 100 ตัว ให้กินอาหารโดยใช้โรติเฟอร์หรือไรแดง ขึ้นอยู่กับระยะของลูกปลาในปริมาณความหนาแน่นโรติเฟอร์ 100 ตัว/มล. หรือไรแดงในปริมาณความหนาแน่น 10 ตัว/มล. ปริมาณอาหารที่ลูกปลากินคำนวณได้จากปริมาณความหนาแน่นของโรติเฟอร์หรือไรแดง ในตู้ปลาที่เปลี่ยนแปลงไปทุก ๆ 2 ชั่วโมง สุ่มนับจำนวนความหนาแน่นของโรติเฟอร์หรือไรแดง พบว่าลูกปลาอายุ 3-6 วัน (ความยาวลำตัวเฉลี่ย 3.02-3.71 มม.) กินโรติเฟอร์เป็นอาหาร ค่าเฉลี่ยจำนวนโรติเฟอร์ที่ลูกปลาอายุ 3 และ 6 วัน กินทั้งวัน ได้แก่ 9 และ 16 ตัว/ลูกปลา ตามลำดับ ลูกปลาอายุ 9 วัน (ความยาวลำตัวเฉลี่ย 4.43 มม.) จะกินโรติเฟอร์และไรแดงเป็นอาหาร ค่าเฉลี่ยจำนวนโรติเฟอร์ที่ลูกปลาอายุ 9 วัน กินทั้งวัน ได้แก่ 19 ตัว/ลูกปลา ค่าเฉลี่ยจำนวนไรแดงที่ลูกปลาอายุ 9 วัน กินทั้งวัน ได้แก่ 10 ตัว/ลูกปลา ตามลำดับ และลูกปลาอายุ 12-15 วัน (ความยาวลำตัวเฉลี่ย 6.11-12.60 มม.) จะกินไรแดงเป็นอาหาร ค่าเฉลี่ยจำนวนไรแดงที่ลูกปลาอายุ 12 และ 15 วัน กินทั้งวัน ได้แก่ 98 และ 113 ตัว/ลูกปลา ตามลำดับ

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Information on the early life history of fish such as yolk absorption, mouth and digestive tract development and starvation of the larvae is needed for optimization of large-scale culture and ultimately for the management of the fish stocks. It is recognized that the critical period of larval rearing begins at the time yolk absorption is completed. If some larvae do not begin to eat during that period, then they become weak and eventually die (Kosutaruk and Watanabe, 1984; Holm, 1986; Eda *et al*., 1994; Amornsakun and Hassan, 1996; Amornsakun *et al*., 1997; Amornsakun, 1999b; Amornsakun *et al*., 2002; Amornsakun *et al*., 2004). Survival of fish larvae is determined by the interplay of various environmental factors, such as temperature, food supply with a suite of species-specific characteristics, egg and larval size, yolk and oil quantity and resorption rates, and time of onset of feeding and feeding behaviour. Larvae can use a varying part of their yolk sac energy content for various activities (Blaxter, 1974; May, 1974).

Mouth size development is very important in the first feeding of larvae to match appropriate prey size. Mouth size at first feeding stage of various larval fish to encounter their prey size has been well documented for a number of cultured fish (Shirota, 1970; Nash *et al*., 1974; Fukuhara, 1986; Doi and Singhagraiwan, 1993; Eda *et al*., 1994).

The growth and survival rate of larval and juvenile fish are determined by various environmental factors, food supply etc. Mass mortality of larval and juvenile fish might occur if the food supply is inadequate (Houde, 1978). The food supply during larval stage is an important factor to achieve high growth and survival rates. The sequence of food in early larval stages differ among species. Freshwater fish are generally given rotifer, *Artemia* or *Moina* as an initial food. At the later stage the larvae or juveniles are fed minced fish, shellfish and shrimp or an artificial pellet. It is obvious that fish of different species require different feeding techniques.

To date, no research on climbing perch regarding the yolk absorption, mouth development in relation to feeding on rotifer, starvation and feeding behaviour in the larval and juvenile has been undertaken.

The purpose of this study was to investigate the period of yolk absorption, the onset of first feeding, mouth development, starvation and feeding behaviour in larval climbing perch. These might provide useful baseline information for optimization of large scale culture and ultimately for the culture management of this fish in future.

Materials and Methods

The experiments were carried out at the facilities of the Fisheries Technology Programme, Faculty of Science and Technology, Prince of Songkla University, Pattani campus, Thailand, from October 1, 2002 to September 30, 2003. Fecundity estimation was made using a gravimetric method (Tarnchalanukit *et al*., 1986). Newly hatched larvae of climbing perch were produced by induced spawning using chemical injection (Suprefact and Motilium). The injection was done using Suprefact 20 µg/kg and Motilium 5 mg/kg. For male and female brooders, the injection was done once. The sexually mature fish were cultured in fiber-glass tank (water volume 300 liters) with the ratio of male and female brooders 2:1. The fishes nesting was prepared by male fishes for spawning activities.

