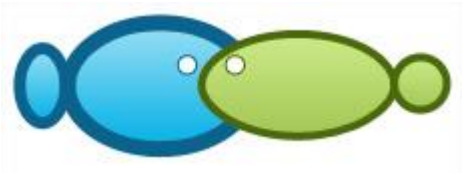

Observation on the embryonic development of Sultan fish, *Leptobarbus hoevenii*

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Abstract. Sultan fish, *Leptobarbus hoevenii*, is a popular freshwater fish species that is important to the aquaculture industry in some Southeast Asian countries, including Malaysia and Thailand. This study examined the embryonic development of *L. hoevenii* in order to fill in the biological knowledge gap and to provide a baseline information to fish farmers for the operations of mass seed production. The fertilized egg of *L. hoevenii* was obtained through natural spawning with the aid of hormones injection. Egg specimens were sampled randomly for the embryonic development examination. At a water temperature of about 28°C, the egg fertilization ratio was 85.4%. The egg developed through the cleavage period, morula and gastrula stages, the segmentation periods, and the complete embryo formed at 18 hrs 11 minutes after fertilization (AF); some newly hatched were already seen at this stage. The egg hatching event completed at 22 hrs and 44 minutes AF, and the egg hatching ratio was 87%. Evaluation of the impacts of the water parameters (including temperature), ambient (e.g. water flow and lighting) and broodstock conditions (e.g. age and nutritional status) on the embryonic development duration in *L. hoevenii* is recommended for future studies.
Key Words: Jelawat, egg incubation, fertilization, hatching, temperature.

Introduction. Sultan fish, *Leptobarbus hoevenii* (also commonly known as Jelawat), is a native cyprinid from some Southeast Asian countries, including Malaysia and Thailand (Mohsin & Ambak 1983; Vidthayanon et al 1997). It is one of the high value freshwater fish species, as its nutritious qualities are recommended for the human consumption (Tee et al 1989). For that reason, the aquaculture of *L. hoevenii* has been established (Meenakarn 1986; Saidin et al 1988). Indeed, in Malaysia, the aquaculture production of *L. hoevenii* has been increasing steadily in 2015–2019 from 923 to 2,100.39 tonnes, with a wholesale value in 2019 worthing approximately USD 5 million (Fisheries Department of Malaysia 2015–2019). Nevertheless, knowledge on the biology of *L. hoevenii* which is important for improving its artificial reproduction techniques is very scarce. Recently, Au et al (2020) has reviewed the feeding and nutrient requirements in different life stages of *L. hoevenii*. The broodstock reproductive characteristics and the early larval development in relation to the first exogenous feeding of *L. hoevenii* also have been reported (Srithongthum et al 2020a,b). In addition, Lim et al (2021) reported the vision-mediated feeding behaviour in the early juvenile *L. hoevenii*, while Mohamad et al (2021) demonstrated the gill modifications in *L. hoevenii* to cope with the high environmental temperature and low pH stress for survival. However, there is still no published information on the embryonic development of *L. hoevenii*.

Embryonic development or embryogenesis is referred to the process by which the embryo forms and develops. In fish, embryonic development starts when the fertilized egg developed through the cleavage period (stages of 2, 4, 8, 16 and 32 cells), stages of

morula, blastula, gastrula, epiboly, embryo formation and finally hatching (Aral et al 2011), while the duration of this process can be varied among different species (Kolm & Ahnesjö 2005). Therefore, the objective of this study was to examine the embryonic development of *L. hoevenii* to fill in the knowledge gap. Such knowledge serves as a baseline information to the fish farmers in their operations of mass seed production of *L. hoevenii*.

Material and Method

Artificial breeding of L. hoevenii. This study was conducted in the Inland Aquaculture Research and Development Regional Center 12, Songkhla, Thailand. The *L. hoevenii* broodstock (1 individual for each female and male) (Figure 1a) were selected from the earthen pond in the center then measured for their total length (TL) and body weight (BW). The TL and BW of the female and male were 39 cm and 800 g, and 38 cm and 850 g, respectively. Subsequently, the fish were injected with Suprefact (20 µg kg^{-1} of body weight) and Motilium (5 mg kg^{-1} of body weight) (Figure 1b) then released into a net cage (60 cm width × 2 m length × 1 m depth, with a 1 inch mesh size), prepared inside a fine mesh cage that been fitted into a 3.5 tons concrete tank (2 m width × 4 m length × 1 m depth) for spawning. Water spray was provided over the net cage to imitate rain fall, and aerations were provided (Figure 1c). The spawning event completed 5 hours after the injection; the broodstock were transferred with the net cage to an earthen pond for recovery, while the spawned egg remained inside the fine mesh cage for incubation.

