Yolk-sac absorption, mouth size development, and first exogenous feeding of Sultan fish, *Leptobarbus hoevenii*

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Abstract. Sultan fish, *Leptobarbus hoevenii* is an important species for aquaculture in several Southeast Asian countries, including Thailand and Malaysia. However, knowledge on its yolk absorption, mouth size development, and first food ingestion timing is still lacking up-to-date. This information on the correct feeding of the *L. hoevenii* larvae are crucial to farmers. The present study hence examined these parameters in the *L. hoevenii*. The newly hatched *L. hoevenii* larvae were obtained through natural spawning with the aid of chemicals injection, and sampled consecutively every 2 hours to measure their yolk volumes, mouth height, and to confirm the ingestion time of the first *Moina* into the larval gut. Also, a starvation experiment was conducted to detect the larval point-of-no-return (PNR). It was found that the yolk sac volume of the newly hatched *L. hoevenii* larvae was 77.51 μm, and it was completely absorbed at 108 hours after hatching (hAH). The larval mouth has first opened at 36 hAH (mouth height 215±22.59 μm) but the larvae only commenced first exogenous feeding on *Moina* (approximately 207 μm in width) at 62 hAH, when its mouth height reached 372.91±79.11 μm. The *L. hoevenii* larvae required about 18 hrs from 62–80 hAH, to adapt themselves to feed on the given *Moina*, and the PNR was estimated to happen at 70–72 hAH. It was recommended that *Moina* should be given to the *L. hoevenii* larvae best within 62–72 hAH, at the rearing water temperature of 27 to 29°C.
Key Words: nutritional transition period, optimum first feeding timing, larval rearing, *Moina*, PNR.

Introduction. Sultan fish, *Leptobarbus hoevenii*, is a cyprinid native to lakes and rivers in Malaysia, Indonesia, Laos, Cambodia, Vietnam, and Thailand (Mohsin & Ambak 1983; Roberts 1989; Rainboth 1996; Vidthayanon et al 1997; Kottelat 2001). According to Tee et al (1989), *L. hoevenii* contains high concentrations of protein, vitamin B, and some minerals, including calcium, phosphorus, and iron, and it is recommended for human consumption. Therefore, it is an important freshwater fish species for the inland fisheries in these countries, and now it has become one of the targeted species for aquaculture, due to the high market demand. Indeed, in Malaysia, the aquaculture production of *L. hoevenii* has steadily increased in 2015–2018 from 923 to 1,771.28 tonnes (Fisheries Department of Malaysia 2015–2018).

The artificial seed production of *L. hoevenii* has been succeeded since the 1980s (Meenakarn 1986; Saidin et al 1988). However, published information on its larval biology, which is essential for the seed production techniques improvement, is still very limited up-to-date. In fish larval rearing, knowledge on the larval early developments in relation to their first exogenous feeding, especially yolk absorption, mouth size development, and the timing of first ingestion, is essential to the farmers to feed the larvae correctly. Based on this information, the optimum feeding timing and the suitable size of food for the larvae can be confirmed (Blaxter & Hempel 1963; Shirota 1970; Nash et al 1974; Bagarinao 1986; Yin & Blaxter 1987; Doi & Singhagraiwan 1993; Eda et al 1994; Amornsakun & Hassan 1996; Amornsakun et al 1997, 2002, 2004, 2005). Unfortunately, such information on *L. hoevenii* is still not available (Au et al 2020).

The present study, therefore, was aimed to examine the yolk absorption, mouth size development, and the timing of first ingestion in *L. hoevenii* larvae. This baseline information can contribute to establish a guideline for the feeding of *L. hoevenii* at early larval stage.

