ORIGINAL ARTICLE

Some aspects in early life stage of Siamese gourami, *Trichogaster pectoralis* (Regan) Larvae

Thumronk Amornsakun[1] Wasan Sriwatana[2] and Ponpanom Promkaew[2]

Abstract

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The sexual maturity of female Siamese gourami, *Trichogaster pectoralis* was studied by determining fecundity and gonadosomatic index (GSI). It was found that the size at sexual maturity of female Siamese gourami was 18.07±1.10 cm (mean±SD) in average total length and 94.20±13.39 g in average body weight. The fecundity was 26,261±5,215.81 ova/fish and gonadosomatic index (GSI) was 10.9±2.1%.

Newly hatched larvae of Siamese gourami 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 1:1. The fertilization rate, hatching out and hatching rate experiments were carried out using a 15-liter aquarium (water volume 10 liters) containing 1,000 eggs. It was found that the eggs were floating and rounded. The fertilized eggs had a diameter of 908.25±39.13 μm. The average fertilization rate was 91.12%, hatching out was 22 hr 10 min and average hatching rate was 83.03% at a water temperature of 27.0-30.5°C. Sampling of the newly-hatched larvae was done at 2-hour

[1]Ph.D. (Aquaculture), Asst. Prof., Department of Technology and Industries, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Pattani, 94000, [2]Pattani Inland Fisheries Development Center, Amphoe Yarang, Pattani 94160, Thailand.
Corresponding e-mail: thumronk@bunga.pn.psu.ac.th
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intervals, when 20 of them were randomly taken and preserved in 10% buffered formalin for later analysis to determine the time of final yolk absorption. Observation using a microscope revealed that newly hatched larvae were 2.73±0.02 mm in total length and had yolk sacs of 153.76±2.49 μm³ in volume. The yolk sacs were completely absorbed within 110 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 60 hr after hatching (3.56±0.04 mm TL), with the mouths measuring 443.64±24.26 μm in height.

The feeding experiments were carried out using a 15-liter glass aquarium (water volume 10 liters) containing 1,000 larvae aged 1.5 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 72 hr of hatching at water temperatures of 27.0-30.5ºC, and measured 503.73±22.57 μm in mouth height. The average number of rotifer in the digestive tract at the start of feeding was 0.57 individual/larva.

A starvation experiment was carried out using a 15-liter glass aquarium (water volume 10 liters) with three replications. Five hundred newly hatched larvae of Siamese gourami were kept without feeding. Larvae started to die at 72 hr and totally died within 156 hr after hatching at water temperature ranging between 27.0 and 30.5ºC.

Key words : fecundity, yolk absorption, mouth development, start of feeding, starvation Siamese gourami, *Trichogaster pectoralis*

บทคัดย่อ

ธำรงค์ อมรสกุล วสันต์ ศิริวัฒนะ และพรพนม พรหมแก้ว

ลักษณะบางประการในระยะวัยอ่อนของปลาสลิด

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ทำการศึกษาปริมาณความดกของไข่ และความสัมพันธ์ระหว่างน้ำหนักตัวกับอวัยวะสืบพันธุ์ (Gonadosomatic index, GSI) ของแม่ปลาสลิดที่สมบูรณ์เพศ พบว่าแม่ปลาสลิดที่สมบูรณ์เพศพร้อมที่จะขยายพันธุ์ มีความยาวลำตัวทั้งสิ้นเฉลี่ย 18.07±1.10 ซม และน้ำหนักลำตัวเฉลี่ย 94.20±13.39 กรัม ไข่ของปลาสลิดเป็นประเภทไข่ลอย ลักษณะกลม มีปริมาณความดกของไข่เฉลี่ย 26,261±5,215.81 ฟอง ความสัมพันธ์ระหว่างน้ำหนักตัวกับอวัยวะสืบพันธุ์ เท่ากับ 10.9±2.1%

