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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; Vidthayanon *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 $\alpha = 0.05$.

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 (Dorostghoal *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:

$$Ni = 0.5Xi+0.25(Xi-1+Xi+1)$$

Description:
Ni: the moving average number of eggs in class range i
Xi: the number of eggs in class range i
Xi-1 and Xi+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.

	n	Total Length (TL)		Body Weight (BW)		GSI		Fecundity (eggs)	
		Min	Max	Min	Max	Min	Max	Min	Max
Female	20	32.2 cm	47.1 cm	350 g	1200 g	1.81%	12.28%	35,476	128,067
Male	20	30.7 cm	45.8 cm	180 g	970 g	1.03%	5.09%	n/a	n/a

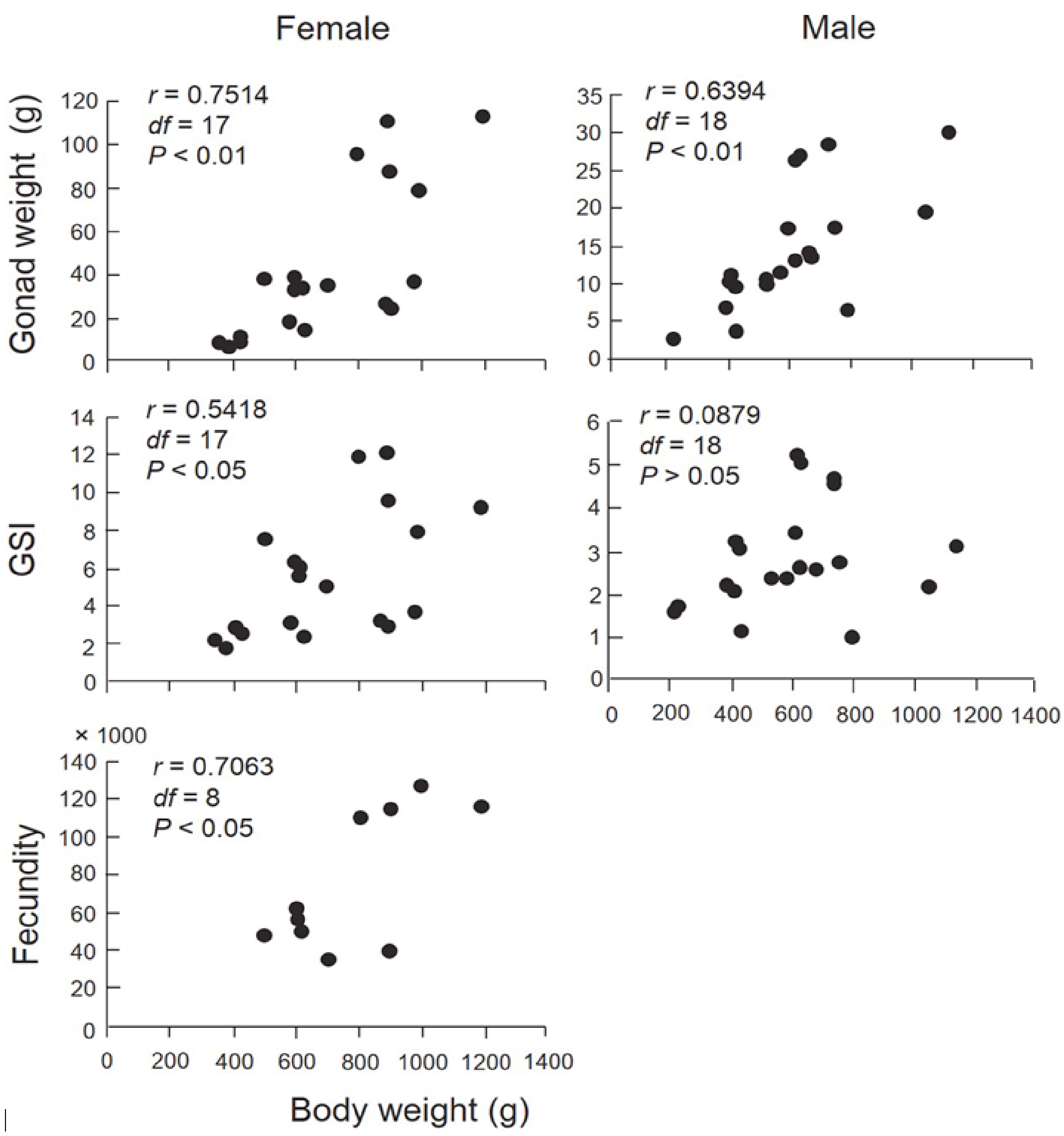

Figure 1. The linear relationships between gonad weight, GSI or fecundity (except for male) and the BW of female and male *L. hoevenii*.