Material and Method

Location of experiment and source of fish samples. This study was conducted in the Inland Aquaculture Research and Development Regional Center 12 in Songkhla, Thailand. The newly hatched larvae of *L. hoevenii* provided by this center were produced through natural spawning, but with the aid of chemical injections to induce spawning. In brief, the selected *L. hoevenii* broodstock, both female and male (sex ratio 1:1), were injected with Suprefact (20 μg kg^{-1}) and Motilium (5 mg kg^{-1}), then released into a fine mesh cage fitted within a concrete tank (3.5 tonnes capacity, 4 m length × 2 m width × 1 m height), prepared for the natural spawning. After the eggs were spawned, the broodstock were removed to another nursery tank for recovery, while the eggs were maintained until hatching and continued to rear within the fine mesh cage. The water temperature in the rearing tank was 27–29°C from morning to evening. Flow-through water was provided to this tank along the course of rearing to maintain the water quality. Subsequently, the larvae were sampled from time to time, to examine and measure for its yolk volume, mouth height, and ingestion ratio on *Moina*, following the methods as described.

Yolk volume and mouth height measurements. The *L. hoevenii* larvae were sampled after hatching and consecutively every 2 hours to measure their yolk volumes and mouth height using a light microscope with a micrometer eyepiece. For each measurement, 20 specimens were used. Subsequently, the yolk volumes were calculated following the formula $4/3 \times \pi \times (R_1/2)^2 \times R_2/2$ by Bagarinao (1986), where R_1=minor axis and R_2=major axis. The mouth height was calculated by multiplying the upper jaw length by $\sqrt{2}$, following Shirota (1970).

Observation of gut content. In order to confirm the first feeding timing of *L. hoevenii*, gut content was analyzed. 500 larvae at 1.5 day after hatching were randomly sampled from the larval stock tank and placed into a 15 L aquaria with 10 L water, and they were fed with *Moina* sp. (approximately 207 μm in width) at the density of 10 individuals mL^{-1}. Subsequently, 20 larvae were randomly sampled from the aquaria consecutively every 2 hours, dissected and observed under a dissecting microscope to confirm the presence of *Moina* in the larval gut (Pechmanee et al 1986). Such experiment was conducted in triplicates hence the data mean was calculated and used as the representative values.

Impact of starvation on larval survival. This experiment was conducted to assess the impact of starvation on the survival of *L. hoevenii* larvae, once its yolk was exhausted completely. In this experiment, 300 newly hatched larvae were randomly sampled from the larval stock tank into a 15 L aquaria with 12 L of water. No *Moina* was provided to the larvae, and the number of dead larvae was measured every 2 hrs to assess its survival ratio, until the larval complete mortality occurred (Fukuhara 1987). This experiment was conducted in triplicates hence the data mean was used as the representative values.

Results and Discussion

This is the first report on the early development of *L. hoevenii* larvae in relation to its first exogenous feeding. Figure 1 shows the yolk absorption, mouth height development, average number of *Moina* ingested, and survival without feeding of the *L. hoevenii* larvae from hatching until 116 hours after hatching (hAH). Larval morphology of the *L. hoevenii* at stages of newly hatched, mouth first opened, prior to first exogenous feeding, active feeding on *Moina*, and complete yolk absorption are shown in Figure 2. At hatching, the yolk volume of *L. hoevenii* was 77.51±9.6 μm^3 (Figure 2A), and it was absorbed rapidly with the larval growth. At 36 hrs after hatching (hAH), the yolk volume was only 20.10±12.7 μm^3, indicating that approximately 74.1% (absorption rate at 2.06% h^{-1}) of it has already been absorbed (Figure 1A, 2B). At this stage, the larval mouth first opened (mouth height 215±22.59 μm) (Figure 1B, 2B), but no *Moina* was observed in the gut (Figure 1C). At 62 hAH (2.6 days), the mouth height was 372.91±79.11 μm, which was approximately 73.5% larger than at 36 hAH, and *Moina* was first observed in the gut of the *L. hoevenii* larvae (Figure 1B, 1C, 2C, 2D), indicating the commencement of the first exogenous feeding. According to Meenakarn (1986), the first feeding of *L. hoevenii* should be provided at day 3 as the larvae will be ready to commence feeding 2 days after hatching (water temperature at 25 to 28°C). Termvidchakorn & Hortle (2013) also reported that *L. hoevenii* larvae in the wild commenced exogenous feeding on zooplanktons within the first 3 days after hatching (water temperature 26-29°C). Therefore, the findings of the present study are in agreement with those by Meenakarn (1986) and Termvidchakorn & Hortle (2013). Nevertheless, a different feeding procedure was mentioned by Truong et al (2003). According to Truong et al (2003), milk powder with *Moina* can be fed to the *L. hoevenii* larvae earlier from one day after hatching. As the particle size range of milk powder is usually 150–200 μm (Sharma et al 2012), it is logical to believe that the first exogenous feeding in the *L. hoevenii* larvae may happen as early as their mouth has first opened at 36 hAH. To elucidate this hypothesis, a further study is necessary to identify the timing of first exogenous feeding in the *L. hoevenii* larvae, based on various sizes of food given.

