ORIGINAL ARTICLE

Some aspects in early life stage of sand goby, *Oxyeleotris marmoratus* Larvae

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Abstract

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Larval sand goby were produced by controlled natural spawning. Fertilized eggs were incubated in an aquarium tank for hatching. 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 solution for later analysis to determine the time of final yolk absorption. Observation using a microscope revealed that newly hatched larvae were 2.39±0.12 mm in total length and had yolk sacs of 55.32±14.85 μm^3 volume. The yolk sacs were completely absorbed within 82 hr after hatching at a water temperature of 27.0-30.5 ^{o}C.

Up until full mouth development (start of feeding), 2-hourly samplings of twenty newly hatched larvae were taken from the aquarium hatching tank for observation of the size of mouth opening. All the larvae had open mouths about 36 hr after hatching (2.86±0.97 mm TL), with the mouths measuring 332.29±17.76 μm in mouth height.

The start-of-feeding experiments were carried out using a 15-liter aquarium (water volume 10 liters) containing 1000 larvae aged 1.5 days post-hatching (just before the mouth opened). They were fed with

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rotifer at a density of 5-10 ind/ml. Twenty larvae were collected at random from the aquarium at 2-hourly intervals, preserved in 10% buffered formalin solution, and then dissected to determine the presence of rotifer in the digestive tract. Digestive tracts fixed 80 hr after hatching at water temperatures of 27.0-30.5 °C., had mouth height of 549.69±47.94 μm. 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 aquarium (water volume 10 liters) with three replications. Two hundred newly hatched larvae of sand goby were kept without feeding. Larvae started to die at 84 hr and totally died within 130 hr after hatching at water temperature ranging from 27.0 to 30.5 °C.

Key words : yolk absorption, mouth development, start of feeding, starvation, sand goby, *Oxyeleotris marmoratus*

บทคัดย่อ

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ลักษณะบางประการในระยะวัยอ่อนของลูกปลาบู่ทราย

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ลูกปลาบู่ทรายผลิตโดยทำการเพาะขยายพันธุ์ปลาโดยวิธีการเลียนแบบธรรมชาติโดยใช้บ่อดิน ไข่ที่ได้รับการผสมพันธุ์นำมาฟักในตู้กระจก สุ่มลูกปลาที่ฟักออกมาใหม่ จำนวน 20 ตัว ทุก ๆ 2 ชั่วโมง เก็บดองในบัฟเฟอร์ฟอร์มาลีน 10% เพื่อใช้ทำการศึกษาการยุบตัวของไข่แดง โดยใช้กล้องจุลทรรศน์ พบว่าลูกปลาที่ฟักออกมาใหม่มีความยาวเฉลี่ย 2.39±0.12 มม. ปริมาตรของไข่แดงเฉลี่ย 55.32±14.85 ลบ.ไมโครเมตร ไข่แดงยุบตัวอย่างสมบูรณ์ประมาณ 82 ชั่วโมง หลังจากฟักออกเป็นตัว ที่อุณหภูมิของน้ำ 27.0-30.5 °C

การพัฒนาของปาก สุ่มลูกปลาจำนวน 20 ตัวจากตู้กระจกที่ใช้สำหรับฟักไข่ ทุก ๆ 2 ชั่วโมง เพื่อทำการศึกษาความสูงของปาก พบว่าที่ 36 ชั่วโมงหลังจากฟักออกเป็นตัว (2.86±0.97 mm TL) ปากของลูกปลาเริ่มเปิด วัดความสูงของปากได้ 332.29±17.76 ไมครอน

ศึกษาการเริ่มกินอาหารของลูกปลาบู่ทรายโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) ใส่ลูกปลาอายุ 1.5 วันหลังจากฟักออกเป็นตัว (ระยะก่อนที่ปากจะเปิด) จำนวนตู้ละ 1000 ตัว โดยให้ลูกปลากินโรติเฟอร์เป็นอาหาร ในอัตราความหนาแน่น 5-10 ตัว/มล. สุ่มลูกปลาจำนวน 20 ตัว จากตู้ปลาที่ใช้ทำการศึกษา ทุก ๆ 2 ชั่วโมง เก็บดองใน บัฟเฟอร์ฟอร์มาลีน 10% พบว่าที่ 80 ชั่วโมงหลังจากฟักออกเป็นตัว ที่อุณหภูมิ 27.0-30.5 °C ความสูงของปากเฉลี่ย 549.69±47.94 ไมครอน ในระบบทางเดินอาหารปรากฏโรติเฟอร์เฉลี่ย 0.57 ตัว/ลูกปลา ซึ่งหมายความถึงการเริ่มกินอาหารของลูกปลา