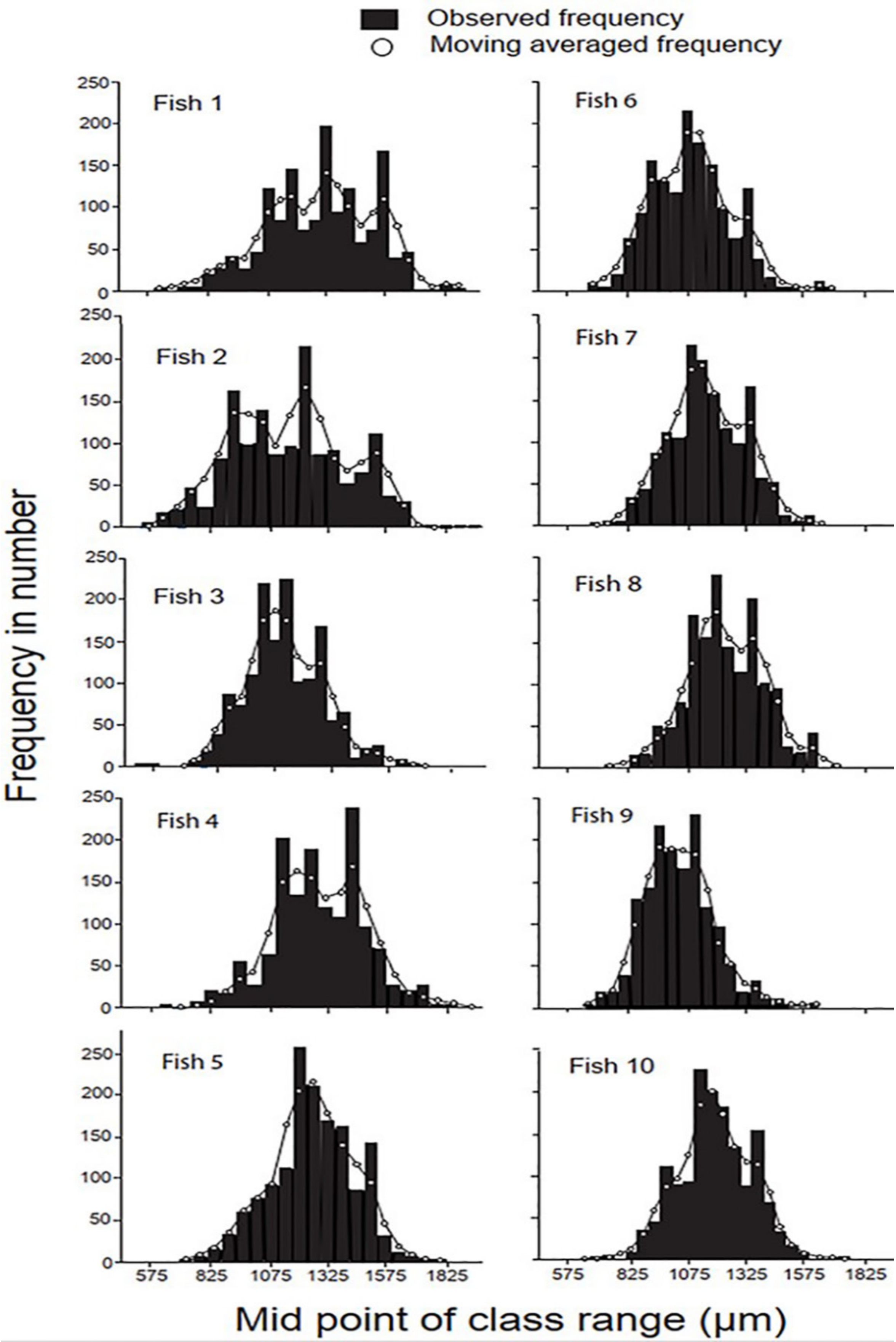

Figure 2. The observed frequency distribution of *L. hoevenii* egg diameter with a class range of 50 µm and their moving averages.