In this study, the *L. hoevenii* larval yolk sac was not completely absorbed (yolk volume 13.93±4.6 μm^3; remained approximately 18% of its initial size) when the larvae commenced first exogenous feeding at 62 hAH (Figure 1A, 1C), its yolk was completely absorbed at 108 hAH (Figure 2E). These results confirmed that the *L. hoevenii* larvae were capable of mixed feeding. Mixed feeding is referred to any combination of nutrient acquirement, endogenous, absorptive or exogenous (Balon 1986). In fact, many freshwater fish species were also reported being capable of mixed feeding, but the duration can be varied among different species. For example, the marble goby, *Oxyeleotris marmoratus* started to feed on rotifer at 80 hAH (yolk volume remained only 6.16% of its initial volume), and its yolk was completely absorbed at about 82 hAH (Amornsakun et al 2002); the red-tail catfish, *Mystus wyckioides* began to feed on *Moina* at 64 hAH (yolk volume remained 13.03%), but the yolk exhausted completely at about 103 hAH (Amornsakun 1999); the Siamese gourami, *Trichogaster pectoralis* commenced feeding on rotifer at 72 hAH (yolk volume remained 32.21%), but its yolk was completely absorbed at 108 hAH (Amornsakun et al 2004); the climbing perch, *Anabas testudineus* commenced first feeding at 32 hAH (yolk volume remained 52.20%), while its yolk was completely absorbed at about 91 hAH (Amornsakun et al 2005). According to Sanderson & Kupferberg (1999), mixed feeding allows fish to continue depending on its endogenous yolk reserve during its adaptation, while learning to feed exogenously. In the present study, the average number of the *Moina* ingested by the *L. hoevenii* slowly increased from 62 to 80 hAH, the number then increased rapidly after 80 hAH (Figure 1C), suggesting that the *L. hoevenii* larvae were adapting themselves to the exogenous feeding of *Moina*. These findings supported the study of Sanderson & Kupferberg (1999).