The fertilization rate, hatching out and hatching rate experiments were carried out using a 15-liter aquarium (water volume 10 liters) containing 7,000-9,000 eggs, and observation of the amount of fertilized eggs at 5 hr after incubation. The fertilization rate was calculated by (number of fertilization eggs/number of eggs) x 100. The time required for the appearance of the first newly-hatched larvae, which would signal hatching out, was recorded. All newly-hatched larvae were collected using a dropper. The hatching rate was calculated by (number of newly-hatched/number of eggs) x 100 (Tarnchalanukit *et al*., 1986). The procedure was carried out with

three replications.

Yolk absorption experiment

The time of yolk absorption and the size of yolk-sacs were determined using a profile projector. Twenty newly-hatched larvae were taken at random at 2-hourly intervals from the rearing aquarium until the yolk sacs were fully absorbed. The specimens were fixed in 10% buffered formalin. Yolk volumes were calculated using the formula $4/3 \times \pi (R1/2)^2 \times R2/2$ (R1, minor axis; R2, major axis) (Fukuhara, 1986).

Mouth development experiment

Up until full mouth development (start of feeding), samples of twenty newly hatched larvae were taken every 2 hours from the rearing aquarium for observation of the size of mouth opening, and measurement of upper jaw length was done using a profile projector. Specimens were fixed in 10% buffered formalin. The mouth height was calculated by multiplying the upper jaw length by $\sqrt{2}$ (Shirota, 1970).

Start of feeding experiment

The experiment was carried out using 15-liter aquaria (water volume 10 liters) containing 1,000 larvae aged 1 days post hatching (just before the mouth opened). They were fed with rotifer (100 μm, width) at a density of 10 individual/ml. Twenty larvae were collected at random from the aquarium at 2-hourly intervals and preserved in 10% buffered formalin. They were then dissected to determine the presence of rotifer in the digestive tract which would signal the time of the start of feeding (Pechmanee *et al*., 1986). The procedure was carried out with three replications.

Starvation experiment

A starvation experiment was carried out using a 15-liter aquarium (water volume 10 liters). Two hundred newly hatched larvae were kept without feeding and mortalities of starved larvae were recorded at 2-hourly intervals until all had died (Fukuhara, 1987). The procedure was carried out in triplicate.

Feed and feeding scheme experiment

The experiments of feed and feeding scheme were conducted in a 15-liter glass aquarium (water volume 10 liters) containing 500 of 2-day old larvae (stage at first feeding). Rotifers were given to 3 to 10-day old larvae with density 100 individual/ml, *Moina* were given to 8 to 15-day old larvae with density 10 individual/ml (rotifer and *Moina* were given to 8 to 10-day old larvae), and pellet were given to 14 to 16-day old larvae (*Moina* and pellet were given to 14 to 15-day old larvae) twice a day (Tarnchalanukit *et al*., 1982; Kungvankij *et al*., 1986; Eda *et al*., 1993). Samples of 20 larvae were randomly collected every day about 1 hour after feeding, then preserved in 5% buffered formalin. Stomach content of preserved larval climbing perch was later determined.

Daily food uptake experiment

To determine the daily food uptake by the larvae and juveniles, three experiments were carried out. All experiments were done in a 15-liter glass aquarium (water volume 10 liters) containing 100 larvae. The larvae were fed with living food organism i.e. rotifer or *Moina* depending on larval stage with density of rotifer 100 individual/ml or with density of *Moina* 10 individual/ml. The amount of food intake was calculated based on changes of food density in the aquarium with and without fish larvae, with 5 replications of water sampling at 2-hour intervals. Aquaria without larvae were set for a control of natural fluctuation in food density (Hassan, 1990). Known numbers of food were added when the density became low.

Results

The size at sexual maturity of female climbing perch were 15.20±1.24 cm (mean±SD) in total length and 61.10±17.32 g in body weight. The fecundity was 24,120.5±3,328.24 ova/fish and gonadosomatic index (GSI) was 10.4±2.5%. The eggs were floating and rounded. The fertilized eggs had a diameter of 830±39 μm. It was found that the fertilization rate in replicates 1, 2 and 3 were 95.26%, 90.50% and 92.25%, respectively. Thus

the average fertilization rate was 92.67%. The hatching out in replicates 1, 2 and 3 were 19 hr 30 min, 20 hr 55 min and 21 hr 5 min, respectively. Thus average hatching out was 20 hr 30 min. The hatching rate in replicates 1, 2 and 3 were 89.67%, 86.67% and 86.00%, respectively. Thus average hatching rate was 87.44 % at the water temperature of 27.0-30.5°C.