ทำการเพาะขยายพันธุ์ปลาด้วยวิธีการผสมเทียมโดยการฉีดสารเคมี ได้แก่ Suprefact ร่วมกับ Motilium เป็นการกระตุ้นให้ไข่พัฒนาเร็วขึ้นและมีการตกไข่ ปล่อยพ่อแม่พันธุ์ปลาในถังไฟเบอร์กลาส (ปริมาตรน้ำ 300 ลิตร) อัตราส่วนระหว่างตัวผู้กับตัวเมีย 1:1 พบว่าไข่ที่ได้รับการผสมกับน้ำเชื้อ มีเส้นผ่าศูนย์กลางเฉลี่ยเท่ากับ 908.25±39.13 μm อัตราการปฏิสนธิของไข่เฉลี่ย 91.12% ระยะเวลาในการฟักไข่ปลา ประมาณ 22 ชั่วโมง 10 นาที และมีอัตราการฟักเฉลี่ย 83.03% ที่อุณหภูมิของน้ำ 27.0-30.5°C สุ่มลูกปลาที่ฟักออกมาใหม่ จำนวน 20 ตัว ทุก ๆ 2 ชั่วโมง เก็บดองในบัฟเฟอร์ฟอร์มาลีน 10% เพื่อใช้ทำการศึกษาการยุบตัวของไข่แดง โดยใช้กล้องจุลทรรศน์ พบว่าลูกปลาที่ฟักออกมาใหม่มีความยาวลำตัวทั้งสิ้น 2.73±0.02 มม. ปริมาตรของไข่แดงประมาณ 153.76±2.49 μm³ ไข่แดงยุบตัวอย่างสมบูรณ์ประมาณ 110 ชั่วโมงหลังจากฟักออกเป็นตัวที่อุณหภูมิของน้ำ 27.0-30.5°C ศึกษาการพัฒนาของปากโดยสุ่มลูกปลาจำนวน 20 ตัวจากตู้กระจกที่ใช้สำหรับฟักไข่ ทุก ๆ 2 ชั่วโมง พบว่าที่ 60 ชั่วโมงหลังจากฟักออกเป็นตัว (3.56±0.04 มม, TL) ปากของลูกปลาเริ่มเปิด วัดความสูงของปากได้ 443.64±24.26 μm

[1]ภาควิชาเทคโนโลยีและการอุตสาหกรรม คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตปัตตานี อำเภอเมือง จังหวัดปัตตานี 94000 [2]ศูนย์พัฒนาประมงน้ำจืดปัตตานี อำเภอยะรัง จังหวัดปัตตานี 94160

ศึกษาการเริ่มกินอาหารของลูกปลาสลิดโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) ใส่ลูกปลาอายุ 1.5 วันหลังจากฟักออกเป็นตัว (ระยะก่อนที่ปากจะเปิด) จำนวนตู้ละ 1,000 ตัว โดยให้ลูกปลากินโรติเฟอร์เป็นอาหาร ในอัตราความหนาแน่น 10 ตัว/มล. สุ่มลูกปลาจำนวน 20 ตัว จากตู้ปลาที่ใช้ทำการศึกษา ทุก ๆ 2 ชั่วโมง เก็บดองในบัฟเฟอร์ฟอร์มาลีน 10% พบว่าที่ 72 ชั่วโมงหลังจากฟักออกเป็นตัว ที่อุณหภูมิ 27.0-30.5°C ความสูงของปาก 503.73±22.57 μm ในระบบทางเดินอาหารปรากฏโรติเฟอร์ ประมาณเฉลี่ย 0.57 ตัว/ลูกปลา ซึ่งหมายความถึงการเริ่มกินอาหารของลูกปลา

ศึกษาการอดอาหารจนตายในลูกปลาสลิดโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) จำนวน 3 ซ้ำ ใส่ลูกปลาที่ฟักใหม่ จำนวนตู้ละ 500 ตัว เลี้ยงโดยไม่ให้อาหาร พบว่าลูกปลาสลิดเริ่มตายที่ 72 ชั่วโมงหลังจากฟักออกเป็นตัว และตายหมดที่ 156 ชั่วโมง ที่อุณหภูมิ 27.0-30.5°C

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). 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 (Blaxter, 1974 and May, 1974). Larvae can use a varying part of their yolk sac energy content for various activities.

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).

To date, no research on Siamese gourami regarding the yolk absorption, mouth development in relation to feeding on rotifer and starvation has been undertaken.

The purpose of this study was to investigate the period of yolk absorption, the onset of first feeding, mouth development and starvation in larval Siamese gourami. 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

Fecundity estimation was made using a gravimetric method (Tarnchalanukit *et al*., 1986). Newly hatched larvae of Siamese gourami were produced by induced spawning using chemical injection (Suprefact and Motilium). The injection was done using Suprefact 15 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 stocking density of 10 fishes/m^2. 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 1,000 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)×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)×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 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 1.5 days post hatching (just before the mouth opened). They were fed with rotifer (100 µm, width) at a density of 10 ind/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.