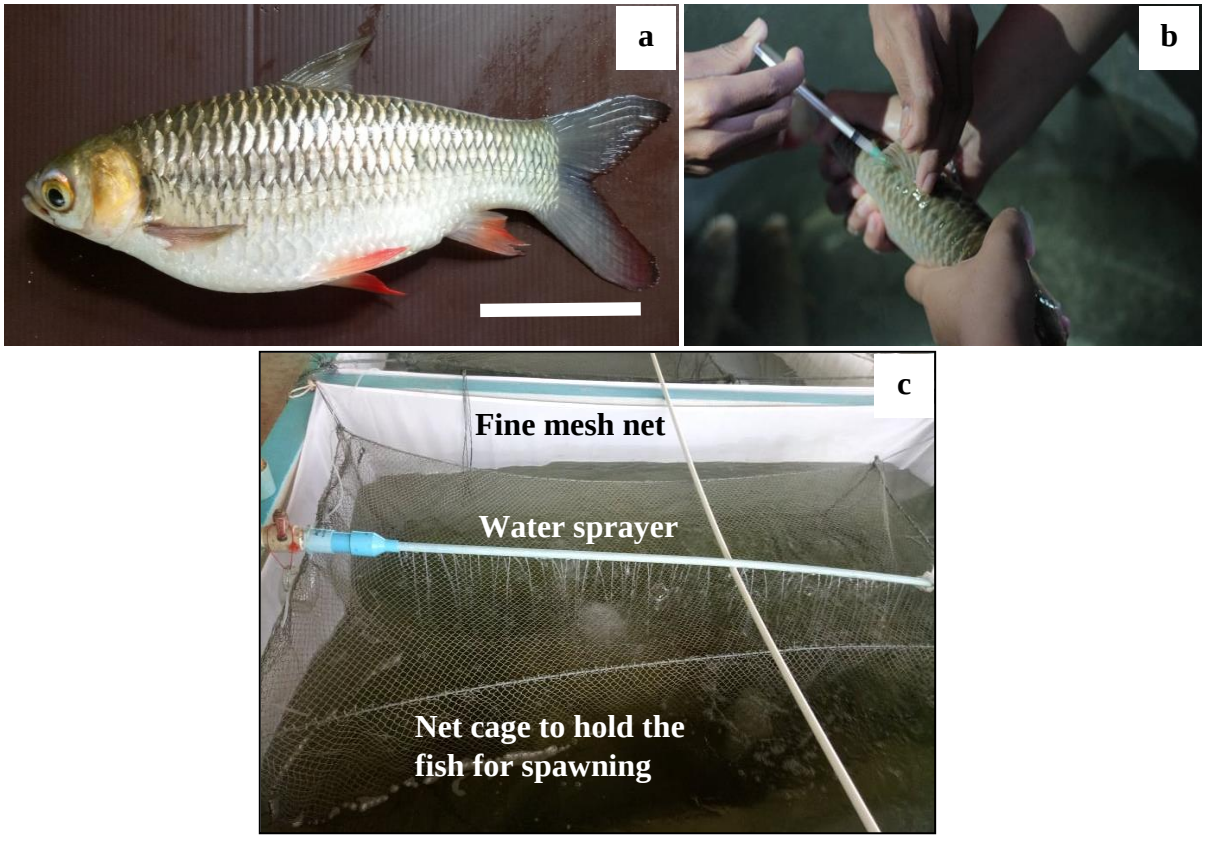

Figure 1. (a) *Leptobarbus hoevenii* broodstock selected for breeding in this study, scale 10 cm; (b) injections of Suprefact and Motilium were done in the *Leptobarbus hoevenii* muscle near to its dorsal fin; (c) concrete tank setup for the breeding and egg incubation of *Leptobarbus hoevenii* (original).

Experimental procedures. The fertilized egg of *L. hoevenii* were sampled from time to time for examination, in order to identify the egg developmental stages, under a compound microscope (Olympus Brand, CHS Model). In each examination, 20 egg specimens were sampled. The egg diameter was measured by an eyepiece with a

micrometer. Through this examination, there were recorded the duration required for the egg to reach each the various developmental stages (Kimmel et al 1995), until hatching. Photos of each developmental stage were also taken through the microscope, using a digital camera (Olympus Brand, Tough Model). From the fish spawning until the egg hatching, the water temperature in the tank was about 28°C. The egg fertilization and hatching ratios were 85.4 and 87%, respectively.