ศึกษาการอดอาหารจนตายในลูกปลาบู่ทรายโดยใช้ตู้ปลาขนาดปริมาตร 15 ลิตร (ปริมาตรน้ำ 10 ลิตร) จำนวน 3 ซ้ำ ใส่ลูกปลาที่ฟักใหม่ จำนวนตู้ละ 200 ตัว เลี้ยงโดยไม่ให้อาหาร พบว่าลูกปลาบู่ทรายเริ่มตายที่ 84 ชั่วโมงหลังจากฟักออกเป็นตัว และตายหมดที่ 130 ชั่วโมง ที่อุณหภูมิ 27.0-30.5 °C

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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 and Amornsakun and Hassan, 1996). 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 and Eda *et al.*, 1994).

To date, no research on sand goby 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 sand goby. These may provide baseline information useful for optimization of large scale culture and ultimately for the culture management of this fish in future.

Materials and Methods

Larvae of sand goby were produced by controlled natural spawning. The sexually mature fish were cultured in earthen ponds with stocking density of 2 fishes/m^2 and fed with fresh fish at 1% of body weight every second day. Concrete plates as fish nesting were prepared for spawing activities and egg observation at the plates was made once a day at 0800 hr. Fertilized eggs were transferred to an aqurium hatching tank. The water depth level in the spawning pond was 1.5 meter and 10% water replacement was carried out daily.

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. 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). The specimens were fixed in 10% buffered formalin solution.

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. The mouth height was calculated by multiplying the upper jaw length by $\sqrt{2}$ (Shirota, 1970). Specimens were fixed in 10% buffered formalin solution.

Start-of-feeding experiment

The experiment was carried out using 15-liter aquaria (water volume 10 liters) containing 1000 larvae aged 1.5 days post hatching (just before the mouth opened). They were fed with rotifer (100 μm, width) at a density of 5-10 individual/ml. Twenty larvae were collected at random from the aquarium at 2-hourly intervals, and preserved in 10% buffered formalin solution. 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

Newly hatched larvae were 2.39±0.12 mm in total length (mean±SD, n = 20), and had yolk sacs of 55.32±14.85 μm^3 volume (mean±SD, n = 20) (Figure 1 and Figure 2). The yolk sacs were completely absorbed within 82 hr (3.4 days) after hatching at water temperatures of 27.0-30.5 ^{o}C. Observation of 20 larvae showed that after 36 hr the yolk size was about 54.68 % of initial size.

All larval mouths were open 36 hr after hatching but were not yet functioned (2.86±0.97 mm TL), and measured 332.29±17.76 µm in mouth height. At 80 hr after hatching, the fish started feeding on the rotifer at which time the yolk sac remained at 6.16 % of its initial volume. Some digestive tracts developed fully within

