Reproductive Characteristics of the Pond-Farmed Sultan Fish
(Leptobarbus hoevenii)

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Abstract
Sultan fish (Leptobarbus hoevenii) is a high value freshwater fish, cultured in some Southeast Asian countries, including Malaysia and Thailand. However, information on its reproductive characteristics is very scarce. This study examined the gonadosomatic index (GSI), fecundity, egg diameter, and determined whether L. hoevenii is a single- or multiple-spawner. Twenty male and female pond-farmed L. hoevenii broodstock were obtained to measure their total length (TL), body weight (BW), and gonad weight to calculate the GSI. Ten females were randomly sampled from the 20 to determine their fecundity. A total of 1,500 eggs were sampled from each female. The egg diameter was measured then its frequency distribution was analyzed to detect the number of egg class group, and to determine whether L. hoevenii is a single- or multiple spawner. The female L. hoevenii examined were 32.2-47.1 cm and 350-1,200 g, while the males were 30.7-45.8 cm and 180-970 g in TL and BW, respectively. All female specimens contained gonads. The potentially smallest mature samples were recorded at 350 g (female) and 180 g (male). GSI for the female and male L. hoevenii were 1.81-12.28 % and 1.03-5.09 %, respectively. The fecundity was 35,467-128,067 eggs, while the highest fecundity was observed in a 1,000 g fish. The observed egg diameter ranged from 500 to 1,855 μm. Two to five groups of egg class were detected, indicating that L. hoevenii is a multiple spawner.

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1. Introduction

The present study was aimed to provide baseline information on the reproductive characteristics of *L. hoevenii*. These reproductive characteristics include the gonadosomatic index (GSI), fecundity, egg diameters, and spawning frequency of *L. hoevenii*. Such information is crucial to the broodstock management and breeding operation of *L. hoevenii* in farms.

The Sultan fish or Jelawat (*Leptobarbus hoevenii*) is a high value cyprinid that can be originally found in some Southeast Asian countries, such as Malaysia, Cambodia, Indonesia, Laos, Vietnam, and Thailand (Mohsin and Ambak, 1983; Roberts, 1989; Rainboth, 1996; Vidhayanon et al., 1997; Kottelat, 2001; Truong et al., 2003). It is an important species for the inland fisheries in these countries as it is rich in protein, some minerals especially calcium, phosphorus, iron, and vitamin B for human consumption (Tee et al., 1989). *L. hoevenii* has recently become a freshwater fish that been targeted for aquaculture. In Malaysia, its aquaculture production has increased from 923 to 2100.39 tonnes in 2015-2019, and the wholesale value in 2019 worth about 21 million Malaysian Ringgit (RM) (Fisheries Department of Malaysia, 2015-2019).

The breeding procedures of *L. hoevenii* can be easily facilitated as its captive breeding has been successfully achieved since the 1980s (Meenakarn, 1986; Saidin et al., 1988; Liao et al., 2000). Recently, the feeding and nutrients requirement of this fish has been studied (Au et al., 2020; Lim et al., 2021). The embryonic and larval development in relation to the first exogenous feeding are also reported (Srithongthum et al., 2020a; 2021). In addition, Mohamad et al. (2021) has reported the modifications of gills in *L. hoevenii* when it strives to survive in the environments with high temperature and low pH.

However, there is still very limited baseline knowledge on its reproduction characteristics, including the gonadosomatic index (GSI), fecundity and egg diameter. It is also unknown whether *L. hoevenii* is a single- or multiple-spawner. Indeed, there are single- or multiple-spawner among the different species of cyprinids (e.g. Rinchard and Kestemont, 1996).

In Songkhla, Thailand, the Inland Aquaculture Research and Development Regional Center 12, supplies *L. hoevenii* seedlings to farmers yearly, through artificial reproduction. In this center, the *L. hoevenii* broodstocks are polycultured with the other cyprinids, mainly the common carp (*Cyprinus carpio*) and Rohu carp (*Labeo rohita*) in the earthen ponds, and they were confirmed in good health condition through the analyses of their length-weight relationships and relative condition factor (Srithongthum et al., 2020b). Therefore, the present study was aimed to determine the GSI, fecundity, egg diameters, and spawning frequency of *L. hoevenii* broodstocks from the Inland Aquaculture Research and Development Regional Center 12. Such information is crucial for the establishment of a guideline in broodstock management and selection for the breeding operation of *L. hoevenii*.