Newly hatched larvae were 2.02±0.20 mm in total length (mean±SD, n=20) with yolk sacs of 111.33±46.19 μm^3 in volume (mean±SD, n=20). The yolk sacs were completely absorbed within 92 hr (3.8 days) (Figure 1) after hatching at water temperatures of 27.0-30.5°C. All larval mouths were open 28 hr after hatching (2.95±0.59 mm TL) and measured 328.42±32.23 μm in mouth

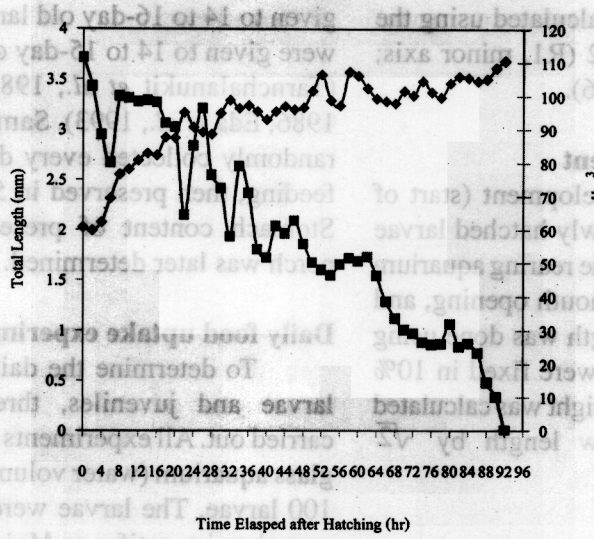

Figure 1. Total lenght (TL) and yolk absorption of larval climbing perch at elapsed time after hatching YV: Yolk volume.

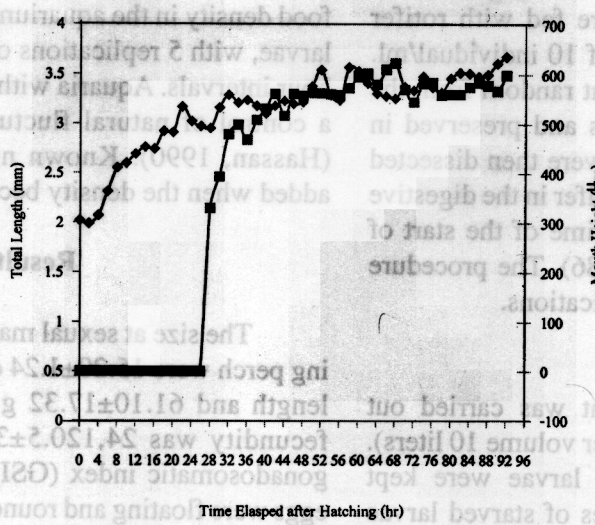

Figure 2. Total lenght (TL) and development of mouth opening of larval climbing perch at elapsed time after hatching MH: Mouth height.

height (Figure 2). At 32 hr after hatching, the fish started feeding on the rotifer which the remaining yolk sac was 52.20% of its initial volume. The digestive tracts developed fully within 32 hr after hatching at water temperatures of 27.0-30.5°C and measured 477.63±47.80 μm in mouth height, and contained numbers of rotifer, indicating that feeding had commenced. Numbers of rotifer in the digestive tract per larva in replicates 1, 2 and 3 were 1.50, 1.70 and 1.3 individual, respectively. Thus the average number of rotifer in the digestive tract at the start of feeding was 1.50 individual/larva. The larvae started to feed on the rotifer at 4 hr after mouth opening, and at 60 hr before yolk absorption (Figure 3).

Without feeding, the climbing perch larvae started to die in all experiments at 216 hr and totally died within 348 hr (14.5 days) after hatching (Figure 4). Water temperature ranged from 27.0 to 30.5°C

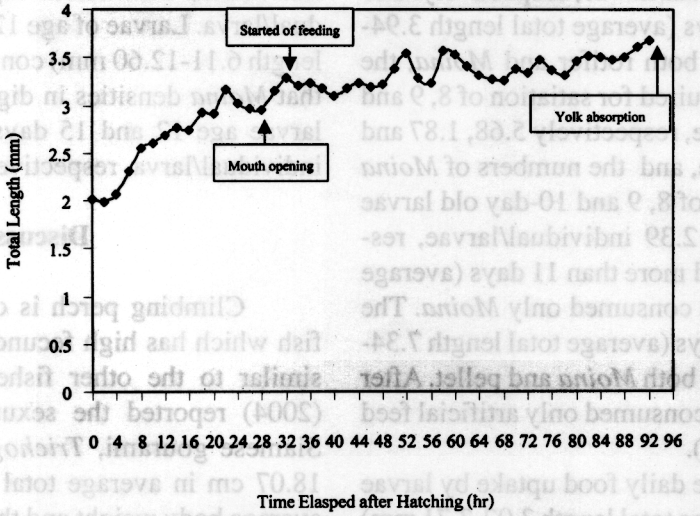

Figure 3. Increase in length of climbing perch larvae at elasped time after hatching
: Mouth opening at 28 hr after hatching
: Started of feeding at 32 hr after hatching
: Yolk absorption at 92 hr after hatching