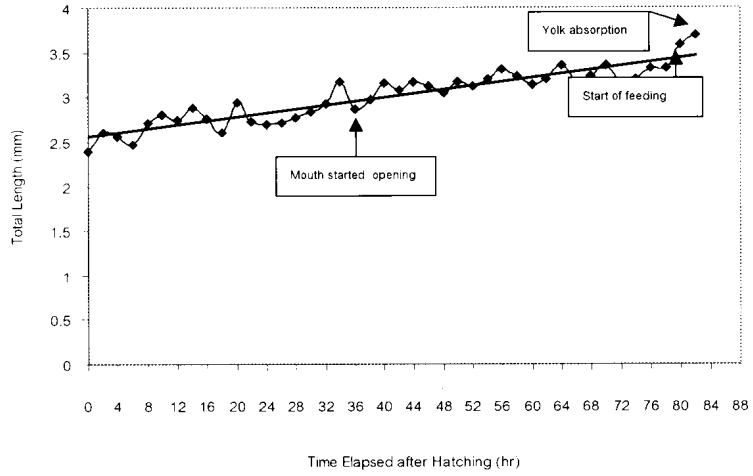

Figure 1. Increase in length of sand goby larvae at elasped time after hatching

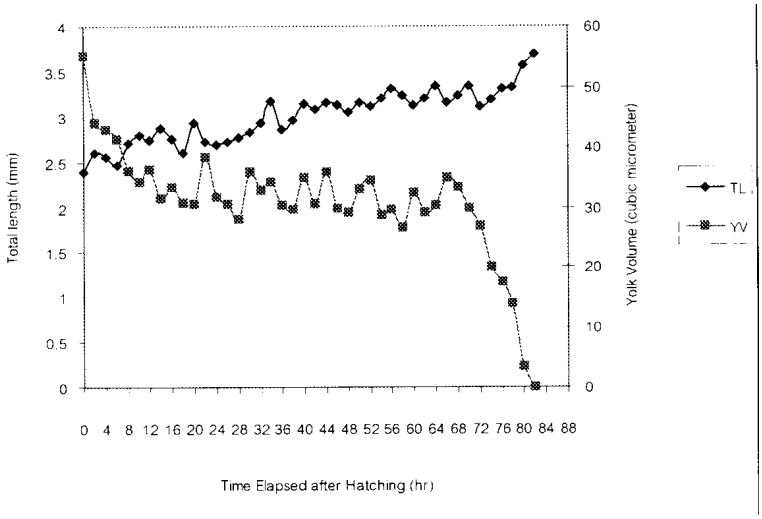

Figure 2. Total length (TL) and yolk absorption of larval sand goby at elapsed time after hatching. YV: Yolk volume

80 hr after hatching at water temperatures of 27.0-30.5 °C, measured 549.69±47.94 µm in mouth height (Figure 3), 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 individual, 0.6 individual 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.

Without feeding, the larval sand goby started to die in all experiments at 84 hr and totally died within 130 hr (5.4 days) after hatching (Figure 4). Water temperature ranged from 27.0 to 30.5 °C.

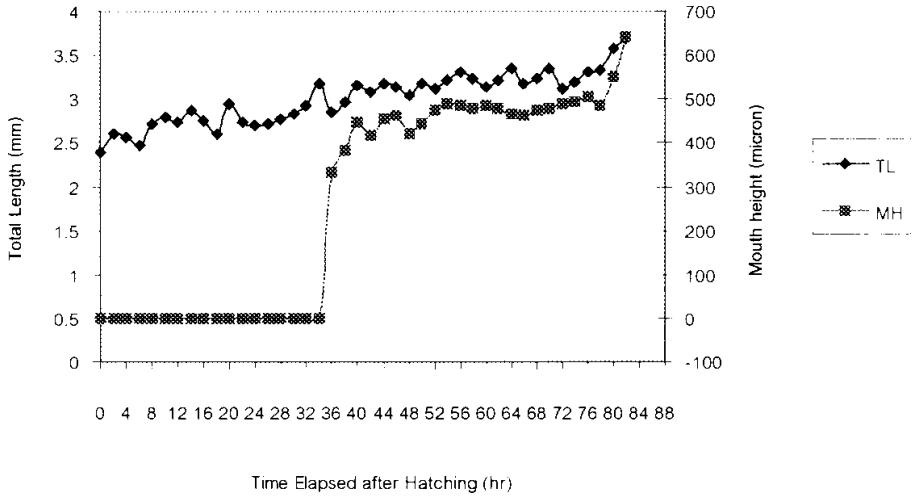

Figure 3. Total length (TL) and development of mouth opening of larval sand goby at elapsed time after hatching. MH: Mouth height