Results

The size at sexual maturity of female Siamese gourami, *Trichogaster pectoralis* were 18.07±1.10 cm (mean±SD) in average total length and 94.20±13.39 g in average body weight. The fecundity was 26,261±5,215.81 ova/fish and gonadosomatic index (GSI) was 10.9±2.1%. The eggs were floating and rounded. The fertilized eggs had a diameter of 908.25±39.13 µm. It was found that the fertilization rate in replicates 1, 2 and 3 were 95.26%, 90.50% and 87.60%, respectively. Thus the average fertilization rate was 91.12%, and hatching out was 22 hr 10 min. The hatching rate in replicates 1, 2 and 3 were 90.90%, 77.92% and 80.29%, respectively. Thus average hatching rate was 83.03% at the water temperature of 27.0-30.5ºC.

Newly hatched larvae were 2.73±0.02 mm in total length (mean±SD, n = 20) with yolk sacs of 153.76±2.49 μm^3 in volume (mean±SD, n = 20). The yolk sacs were completely absorbed within 110 hr (4.5 days) (Figure 1) after hatching at water temperatures of 27.0-30.5ºC. All larval mouths were open 60 hr after hatching (3.56±0.04 mm TL) and measured 443.64±24.26 µm in mouth height (Figure 2). At 72 hr after hatching, the fish started feeding on the rotifer at which time the remaining yolk sac was 32.21% of its initial volume. The digestive tracts developed fully within 72 hr after hatching at water temperatures of 27.0-30.5ºC and measured 503.73±22.57 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 0.6, 0.6 and 0.5 individual, respectively. Thus the average number of rotifer in the digestive tract at the start of feeding was 0.57 individual/larva. The Siamese gourami larvae started to feed on the rotifer at 12 hr after mouth opening, and at 38 hr before yolk absorption (Figure 3).

Without feeding, the larval Siamese gourami started to die in all experiments at 72 hr and totally

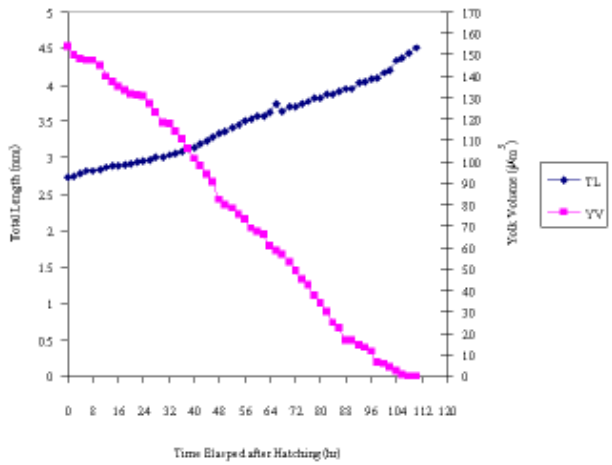

Figure 1. Total length (TL) and yolk absorption of larval Siamese gourami at elapsed time after hatching. YV: Yolk volume

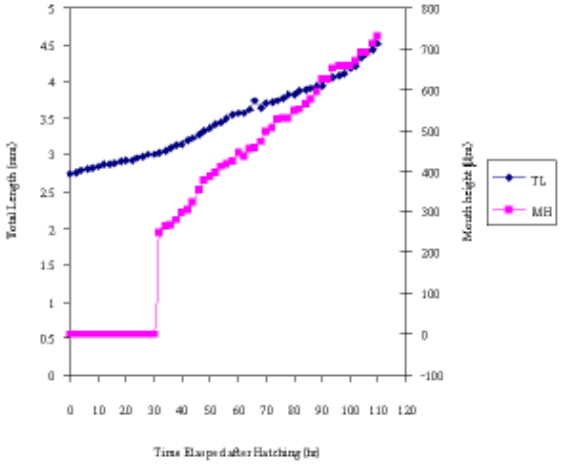

Figure 2. Total length (TL) and development of mouth opening of larval Siamese gourami at elapsed time after hatching. MH: Mouth height