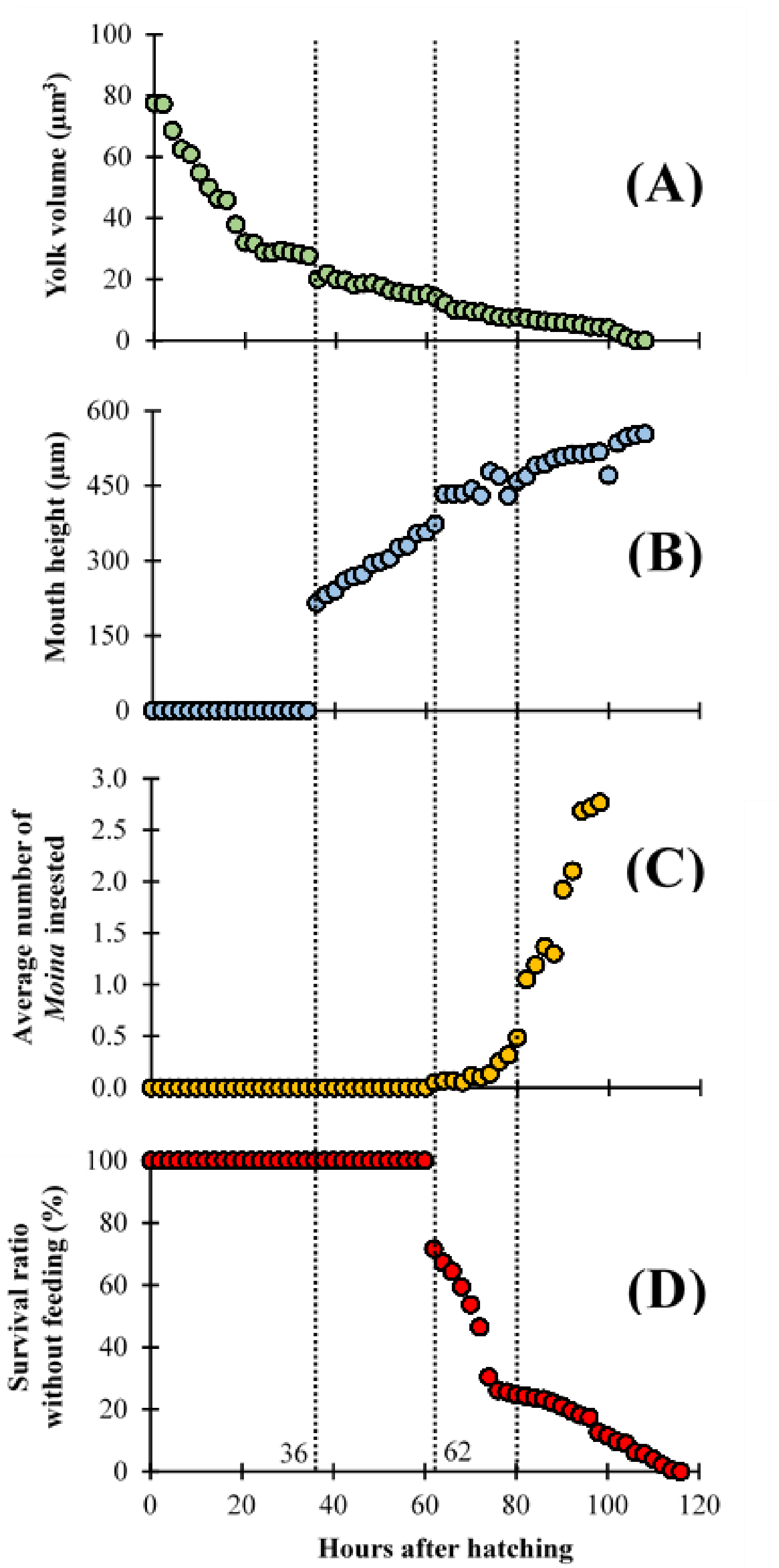

Figure 1. The yolk-sac absorption (A), mouth height development (B), average number of *Moina* ingested (C), and survival ratio without feeding of the *Leptobarbus hoevenii* from hatching until 116 hAH. Each plot in (A) and (B) shows the mean values from 20 specimens observed. In (C), each plot represents the mean values from 60 specimens observed. In (D), each plot shows the mean values observed from triplicate groups of larvae.