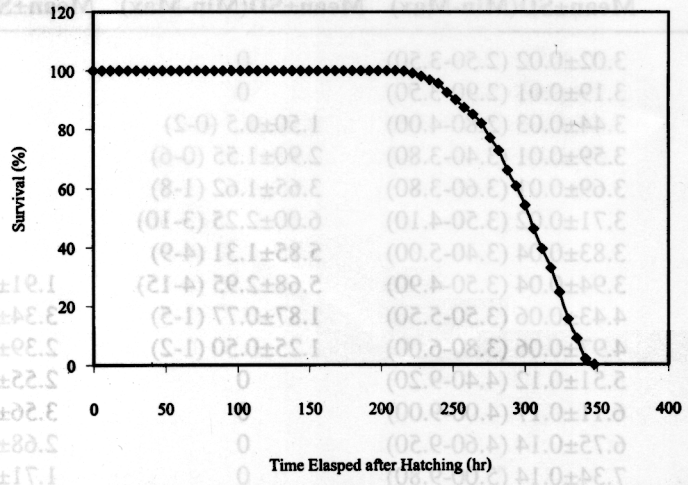

Figure 4. Survival of larval climbing perch after hatching without feeding at 27.0-30.5°C.

It was found that larvae of age 3-10 days (average total length 3.02-4.97 mm) consumed rotifer. The numbers of rotifer required for satiation of 3, 4, 5, 6, 7, 8, 9 and 10-day old larvae were 1.5, 2.9, 3.65, 6.0, 5.85, 5.68, 1.87 and 1.25 individual/larvae, respectively. The larvae of age 8-15 days (average total length 3.94-12.60 mm) consumed *Moina*. The numbers of *Moina* required for satiation of 8, 9, 10, 11, 12, 13, 14 and 15-day old larvae were 1.91, 3.34, 2.39, 2.55, 3.56, 2.68, 1.71 and 1.68 individual/larvae, respectively. The larvae of age 8-10 days (average total length 3.94-4.97 mm) consumed both rotifer and *Moina*, the numbers of rotifer required for satiation of 8, 9 and 10-day old larvae were, respectively 5.68, 1.87 and 1.25 individual/larvae, and the numbers of *Moina* required for satiation of 8, 9 and 10-day old larvae were 1.91, 3.34 and 2.39 individual/larvae, respectively. Larvae aged more than 11 days (average total length 5.51 mm) consumed only *Moina*. The larvae of age 14-15 days (average total length 7.34-12.60 mm) consumed both *Moina* and pellet. After 16-day of age, larvae consumed only artificial feed (Table 1 and Figure 5).

It was found the daily food uptake by larvae of age 3-6 days (average total length 3.02-3.71 mm) were only rotifer. The average densities of rotifer in digestive tract per day of larval age 3 and 6 days old were 9 and 16 individual/larva, respectively. The 9-days old larvae (average total length 4.43 mm) consumed both rotifer and *Moina*, the average rotifer densities in digestive tract in a day were 19 individual/larva while the average *Moina* densities in digestive tract were 10 individual/larva. Larvae of age 12-15 days (average total length 6.11-12.60 mm) consumed only *Moina*, and that *Moina* densities in digestive tract 10 individual/larva. Larvae of age 12-15 days (average total length 6.11-12.60 mm) consumed only *Moina*, and that *Moina* densities in digestive tract in a day of larvae age 12 and 15 days old were 98 and 113 individual/larva, respectively (Figure 6).

Discussion

Climbing perch is one kind of freshwater fish which has high fecundity. It was found to be similar to the other fishes. Amornsakun *et al*. (2004) reported the sexual maturity of female Siamese gourami, *Trichogaster pectoralis*, was 18.07 cm in average total length and 94.20 g in average body weight and the fecundity was 26,261

Table 1. Amount of food uptake in a day by climbing perch in each stage

Larval age (days)	Total length (mm)	Rotifer (ind)	*Moina* (ind)
	Mean±SD(Min-Max)	Mean±SD(Min-Max)	Mean±SD(Min-Max)
1.	3.02±0.02 (2.50-3.50)	0	0
2.	3.19±0.01 (2.90-3.50)	0	0
3.	3.44±0.03 (2.80-4.00)	1.50±0.5 (0-2)	0
4.	3.59±0.01 (3.40-3.80)	2.90±1.55 (0-6)	0
5.	3.69±0.01 (3.60-3.80)	3.65±1.62 (1-8)	0
6.	3.71±0.02 (3.50-4.10)	6.00±2.25 (3-10)	0
7.	3.83±0.04 (3.40-5.00)	5.85±1.31 (4-9)	0
8.	3.94±0.04 (3.50-4.90)	5.68±2.95 (4-15)	1.91±1.64 (1-6)
9.	4.43±0.06 (3.50-5.50)	1.87±0.77 (1-5)	3.34±1.43 (1-8)
10.	4.97±0.06 (3.80-6.00)	1.25±0.50 (1-2)	2.39±0.78 (1-4)
11.	5.51±0.12 (4.40-9.20)	0	2.55±1.15 (1-5)
12.	6.11±0.17 (4.00-9.00)	0	3.56±1.51 (1-7)
13.	6.75±0.14 (4.60-9.50)	0	2.68±1.34 (1-7)
14.	7.34±0.14 (5.00-9.80)	0	1.71±0.92 (1-4)
15.	12.60±0.41 (9.00-23.0)	0	1.68±1.12 (0-4)