2. Materials and Methods

2.1 Determination of GSI and Fecundity

In December 2018, forty *L. hoevenii* broodstocks (20 individuals of each sex) were randomly sampled from the earthen ponds in the Inland Aquaculture Research and Development Regional Center 12, Songkhla, Thailand. The total length (TL) and body weight (BW) of each fish were measured. Subsequently, the fish were immersed into ice-water, dissected, and the gonads were extracted. The gonads (ovary or testis) were weighed, and the GSI of each fish was estimated by dividing the ovary or testis weight by its body weight (both are in g) and multiplied with 100% (Amornsakun et al., 2011). Among the females, 10 of them were randomly sampled to measure their fecundity, using the gravimetric method. The number of egg that weighed approximately 1% of the ovary weight was counted, then the number was multiplied with the ovary weight (Amornsakun et al., 2011). Subsequently, the fish body weight was plotted against the gonad weight, GSI, or fecundity to determine the strength of linear associations (r) among these parameters, using the MS-Excel computer software. Significant level was at α = 0.05.

2.2 Measurement of Egg Diameter and Frequency Distribution Analysis

It is important to know the egg diameter at mature stages since the increase in egg diameter is associated with egg development (Dorostgoaal et al., 2009; Plaza et al., 2011; Milton et al., 2018). In order to measure the egg diameters, 1,500 eggs were sampled from the ovary of each fish (500 eggs each from the anterior, middle, and posterior parts of the ovary), and measured under a light microscope using an eyepiece micrometer scale to 25 µm. The frequency of the egg diameters was plotted against the egg diameter, then the weighted moving averages was applied to smooth out class range fluctuations and highlight components in polymodal frequency distributions. The moving average formula for this calculation was based on Kawamura et al. (2009) as follows:
\[ N_i = 0.5X_i + 0.25(X_{i-1} + X_{i+1}) \]

**Description:**

\( N_i \): the moving average number of eggs in class range \( i \)

\( X_i \): the number of eggs in class range \( i \)

\( X_{i-1} \) and \( X_{i+1} \): the numbers of eggs in class range \( i-1 \) and \( i+1 \)

As the frequency distributions of the egg diameters are usually skewed and polymodal and the modes can be corresponded to individual diameter-classes, Bhattacharya’s (1967) method was used to separate the normal distribution components from the frequency distribution. Following Bhattacharya’s method, let \( y(x) \) denote the observed frequency in the class with \( x \) as its mid-point and let \( h \) denote the class interval. The log differences, \( \Delta \log y = \log y(x + h) - \log y(x) \), were plotted against \( x \), and the regions where the graph appeared as a straight line with a negative slope was determined. The number of such regions is the number of the normal distribution components (Bhattacharya, 1967).

**Table 1.** The body measurements, GSI, and fecundity of the *L. hoevenii* examined in the present study.

|         | Total Length (TL) | Body Weight (BW) | GSI       | Fecundity (eggs) |
|---------|-------------------|------------------|-----------|------------------|
|         | Min (cm)          | Max (cm)         | Min (g)   | Max (g)          | Min (%) | Max (%) | Min     | Max     |
| Female  | 20                | 32.2 cm          | 350 g     | 1200 g           | 1.81%    | 12.28%  | 35,476  | 128,067 |
| Male    | 20                | 30.7 cm          | 180 g     | 970 g            | 1.03%    | 5.09%   | n/a     | n/a     |

**Figure 1.** The linear relationships between gonad weight, GSI or fecundity (except for male) and the BW of female and male *L. hoevenii*.
Figure 2. The observed frequency distribution of *L. hoevenii* egg diameter with a class range of 50 μm and their moving averages.
Figure 3. Graph of logarithmic differences of the class-frequencies against the mid-point of the classes, following Bhattacharya’s (1967) method.
Normal probability plot analysis is another way for the separation of normal components from a poly-modal frequency distribution. The cumulative % frequencies of the observed relative frequencies % were plotted on the normal probability paper and regions where parts of graph seemed like a straight line with positive slope were determined (Forthofer et al., 2007). Based on Bhattacharyya’s method and the normal probability plot analysis, it could be confirmed whether L. hoevenii was a single- or multiple-spawner (Soetignya et al., 2017).

3. Results and Discussion

According to the observation of Termvidcha-korn and Hortle (2013) on wild specimens of L. hoevenii, fish that weighed 500-600 g were mature, and the 1,000 g mature female may carry 50,000-70,000 eggs. In the present study, the ranges of TL and BW in the female L. hoevenii were 32.2-47.1 cm and 350-1200 g, respectively (Table 1). All females contained ovaries and the GSI was in the range of 1.81-12.28%. The fecundity was 35,467-128,067 eggs; the lowest fecundity was observed in a 700 g fish (GSI 5.1%), while the highest one was observed in a 1,000 g fish. On the other hand, the TL and BW of the male fish ranged from 30.7-45.8 cm and 180-970 g, respectively (Table 1). All males contained testes and the GSI was 1.03-5.09% (Table 1).