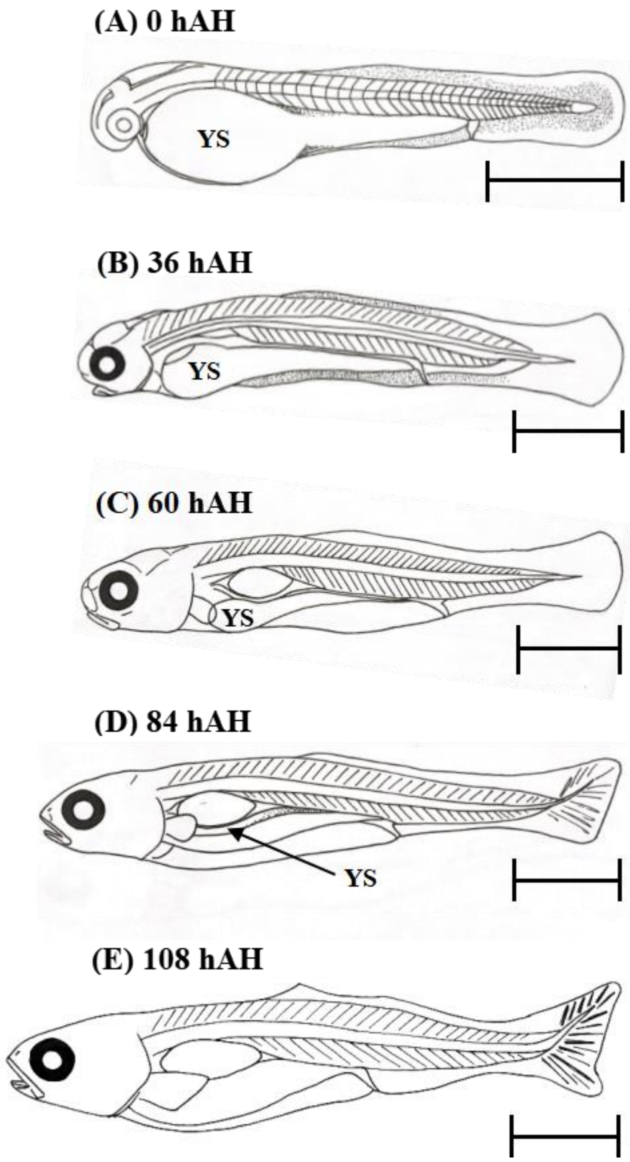

Figure 2. The larval morphology of *Leptobarbus hoevenii* at the stages newly hatched (A), mouth first opened (B), prior to the first exogenous feeding on *Moina* (C), active feeding on *Moina* (D), and completion of yolk absorption (E). Scales: 1 mm; YS: yolk-sac.

The point-of-no-return (PNR) is defined as the threshold point during progressive starvation when 50% of the fish larvae are still alive but they are too weak to feed even if food become available, and this point is also called as the "irreversible starvation" (Blaxter & Hempel 1963). In the present study, mortality occurred drastically when food was not provided to the larvae from 62 to 80 hAH (survival ratio dropped from 100% to 24.7%) (Figure 1D), and the PNR of the *L. hoevenii* larvae was estimated to happen at 70–72 hAH, when the survival ratio remained at 53.7±18% and 46.7±17%, respectively. Therefore, it is suggested that food should be given to the *L. hoevenii* larvae best no later than 70 hAH. Nevertheless, the effect of delayed feeding to the survival of *L. hoevenii* larvae should be examined in a future study to elucidate this hypothesis.

Conclusions. It was concluded that the yolk sac volume of the newly hatched *L. hoevenii* larvae was 77.51 μm; it was absorbed rapidly until the larval mouth has first opened at 36 hAH, and was completely absorbed at 108 hAH. At 62 hAH, the *L. hoevenii* larvae commenced the first exogenous feeding on *Moina* (approximately 207 μm in width) when their mouth height reached 372.91±79.11 μm. The *L. hoevenii* larvae required about 18 hrs from 62–80 hAH, to adapt themselves to feed on the given *Moina*, the PNR was estimated to happen at 70–72 hAH. It was recommended that *Moina* should be given to the *L. hoevenii* larvae best within 62–72 hAH at the rearing water temperature of 27 to 29°C.

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