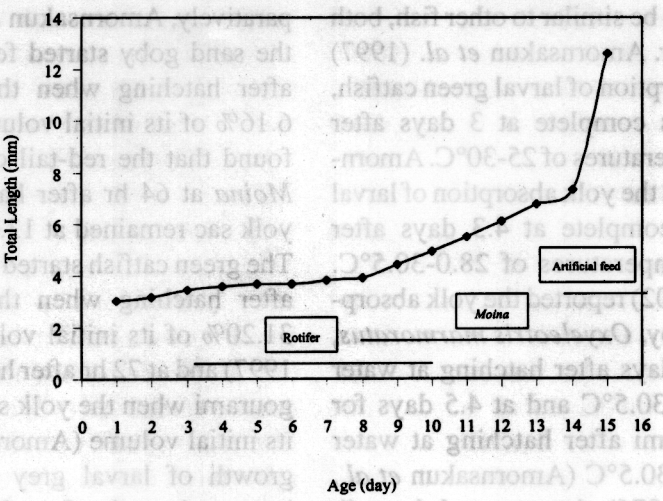

Figure 5. Mean total length of larval climbing perch according to age and feeding scheme.

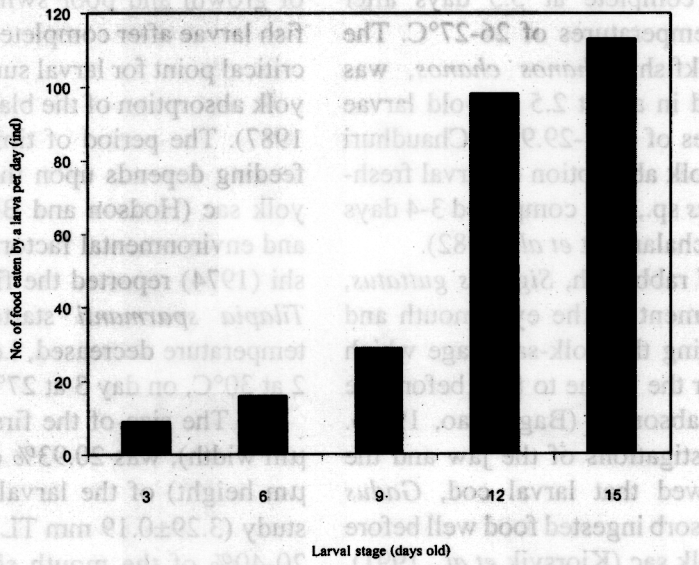

Figure 6. Number of food eaten in a day by larval climbing perch.

ova/fish. The fertilized eggs of the climbing perch have a diameter of 830 μm which is close to Siamese gourami (908.25 μm in a diameter) but smaller than the red-tail catfish, *Mystus wyckioides* (2,278.80 μm in a diameter) (Amornsakun, 1999b; Amornsakun *et al.*, 2004). The gonadosomatic index (GSI) of mature freshwater fishes were reported as 8-10% (Na nakorn, 1995). Amornsakun *et al.* (2004) reported the gonadosomatic index of mature Siamese gourami as 10.9%. Female climbing perch in this study were mature and the gonadosomatic index (GSI) was found to be 10.4%.

The period of hatching out was 20 hr 30 min at the water temperature of 27.0-30.5°C. This is similar to the other fishes such as green catfish, red-tail catfish and Siamese gourami, which have hatching-times out of 18 hr, 23 hr 40 min and 22 hr 10 min, respectively, at the water temperature 27.0-30.5°C (Amornsakun, 1999a; Amornsakun, 1999b; Amornsakun *et al.*, 2004).

The yolk absorption period for newly-hatched larval climbing perch (3.8 days after

hatching) was found to be similar to other fish, both marine and freshwater. Amornsakun *et al*. (1997) reported the yolk absorption of larval green catfish, *Mystus nemurus*, was complete at 3 days after hatching at water temperatures of 25-30°C. Amornsakun (1999c) reported the yolk absorption of larval red-tail catfish was complete at 4.3 days after hatching at water temperatures of 28.0-30.5°C. Amornsakun *et al*. (2002) reported the yolk absorption of larval sand goby, *Oxyeleotris marmoratus*, was complete at 3.4 days after hatching at water temperatures of 27.0-30.5°C and at 4.5 days for larval Siamese gourami after hatching at water temperatures of 27.0-30.5°C (Amornsakun *et al*., 2004). Houde *et al*. (1976) also reported the yolk absorption of larval white mullet, *Mugil* curema Valenciennes, was complete at 3.5 days after hatching at water temperatures of 26-27°C. The yolk of larval milkfish, *Chanos chanos*, was completely absorbed in about 2.5 day-old larvae at water temperatures of 26.4-29.9°C (Chaudhuri *et al*., 1978). The yolk absorption of larval freshwater catfish, *Clarias* sp., was completed 3-4 days after hatching (Tarnchalanukit *et al*., 1982).