In the present study, the correlation coefficient (r) of the ovary weight (r = 0.75414, P < 0.01), GSI (r = 0.5418, P < 0.05) or fecundity (r = 0.7063, P < 0.05) versus the BW in the female L. hoevenii were significantly high (Figure 1). These findings suggested that the larger sized L. hoevenii females (in BW) tended to have better reproductive performance. Based on this outcome, farmers are recommended to always select larger female broodstock (heavier BW) for seed production purpose, in addition to the other common selection criteria, such as the enlarged and rounded belly. On the other hand, unlike the female L. hoevenii, the correlation coefficient (r = 0.6394) between the testes weight and BW of the male L. hoevenii was significantly high (P < 0.01) but that of the GSI versus BW was not (r = 0.0879, P > 0.05). In fact, the GSI values of many male cyprinids did not fluctuate much even during the spawning season as observed in the wild (Muchlisin et al., 2010; Kiran, 2015; Esmaeili et al., 2017). These results suggested that any sizes of the male L. hoevenii can be selected for breeding purpose, as long as the fish has reached the body size of being sexually mature.

The egg diameter observed in the present study was highly variable, ranging from 500 to 1,855 μm. In the moving averages of the frequency distribution of the egg diameter with a class range of 50 μm, the polymodal frequency distribution was evident (Figure 2). In the graphs of logarithmic differences of the class-frequencies against the mid-point of the classes (Bhattacharyya’s method), there were 4 to 5 regions where the graph looks like a straight line with negative slope, except for Fish 5, Fish 9, and Fish 10, where only 2 to 3 of such regions were observed (Figure 3). In the graphs of normal probability plot analysis, the regions where parts of graph appear like a straight line with a positive slope are shown by straight lines, and the cumulative % frequency distributions comprised two (Fish 8), three (Fish 5, Fish 9, Fish 10), four (Fish 2, Fish 3, Fish 4, Fish 6, Fish 7), and five (Fish 1) normal components (Figure 4).

In general, 2-5 modes were found in the polymodal egg diameter frequency distribution of L. hoevenii following Bhattacharyya’s (1967) method, while 2-5 normal modes were also found following the normality probability plot analysis. Such findings were similar to those reported by Soetignya et al. (2017). Soetignya et al. (2017) examined the ovarian development of 124 females of a cyprinid Hampala bimaculata and reported that the polymodal egg diameter frequency distribution showed three modes, which is the typical trait of multiple-spawner. According to Hong et al. (2006), Chen and Tzeng (2009), and Nunez and Duponchelle (2009), the presence of developing oocytes of different sizes in spawned stage characterizes the beginning of one or more new spawning cycles until the end of the breeding season, which differentiates multiple-spawner from single-spawner.
Thus, the results indicated that *L. hoevenii* is a multiple-spawner. In a hatchery, it is strongly recommended that *L. hoevenii* is bred through natural spawning with or without chemicals/hormonal injection, rather than hand-stripping and fertilizing the eggs artificially. The latter method will result in very poor fecundity as the fish will ovulate only a small proportion of their eggs while the remaining eggs may be destroyed during the stripping process (Mylonas and Zohar, 2009).

4. Conclusion

The GSI and fecundity of the female *L. hoevenii* were 1.81-12.28 % and 35,467-128,067 eggs, respectively. The GSI of the male *L. hoevenii* was 1.03-5.09 %. This study first reported the potentially smallest maturity sizes for the female and male *L. hoevenii* at 350 g and 180 g, respectively. Also, the maximum fecundity of *L. hoevenii* was found for the first time to be >100,000 eggs. The *L. hoevenii* egg diameters ranged from 500 to 1855 μm. Two to five egg class groups were detected hence it was confirmed that this fish is a multiple-spawner.

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Authors’ Contributions

All authors have contributed to the final manuscript. The contribution of each author is as follows,
SS, HLA, TA and PM; conducted the measurements and collected the data. WJM and NFAH; managed the data, devised the main conceptual ideas and critically improved the writing of this manuscript draft. GK and LSL; analyzed the data and draft this manuscript. All authors discussed the results and contributed to the final manuscript.

**Conflict of Interest**

The authors declare that they have no competing interests.