Results and Discussion. This is the first report on the embryonic development of *L. hoevenii*. In this study, the egg fertilization and hatching ratios of *L. hoevenii* were considerably high (>80%), in a water temperature of about 28°C. The embryonic developmental process examined in this study was assumed to be valid and reliable. The embryonic development of *L. hoevenii* is shown in Figure 2, and the timings for reaching the different developmental stages are summarized in Table 1.

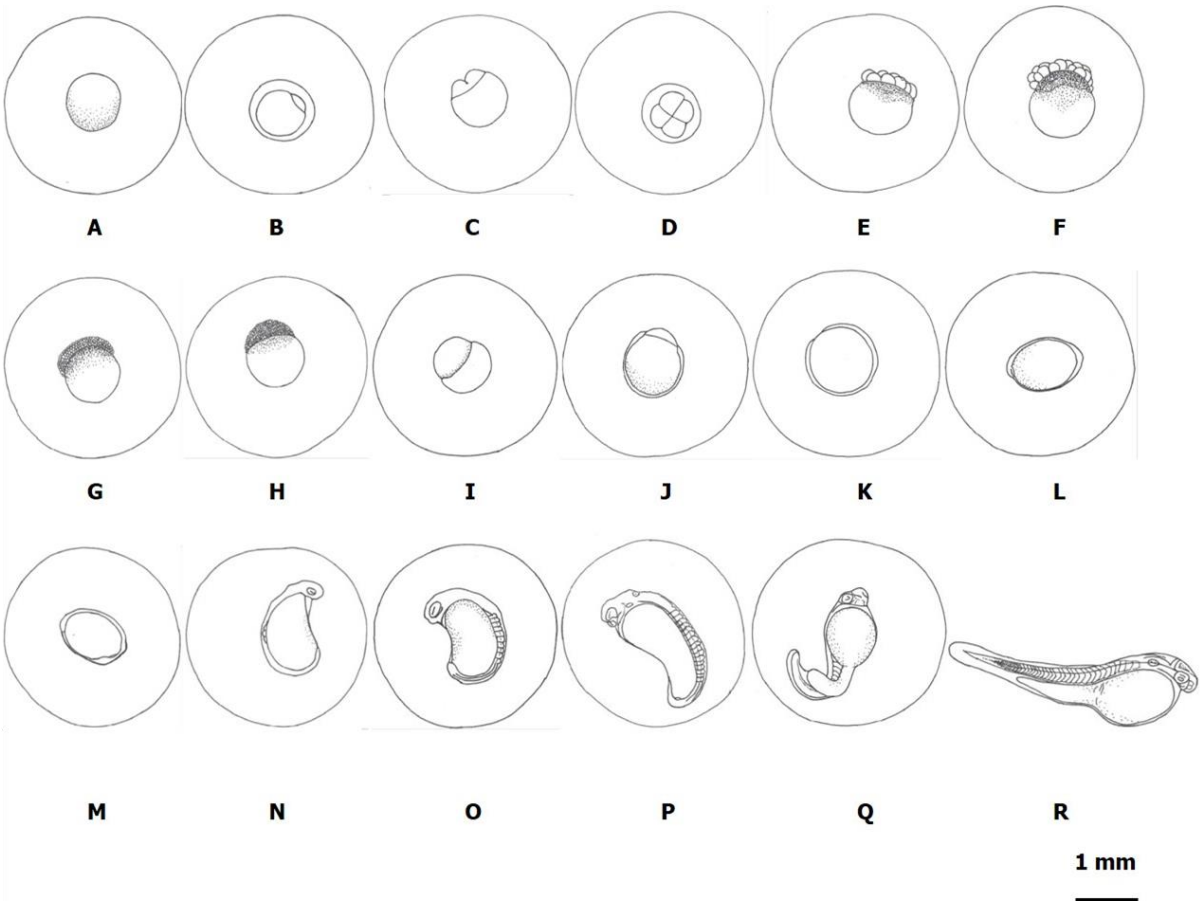

Figure 2. Embryonic developmental stages of *Leptobarbus hoevenii* observed in the present study. A-Fertilized egg; B-1 cell stage; C-2 cells stage; D-4 cells stage; E-8 cells stage; F-16 cells stage; G-Early morula; H-Late morula; I-Late blastula/ gastrula; J-75% epiboly stage; K-95% epiboly stage; L-100% epiboly stage; M-Tail bud formed; N-3-somite; O-10-somite; P-17-somite; Q-Embryo formed; R-Newly hatched larvae.