The larvae of rabbitfish, *Siganus guttatus*, have rapid development of the eye, mouth and alimentary tract during the yolk-sac stage which makes it possible for the larvae to feed before the yolk is completely absorbed (Bagarinao, 1986). Morphological investigations of the jaw and the digestive tract showed that larval cod, *Gadus morhua*, is able to absorb ingested food well before exhaustion of the yolk sac (Kjorsvik *et al*., 1991). In this study, through microscopic observation, it was found that after 28 hr about 68.58% of yolk remained and the mouths (328.42±32.23 μm in mouth height) of all larval climbing perch had already opened but were not yet functioning. The yolk sac remaining at the time first of feeding of climbing perch larvae was more than that in sand goby and red-tail catfish but similar to green catfish and Siamese gourami. The result of the present study reveals that climbing perch larvae start to feed supplied rotifer at 32 hr after hatching (477.63 ±47.80 μm, mouth height) with their remaining yolk sacs at 52.20% of the initial volume. Comparatively, Amornsakun *et al*. (2002) reported that the sand goby started feeding on rotifer at 80 hr after hatching when the yolk sac remained at 6.16% of its initial volume. Amornsakun (1999c) found that the red-tail catfish started feeding on *Moina* at 64 hr after hatching at which time the yolk sac remained at 13.03% of its initial volume. The green catfish started feeding on *Moina* at 52 hr after hatching when the yolk sac remained at 31.20% of its initial volume (Amornsakun *et al*., 1997) and at 72 hr after hatching for larval Siamese gourami when the yolk sac remained at 32.21% of its initial volume (Amornsakun *et al*., 2004). The growth of larval grey mullet, *Mugil cephalus*, increased on the first day, which coincides with rapid yolk absorption (Kuo *et al*., 1973). Reduction of growth and poor swimming activity of unfed fish larvae after complete yolk absorption, led to a critical point for larval survival in association with yolk absorption of the black sea bream (Fukuhara, 1987). The period of time from hatching to first feeding depends upon the nutrients stored in the yolk sac (Hodson and Blunt, 1986; Ware, 1975) and environmental factors (Houde, 1974). Ishibashi (1974) reported the first feeding of the larval *Tilapia sparmanii* started later as the water temperature decreased, i.e. they took food on day 2 at 30°C, on day 3 at 27°C and on day 6 at 24°C.

The size of the first live food, rotifer (100 μm width), was 20.93% of mouth height (477.63 μm height) of the larval climbing perch in this study (3.29±0.19 mm TL). It is close to the range 20-40% of the mouth size in various fishes as reported by Ito and Suzuki (1977), Hunter (1980), Amornsakun *et al*. (1997), Amornsakun (1999c), Amornsakun *et al*. (2002) and Amornsakun *et al*. (2004). Larval climbing perch is considered as a fish species difficult to rear in early life stages as a result of certain food organisms with appropriate size to larval mouth (20.93% of mouth height).

The mouth height at the start of feeding (477.63 μm) of larval climbing perch is similar to green catfish, red-tail catfish, sand goby and Siamese gourami but the time of its first feeding is earlier than time of green catfish, red-tail catfish, sand goby and Siamese gourami. Green catfish

started to feed on *Moina* when the mouth height was 553 µm (40.65% of mouth height) at 52 hr after hatching. Red-tail catfish started to feed on *Moina* when the mouth height was 534 µm (45.26% of mouth height) at 64 hr after hatching. Sand goby started to feed on rotifer when the mouth height was 549 µm (18.70% of mouth height) at 80 hr after hatching and started to feed on rotifer when the mouth height was 503 µm (19.85 % of mouth height) at 72 hr after hatching for larval Siamese gourami (Amornsakun *et al*., 1997; Amornsakun, 1999c, Amornsakun *et al*., 2002; Amornsakun *et al*., 2004). On the contrary, the mouth height at first feeding of larval climbing perch is greater than that of rabbitfish and grouper. Juario *et al*. (1985) reported that the mouth of the larval rabbitfish, *Siganus guttatus* (Bloch), was about 125 µm wide when feeding started 2 days after hatching on rotifers. Maneewong *et al*. (1986) reported the mouth size of the larval grouper, *Epinephelus malabaricus* (Bloch and Schneider), was 169.7±16.1 µm when it was first able to consume rotifers with size of 91-100 µm width. Mouth size appears to be the limiting factor in juvenile fish feeding on both natural and pellet diets (Hyatt, 1979). Nash *et al*. (1974) reported the mouths of larval grey mullet was open when the jaws are becoming ossified and eye pigment is sufficiently developed. Larvae with small mouths grew more slowly than those with larger ones (Shirota, 1970; Arumugum and Geddes, 1987). The mouth height of the larval climbing perch was related to total length. The same relationship between mouth height and total length was also found in larval perch, *Perca fluviatilis* (Guma, 1978).