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**References**

Al Hafedh, Y. S., Siddiqui, A. Q., & Al-Saiady, M. Y. (1999). Effects of dietary protein levels on gonad maturation, size and age at first maturity, fecundity and growth of nile tilapia. *Aquaculture International*, 7:319-332.

Amornsakun, T., Sriwatana, W., & Promkaew, P. (2011). Some aspects in early life stage of snake head fish, *Channa striatus* larvae. *Songklanakarin Journal of Science and Technology*, 33(6):671-677.

Au, H. L., Lim, L. S., Amornsakun, T., Rahmah, S., Liew, H. J., Musikarun, P., Promkaew, P., Mok, W. J., Kawamura, G., Yong, A. S. K., Shapawi, R., & Mustafa, S. (2020). Feeding and nutrients requirement of Sultan fish, *Leptobarbus hoevenii*: A review. *International Journal of Aquatic Science*, 11(1):3-12.

Bhattacharya, C. G. (1967). A Simple Method of Resolution of a Distribution into Gaussian Components. *Biometrics*, 23(1):115-135.

Chen, K. Y., & Tzeng, W. N. (2009). Reproductive Mode of the Blue-striped Angelfish *Chaetodontoplus septentrionalis* in Northeastern Taiwan. *Zoological Studies*, 48:468-476.

Dorostghoul, M., Peyghan, R., Rapan, F., & Khalili, L. (2009). Macroscopic and microscopic studies of annual ovarian maturation cycle of shirbot *Barbus grypus* in Karoon river of Iran. *Iranian Journal of Veterinary Research*, 10(2):172-179.

Esmaeili, H. R., Choobineh, R., Zareian, H., & Gholamhosseini, A. (2017). Life history traits and gonad histology of an endemic cyprinid fish, mond spotted barb, *Capoeta mandica* from Southern Iran. *Caspian Journal of Environmental Sciences*, 15:97-112.

Fisheries Department of Malaysia. (2015–2019). Annual fisheries statistics. https://www.dof.gov.my/index.php/pages/view/82. (Accessed 20 March 2021).

Forthofer, R. N., Lee, E. S., & Hernandez, M. (2007). Biostatistics: a guide to design, analysis and discovery (2nd Ed.). USA: Elsevier Academic Press.

Honj, R. M., Vaz-dos-Santos, A. M., & Rossi-Wongschowsk, C.L.D.B. (2006). Identification of the stages of ovarian maturation of the Argentine hake *Merluccius hubbsi* Marini, 1933 (Teleostei: *Merlucciidae*): advantages and disadvantages of the use of the macroscopic and microscopic scales. *Neotropical Ichthyology*, 4(3):329-337.

Kawamura, G., Noahara, T., Tanaka, Y., Nishi, T., & Anraku, K. (2009). Near-ultraviolet radiation guides the emerged hatchlings of loggerhead turtles *Caretta caretta* (Linnaeus) from a nesting beach to the sea at night. *Marine and Freshwater Behaviour and Physiology*, 42(1):19-30.

Kirian, B. R. (2015). Study of gonado-somatic index of cyprinid fish, *Salmostoma untrahi* (Day) from Bhadra Reservoir, Karnataka. *International Journal of Research in Environmental Science*, 1:6-10.

Kottelat, M. (2001). Fishes of Laos. Colombo: WHT Publications Ltd.

Liao, I. C., Su, H. M., & Chang, E.Y. (2000). Techniques in finfish larviculture in Taiwan. *Aquaculture*, 200:1-31.

Lim, L. S., Amornsakun, T., Au, H. L., Tuzan, A. D., Liew, H. J., Mukai, Y., Mustafa, S., & Kawamura, G. (2021). Vision-mediated feeding behaviour of early juvenile Sultan fish, *Leptobarbus hoevenii*. *Aquaculture Research*, 52(4):1784-1787.

Luquet, P., & Watanabe, T. (1986). Interaction “nutri-
tion-reproduction” in fish. *Fish Physiology and Biochemistry*, 2:121-129.

Meenakarn, S. (1986). Induced spawning on *Leptobarbus hoevenii* (Bleeker) carried out in Jambi, Indonesia. Collaborative project of The Directorate General of Fisheries, Indonesia and the United States Agency for International Development (Final report of Small-Scale Fisheries Development Project. Cage culture and seed production sub-project. USAID/ARD Project no. 497-0286). Jakarta: The Directorate General of Fisheries.

Milton, J., Bhat, A. A., Haniffa, M. A., Hussain, S. A., Rather, I. A., Al-Anazi, K. M., Hailan, W. A. Q., & Farah, M. A. (2018). Ovarian development and histological observations of threatened dwarf snakehead fish, *Channa gachua* (Hamilton, 1822). *Saudi Journal of Biological Sciences*, 25:149-153.