In brief, the egg of *L. hoevenii* has developed into 1 cell stage 20 minutes after fertilization (AF). In 2 hrs 52 minutes AF, the embryonic development has progressed through the stages of 2, 4, 8 and 16 cells to the early morula stage and reached the late morula stage. The egg has entered the gastrula stage in 6 hrs 53 minutes AF and this stage completed when the tail bud was formed, 8 hrs 37 minutes AF. Then, it continued to develop through the segmentation period (stages of 3-, 10- and 17 -somites), and the embryo was formed 18 hrs 11 minutes AF. At this stage, some newly hatched larvae were already seen in the incubation tank. All *L. hoevenii* larvae hatched out from the egg

after 22 hrs and 42 minutes of incubation time. Such embryonic developmental pattern was similar with those described for most of the freshwater fish species (Aral et al 2011). The findings of this study on the timing of egg hatching were similar with those reported by Meenakarn (1986), but different from those of Truong et al (2003).

Table 1

Embryonic developmental stages of *Leptobarbus hoevenii* with its timing and duration

Stages	*Timing*	*Duration*
A) Fertilized egg	0:00	
B) 1 cell	0:20	20 min
C) 2 cells	0:50	30 min
D) 4 cells	1:20	30 min
E) 8 cells	1:45	25 min
F) 16 cells	1:47	2 min
G) Early morula	1:56	9 min
H) Late morula	2:52	56 min
I) Late blastula/Gastrula	6:53	4 hrs 1 min
J) 75% epiboly stage	7:17	24 min
K) 95% epiboly stage	7:45	28 min
L) 100% epiboly stage	8:03	18 min
M) Tail bud formed	8:37	34 min
N) 3-somite	10:02	1 hr 25 min
O) 10-somite	10:15	13 min
P) 17-somite	14:13	3 hrs 58 min
Q) Embryo is formed/some larvae hatched	18:11	3 hrs 58 min
R) Egg hatching completed	22:44	4 hrs 33 min

In the report of Meenakarn (1986), the egg hatching of *L. hoevenii* occurred 15–18 hrs AF, at a water temperature of 25–28°C, but the informations on the egg fertilization and hatching ratios were not mentioned. On the other hand, Truong et al (2003) reported that the *L. hoevenii* larvae hatched out from the egg approximately 13 hrs AF, at a water temperature of 29–30°C, but the egg fertilization ratios were only 5–72.5% in 10 breeding trials (no information on the hatching ratio). The short egg incubation duration in the case of Truong et al (2003) could be due to the higher water temperature, of 29–30°C, which could accelerate the embryonic development, but could also cause mortality to the *L. hoevenii* egg, as mentioned by Meenakarn (1986). Apparently, the findings of the present study, together with those of Meenakarn (1986), indicated that 28°C could be the optimal water temperature for the incubation of *L. hoevenii* egg. Further study should be conducted to elucidate this hypothesis.

Conclusions. The embryonic developmental pattern of *L. hoevenii* was similar with those described on most of the freshwater fish species. The *L. hoevenii* embryo formed 18 hrs 11 minutes AF. Some newly hatched larvae were already seen in the tank at this stage. All *L. hoevenii* larvae hatched out from the eggs 22 hrs 42 min AF at a water temperature of about 28°C.

Acknowledgements. This study was financially supported by the research grants - SAT6103004S and GUG0380-1/2019, provided by the Prince of Songkla University, Pattani campus, and the Research and Innovation Management Center of Universiti Malaysia Sabah, respectively. The first author thanks the Prince of Songkla University (PSU) and the Universiti Malaysia Sabah (UMS) for the scholarships (PSU, the Discipline of Excellence – DoE scholarship; UMS, the Postgraduate Aid Scheme – SBP) which supported her study in the PSU-UMS Dual Master Degree Program.

Conflict of interest. The authors declare no conflict of interest.

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Received: 02 March 2021. Accepted: 12 May 2021. Published online: 24 May 2021.
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How to cite this article:
Srithongthum S., Au H. L., Amornsakun T., Musikarun P., Fatihah S. N., Halid N. F. A., Lim L. S., 2021 Observation on the embryonic development of Sultan fish, *Leptobarbus hoevenii*. AACL Bioflux 14(3):1359-1364.