Rotifer could be a good live food for larval climbing perch because of its suitable size and ease of culture. The number of rotifer in the digestive tract at the start of feeding was 1.5 individual /larva. This is similar to those reported in milkfish, green catfish and red-tail catfish, but it is more than reported in Siamese gourami. Eda *et al*. (1990) reported that the gut of milkfish larvae (3.57-3.81 mm, TL) was first found to contain rotifers 80 hr after hatching. The number of rotifers in the milkfish gut ranged from 1-4 individual/ larva. Amornsakun *et al*. (1997) reported that the gut of green catfish larvae was first found to contain *Moina* 52 hr after hatching. The number of *Moina* in the green catfish gut was 1.8 individual/ larva. Amornsakun(1999c) reported that the gut of red-tail catfish larvae was first found to contain *Moina* 64 hr after hatching. The number of *Moina* in the red-tail catfish gut was 1.3 ind/larva. And the gut of Siamese gourami larvae was first found to contain rotifer 72 hr after hatching with the number of rotifer of 0.57 individual/larva (Amornsakun *et al*., 2004).

Without feeding, the larval climbing perch become debilitated and eventually started to die off in all experiments at 216 hr after hatching. A catastrophic mortality of 50% was observed at 300 hr after hatching and all died off within 348 hr after hatching at water temperatures of 27.0-30.5°C. Larvae can tolerate feeding delay up to a certain point depending on the amount of yolk, temperature and other species-specific characteristics (May, 1974; Hunter, 1980; Holm,1986). Mortality of larvae is more than the other species about 5 to 7 days after hatching, depending on the species. For example, mortality of northern anchovy, *Engraulis mordax*, was observed on the sixth day after hatching (Lasker *et al*., 1970), on the seventh day after hatching for the grey mullet (Kuo *et al*., 1973), on the sixth day after hatching for the milk fish (Chaudhuri *et al*., 1978), on the fifth to seventh day after hatching for the larval dragonets, *Repomucenus* sp. (Eda *et al*., 1993). In the seabass, *Lates calcarifer*, mortality was observed on the fifth day after hatching (Hassan and Amornsakun, 1996). In the Siamese gourami mortality was observed on the sixth day after hatching (Amornsakun *et al*., 2004). Larval climbing perch is able to slightly maintain its survival through starvation (14.5 days after hatching) although its yolk sac is small (111.33 μm^3). Ishibashi (1974) observed that the yolk sac of unfed *Tilapia sparmanii* larvae was absorbed faster than that of fed larvae. Larvae of climbing perch are like other larvae in that after yolk is completely absorbed their mortality becomes pronounced,

particularly 10 days after absorption, longer than green catfish, which was 4 days after absorption (Amornsakun *et al*., 1997) and 2 days after absorption for Siamese gourami (Amornsakun *et al*., 2004). Lasker *et al*. (1970) experimented on delayed feeding period of *Engraulis mordax* and found in a catastrophic mortality after 2.5 days of complete absorption. The unfed larvae grow slowly, swim weakly, eventually falling to the bottom of the tank and dying.

The food supply during the larval stage is an important factor in achieving high survival and growth rates. Mass mortality of larval and juvenile fish will often occur if the food supply is inadequate (Houde, 1978). Different species require different sequential food during the early life stages. Most freshwater fish are given rotifer or *Moina* as a first feeding (Tarnchalanukit *et al*., 1982; Tawaratmanikul, 1988; Vatcharakornyothin *et al*., 1988), and artificial feeds for juveniles are generally in the form of fine crumbles of appropriate particle size. The larval climbing perch is no exception.

The type of feeding for larval climbing perch cultured such as rotifer, *Moina* and artificial feed is similar to the other fishes, but time for feeding may be different. It was found that larval climbing perch of age 3-10 days (average total length 3.02-4.97 mm) consumed rotifer. The larvae of age 8-15 days (average total length 3.94-12.60 mm) consumed *Moina*, and those larvae of age 8-10 days (average total length 3.94-4.97 mm) consumed both rotifer and *Moina*. The larvae of age 11-day (average total length 5.51 mm) consumed only *Moina*. The larvae of age 14-15 days (average total length 7.34-12.60 mm) consumed both *Moina* and artificial feed. And after 16 days, larvae consumed only artificial feed. Watanabe *et al*. (1983) described the food regimes used most extensively in the larvae of various fish production in Japan. In newly hatched fish greater than 2.3 mm of body length, rotifers were exclusively given as an initial feed, when fish reached 7 mm or more, marine copepods such as *Tigriopus, Acartia, Oithona* and *Paracalanus* were given. Brine shrimp, *Artemia salina*, were frequently used for the larvae of many species during shortages of marine copepods. Larvae larger than 10 to 11 mm were fed minced fish, shellfish and shrimp or an artificial diet. Tsukashima and Kitajima (1981) reported the rearing of larval and juvenile filefish, *Stephanolepis cirrhifer*, up to the stage of young fish. They were fed rotifer, *Tigriopus japonicus, Artemia* and subsequently fish meat. Tarnchalanukit *et al*. (1982) reported that *Clarias batrachus* of age 2-15 days were fed on *Moina*, and fed with a commercial catfish pellet when they reached 10 days-old. Chawpaknam *et al*. (1990) reported that fry nursing of two-spot glass catfish, *Ompok bimaculatus*, of age 3-15 days fed with *Moina* showed a better growth and higher survival rates than those fed with egg custard. The climbing perch of 3-15 days-old (average total length 3.02-12.60 mm) consumed zooplankton such as rotifer and *Moina* at this age the young climbing perch is classified as larvae stage. And after 16 days-old, larvae consumed only artificial feed, that is classified as juvenile stage. The time for juvenile-stage development is similar to green catfish but it is earlier than sand goby and Siamese gourami. Larval green catfish of age 2-10 days is classified as larval stage which consumed *Moina* only, and the feed completely changed to commercial pellet when they were 16 day-old which is classified as juvenile stage (Amornsakun *et al*.,1997; Amornsakun *et al*., 1998a). The sand goby of age 3-27 days is classified as larval stage which consumed zooplankton such as rotifer and *Artemia* at the start of feeding, and 30-45 days-old larvae is classified as juvenile stage which consumed *Moina* and grinding fish (Amornsakun *et al*., 2003a; Amornsakun *et al*., 2003b). The Siamese gourami of age 3-13 days (average total length 3.88-8.27 mm) consumed only rotifer, the 13-18 days-old larvae (average total length 8.27-8.36 mm) consumed rotifer and *Moina*, the 18-25 days-old larvae is classified as larval stage (average total length 8.36-9.67 mm) which consumed only *Moina* and the 25-30 days-old larvae is classified as juvenile stage (average total length 9.67-11.26 mm.) which consumed *Moina* and artificial feed (Amornsakun *et al*., 2004).