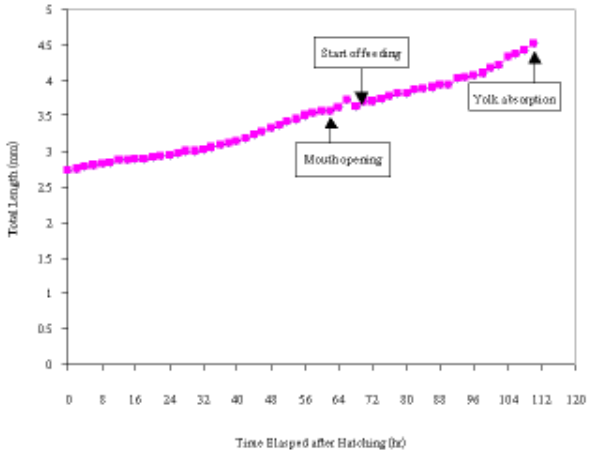

Figure 3. Increase in length of Siamese gourami larvae at elasped time after hatching
: Mouth opening at 60 hr after hatching
: Started of feeding at 72 hr after hatching
: Yolk absorption at 110 hr after hatching

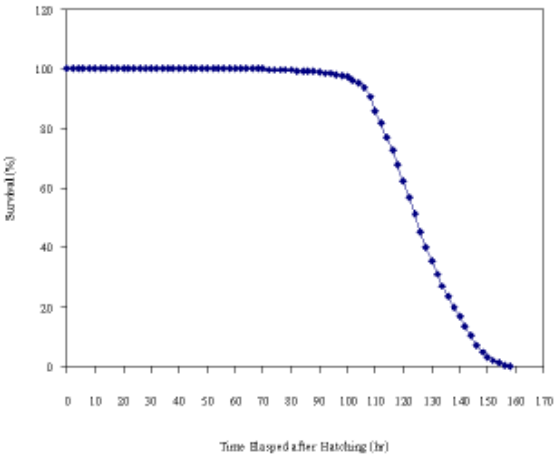

Figure 4. Survival of larval Siamese gourami after hatching without feeding at 27.0-30.5ºC

died within 156 hr (6.5 days) after hatching (Figure 4). Water temperature ranged from 27.0 to 30.5ºC.

Discussion

Siamese gourami, *Trichogaster pectoralis* is one kind of freshwater fish which has high fecundity. It was found to be similar to the other fishes. Amornsakun (2001) reported the sexual maturity of female climbing perch, *Anabas testudineus*, was 15.2 cm in average total length and 61.1 g in average body weight and the fecundity was 24,120 ova/fish. The fertilized eggs of the Siamese gourami have a diameter of 908.25

μm which is similar to climbing perch (830 μm in a diameter) but smaller than the red-tail catfish, *Mystus wyckioides* (2,278.80 μm in a diameter) (Amornsakun, 1999b and Amornsakun, 2001). The female of Siamese gourami in this study was maturity because gonadosomatic index (GSI) was 10.9%. It was found to be similar to climbing perch. Amornsakun (2001) reported the gonadosomatic index of climbing perch was 10.4 %. The gonadosomatic index (GSI) of freshwater fishes were maturity to be found 8-10 % (Na nakorn, 1995).

The time of hatching out was 22 hr 10 min at the water temperature of 27.0-30.5ºC. This is similar to the other fishes such as green catfish, red-tail catfish and climbing perch, which have hatching-times out of 18 hr, 23 hr 40 min and 21 hr, respectively, at the water temperature 27.0-30.5ºC (Amornsakun, 1999a; Amornsakun, 1999b; Amornsakun, 2001).

The yolk absorption period for newly-hatched larval Siamese gourami (4.5 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 25-30ºC. Amornsakun (1999c) reported the yolk absorption of larval red-tail catfish, *Mystus wyckioides*, was complete at 4.3 days after hatching at water temperatures of 28.0-30.5ºC and at 3.4 days for larval sand goby, *Oxyeleotris marmoratus*, after hatching at water temperatures of 27.0-30.5ºC (Amornsakun *et al*., 2002). 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 26-27ºC. The yolk of larval milkfish, *Chanos chanos*, was completely absorbed in about 2.5 day-old larvae at water temperatures of 26.4-29.9ºC (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 60 hr about 49.93% of yolk remained and the mouths (443.64±24.26 μm in mouth height) of all larval Siamese gourami had already opened but were not yet functioning. The yolk sac remaining at the time first of feeding of Siamese gourami larvae was more than that in sand goby and red-tail catfish but similar to green catfish. The result of the present study reveals that Siamese gourami larvae start to feed supplied rotifer at 72 hr after hatching (503.73±22.57 μm, mouth height) with their remaining yolk sacs at 32.21% 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). The growth of larval grey mullet, *Mugil cephalus*, increase on the first day, which coincide 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 length of time from hatching to first feeding depends upon the nutrients stored in the yolk sac (Hodson and Blunt, 1986 and 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 30ºC, on day 3 at 27ºC and on day 6 at 24ºC.