Mohamad, S., Liew, H. J., Zainuddin, R. A., Rahmah, S., Waiho, K., Ghaffar, M. A., Nhan, H. T., Loh, J. Y., Lim, L. S., Chang, Y., Liang, L., & De Boeck, G. (2021). High environmental temperature and low pH stress alter the gill phenotypic plasticity of Hoven’s carp *Leptobarbus hoevenii*. *Journal of Fish Biology*, 99(1):206-218.

Mohsin, M. A. K., & Ambak, M.A. (1983). Freshwater fishes of Peninsular Malaysia. Kuala Lumpur: Percetakan Nan Yang Muda Sdn. Bhd.

Muchlisin, Z. A., Musman, M., & Siti Azizah, M.N. (2010). Spawning seasons of *Rasbora tawaresnis* (Pisces: Cyprinidae) in Lake Laut Tawar, Aceh Province, Indonesia. *Reproductive Biology and Endocrinology*, 8:49.

Mylonas, C., & Zohar, Y. (2009). Controlling fish reproduction in aquaculture. In G. Burnell & G. Alan (Eds.), New technologies in aquaculture: improving production efficiency, quality and environmental management. (pp. 110-130). USA: CRC Press.

Nunez, J., & Duponchelle, F. (2009). Towards a universal scale to assess sexual maturation and related life history traits in oviparous teleost fishes. *Fish Physiology and Biochemistry*, 35:167-180.

Plaza, G., Espejo, V., Almanza, V., & Claramunt, G. (2011). Female reproductive biology of the silverside *Odontesthes regia*. *Fisheries Research*, 111:31-39.

Rainboth, W. J. (1996). Fishes of the Cambodian Mekong: FAO species identification field guide for fishery purposes. Rome: Food and Agriculture Organization of the United Nations.

Rinchard, J., & Kestemont, P. (1996). Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. I. Morphological and histological features. *Journal of Fish Biology*, 49:883-894.

Roberts, T. R. (1989). The freshwater fishes of Western Borneo (Kalimantan Barat, Indonesia). *Memoirs of the California Academy of Sciences*, 14:1-210.

Saidin, T. B., Othman, A. A. B., & Sulaiman, M. Z. B. (1988). Induced spawning techniques practiced at Batu Berendam, Melaka, Malaysia. *Aquaculture*, 74:23-33.

Soetignya, W. P., Suryobroto, B., Kamai, M. M., & Boediono, A. (2017). Ovarian development and spawning of Bornean endemic fish *Hampala bimaculata* (Popta, 1905). *AACL Bioflux*, 10(5):1376-1385.

Srithongthum, S., Au, H. L., Amornsakun, T., Chesoh, S., Jantarat, S., Suzuki, N., Takeuchi, Y., Hassan, A., Kawamura, G., & Lim, L. S. (2020a). Yolk-sac absorption, mouth size development, and first exogenous feeding of Sultan fish, *Leptobarbus hoevenii*. *AACL Bioflux*, 13(3):1320-1327.

Srithongthum, S., Amornsakun, T., Musikarun, P., Promkaew, P., Au, H. L., Kawamura, G., Lal, M. T. M., & Lim, L. S. (2020b). Length-weight relationship and relative condition factor of the Sultan fish, *Leptobarbus hoevenii* broodstock farmed in earthen ponds. *Egyptian Journal of Aquatic Biology and Fisheries*, 24(3):3-59.

Srithongthum, S., 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.

Tee, E. S., Siti Mizura, S., Kuladevan, R., Young, S. I., Khor, S. C., & Chin, S. K. (1989). Nutrient composition of Malaysian freshwater fishes. *Proceedings of the Nutrition Society of Malaysia*, 4:63-73.

Termvidchakorn, A., & Hortle, K.G. (2013). A guide to larvae and juveniles of some common fish species.
from the Mekong River Basin. MRC Technical Paper no. 38. Phnom Penh: Mekong River Commission.

Truong, D.V., Thanh, N. M., Bao, H. Q., Vinh, T. T., Khoi, P. D., Van, N. T. H., & Trong, T. Q. (2003). Artificial propagation of Hoeven’s slender carp (Leptobarbus hoevenii). In Proceedings of the 6th Technical Symposium on Mekong Fisheries. (pp. 89-96). Lao PDR: Mekong River Commission.

Vidthayanon, C., Karnasuta, J., & Nabhitabhata, J. (1997). Diversity of freshwater fishes in Thailand. Bangkok: Office of Environmental Policy and Planning.