For rearing larval and juvenile on daily food

uptake are very important, it was concluded that the daily food uptake by larval climbing perch of age 3-15 days fed with rotifer or *Moina* depending on larval stage. The mean numbers of rotifer eaten in a day by 3, 6 and 9-day old larvae were 9, 16 and 19 individual/larvae, respectively. Rotifer were eaten in a day by fish larvae in the range of 9-19 individual/larvae. And the mean numbers of *Moina* eaten in a day by 9, 12 and 15-day old larvae were 10, 98 and 113 individual/larvae, respectively. *Moina* were eaten in a day by fish larvae in the range of 10-113 individual/larvae. This increases with larval age as shown in Figure 6. The numbers of prey consumed showed a similar trend to those of other fish. Amornsakun *et al*. (1998b) reported that *Moina* were eaten in a day by fish larvae of green catfish, *Mystus nemurus*, in the range of 61.56-421.74 individual/larvae. Tawaratmanikul *et al*. (1988) reported that the studies on numbers of *Moina* taken in a day by giant catfish, *Pangasianodon gigas*, larvae for the 2-3 day, 5-6 day and 8-9 day old larvae were 64, 396 and 341 individual, respectively. Hassan (1990) reported the amount of food taken in a day by larval and juvenile mullet, *Liza haematocheila*. A 23-day old larva and a 28-day old larva consumed about 1,900 and 3,300 rotifers a day, respectively, and a 31-day old larva and a 42-day old larva consumed about 440 and 2160 *Artemia nauplii* a day, respectively. Bryant and Matty (1980) reported optimum *Artemia* feeding rate for carp larvae, *Cyprinus carpio*, to be 200-250 % of their body weight on *Artemia nauplii* per day. It is very important to determine the suitable amount of food consumed by a larva per day at each stage of its sequential growth. Suitably estimating the amount food needed for a certain number of cultured larvae or juveniles will ensure better growth and survival as well as avoid unnecessary food wastage.

It was concluded that the size at sexual maturity of female climbing perch was 15.20±1.24 cm (mean±SD) in total length and 61.10±17.32 g in body weight. The eggs were floating and rounded. The fertilized eggs had a diameter of 830±39 µm. The fecundity was 24,120.5±3,328.24 ova/fish and gonadosomatic index (GSI) was 10.4 ±2.5%. The total length of newly hatched larvae of climbing perch were 2.02±0.20 mm with yolk sacs of 111.33±46.19 μm^3 in volume. The yolk sacs were completely absorbed within 92 hr after hatching. All larval mouths were open at 28 hr after hatching with the height 328.42±32.23 µm. And at 32 hr after hatching (477.63±47.80 µm in mouth height), the fish started feeding on the rotifer with the number of 1.5 individual/larva at water temperatures of 27.0-30.5°C. The starved larvae started to die at at 216 hr and totally died within 348 hr after hatching at water temperature ranged between 27.0 to 30.5°C. The food uptake by larval climbing perch of age 3-15 days were rotifer or *Moina* depending on larval stage. The mean numbers of rotifer eaten in a day by 3, 6 and 9-day old larvae were 9, 16 and 19 individual/larvae, respectively. Rotifer were eaten in a day by fish larvae in the range of 9-19 individual/larvae. And the mean numbers of *Moina* eaten in a day by 9, 12 and 15-day old larvae were 10, 98 and 113 individual/larvae, respectively. *Moina* were eaten in a day by fish larvae in the range of 10-113 individual/larvae.

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