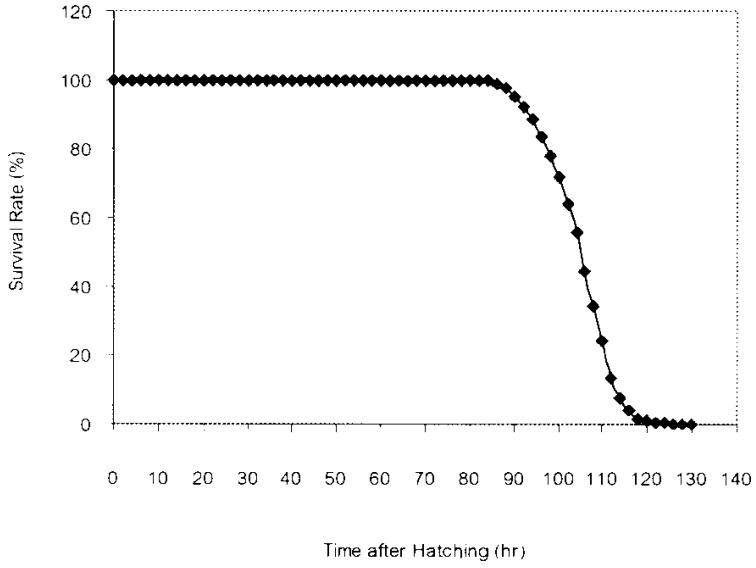

Figure 4. Survival rate of larval sand goby after hatching without feeding at 27.0-30.5 °C

Discussion

The yolk absorption period for newly-hatched larval sand goby (3.4 days after hatching) was found to be similar to that of a number of fish, both marine and freshwater types. Amornsakun *et al*. (1997) reported the yolk absorption of larval green catfish, *Mystus nemurus*, as being complete at 3 days after hatching at water temperatures of 25-30 °C. Houde *et al*. (1976) reported the yolk absorption of larval white mullet, *Mugil curema* Valenciennes, as being 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 36 hr about 54.68% of yolk remained and the mouths of all larval sand goby had already opened but were not yet functioning. The yolk sac remaining at the time first feeding of sand goby larvae was less than that in green catfish and red-tail catfish. The result of the present study reveals that sand goby larvae start to feed supplied rotifer at 80 hr after hatching (549.69±47.94 µm, mouth height) when their yolk sacs remain at 6.16% of its initial volume. Comparatively, Amornsakun *et al*. (1997) reported that the green catfish started feeding on *Moina* at 52 hr after hatching when the yolk sac remained at 31.20 % of its initial volume. 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 (Amornsakun, 1999). The larval grey mullet, *Mugil cephalus*, growth curve consisted of an increase on the first day, which coincided 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 in the larval life 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 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 18.7 % of mouth height (549 µm height) of the larval sand goby in this study (3.58±0.61 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) and Amornsakun (1999). Larval sand goby is considered as a fish species difficult to rear in early life stages since certain food organisms with appropriate size suitable to larval mouth (18.7 % of mouth height).

The start of feeding, with a mouth height (549 µm) of larval sand goby is similar to green catfish and red-tail catfish but the time of first feeding is later than in those fish. 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 (Amornsakun *et al*,.1997 and Amornsakun, 1999). On the contrary, the mouth height at first feeding of larval sand goby 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*, 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 sand goby 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 sand goby 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. 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 hatch. The number of rotifers in the milkfish gut ranged from 1-4 individual/larva.

Without feeding, the larval sand goby become debilitated and eventually started to die off in all experiments at 84 hr after hatching. A catastrophic mortality by 50 % was observed at 106 hr after hatching and all died off within 130 hr after hatch 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 and Holm, 1986). Mortality of unfed sand goby larvae is similar to that of other starved fishes about 5 to 7 days after hatching, depending on the species. For example, in the northern anchovy, *Engraulis mordax*, mortality was observed on the sixth day after hatching (Lasker *et al*., 1970), in the grey mullet, *Mugil cephalus*, on the seventh day after hatching (Kuo *et al*., 1973), in the milk fish, *Chanos chanos*, on the sixth day after hatching (Chaudhuri *et al*., 1978), in the larval dragonets, *Repomucenus* sp., on the fifth to seventh day after hatching (Eda *et al*., 1993) and in the seabass, *Lates calcarifer*, mortality was observed on the fifth day after hatching (Hassan and Amornsakun, 1996). Larval sand goby is able to slightly maintain its survival through starvation (5.4 days after hatching), shorter than red-tail catfish (8 days after hatching), possibly owing to its large yolk sac (1443.17 μm^3) (Amornsakun, 1999 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 sand goby are like other larvae in that after yolk is completely absorbed their mortality becomes pronounced, particularly 2 days after absorption. 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.

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