The size of the first live food, rotifer (100 μm width), was 19.85 % of mouth height (503 μm

height) of the larval Siamese gourami in this study (3.71±0.01 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) and Amornsakun *et al*. (2002). Larval Siamese gourami is considered as a fish species difficult to rear in early life stages since certain food organisms with appropriate size suitable to larval mouth (19.85% of mouth height).

The mouth height at the start of feeding (503 μm) of larval Siamese gourami is similar to green catfish, red-tail catfish and sand goby but the time of its first feeding is later than time of green catfish and red-tail catfish but earlier than time of sand goby. Green catfish and red-tail catfish started to feed on *Moina* when the mouth heights were 553 μm at 52 hr after hatching and 534 μm at 64 hr after hatching, respectively. Sand goby started to feed on rotifer when the mouth heights were 549 μm at 80 hr after hatching. (Amornsakun *et al*., 1997; Amornsakun, 1999c and Amornsakun *et al*., 2002). On the contrary, the mouth height at first feeding of larval Siamese gourami 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 mullet, *Mugil cephalus*, 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 and Arumugum and Geddes, 1987). The mouth height of the larval Siamese gourami was linearly 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 Siamese gourami because of its suitable size and ease of culture. The number of rotifer in the digestive tract at the start of feeding was 0.57 ind/larva. This is less than reported in milkfish, green catfish and red-tail catfish. Eda *et al*. (1990) reported that the gut of milkfish, *Chanos chanos*, 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 ind/larva. Amornsakun *et al*. (1997) reported that the gut of green catfish, *Mystus nemurus*, larvae was first found to contain *Moina* 52 hr after hatching. The number of *Moina* in the green catfish gut was 1.8 ind/larva. And the gut of red-tail catfish, *Mystus wyckioides*, larvae was first found to contain *Moina* 64 hr after hatching with the number of *Moina* of 1.3 ind/larva (Amornsakun, 1999c).

Without feeding, the larval Siamese gourami become debilitated and eventually started to die off in all experiments at 72 hr after hatching. A catastrophic mortality of 50 % was observed at 124 hr after hatching and all died off within 156 hr after hatching at water temperatures of 27.0-30.5ºC. 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 and Holm,1986). Mortality of unfed Siamese gourami larvae is similar to that of other starved fishes 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, *Mugil cephalus* (Kuo *et al*., 1973), on the sixth day after hatching for the milk fish, *Chanos chanos* (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). Larval Siamese gourami is able to slightly maintain its survival through starvation (6.5 days after hatching), shorter than red-tail catfish (8 days after hatching), possibly owing to its large yolk sac

(1,443.17 μm^3) (Amornsakun, 1999c and Amornsakun, 2000). Ishibashi (1974) observed that the yolk sac of unfed *Tilapia sparmanii* larvae was absorbed faster than that of fed larvae. Larvae of Siamese gourami are like other larvae in that after yolk is completely absorbed their mortality becomes pronounced, particularly 2 days after absorption, shorter than green catfish, which was 4 days after absorption (Amornsakun *et al*., 1997). 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.

It was concluded that the total length of newly hatched larvae of Siamese gourami were 2.73±0.02 mm with had yolk sacs of 153.76±2.49 μm^3 in volume. The yolk sacs were completely absorbed within 110 hr after hatching. All larval mouths were open at 60 hr after hatching with the height 443.64±24.26 µm. And at 72 hr after hatching (503.73±22.57 µm in mouth height), the fish started feeding on the rotifer at water temperatures of 27.0-30.5ºC. The starved larvae started to die at at 72 hr and totally died within 156 hr after hatching at water temperature ranged between 27.0 to 30.5ºC.

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