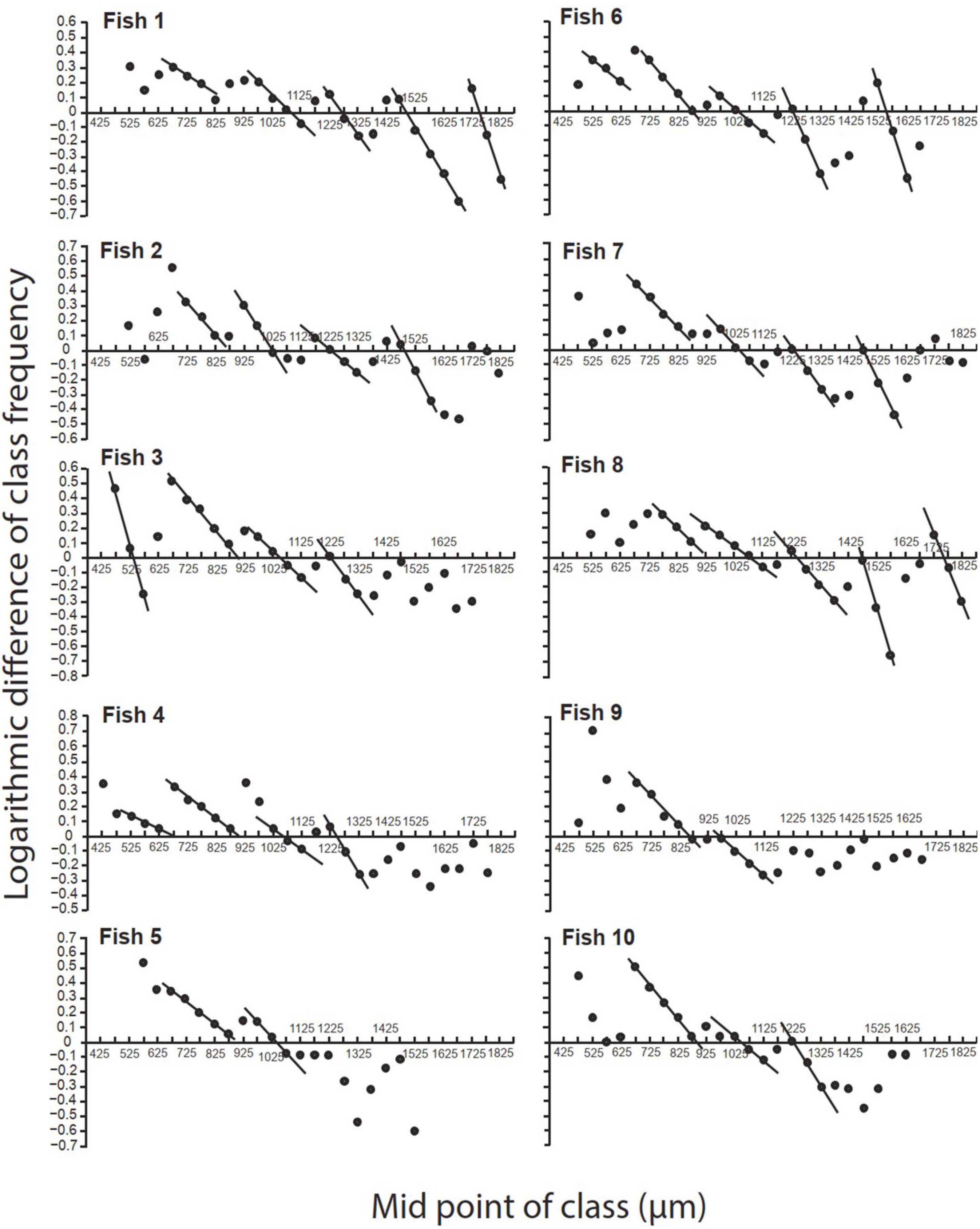

Figure 3. Graph of logarithmic differences of the class-frequencies against the mid-point of the classes, following Bhattacharya's (1967) method.

Normal probability plot analysis is another way for the separation of normal components from a polymodal 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 Bhattacharya'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 Termvidchakorn 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 5.11 %), 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). Apparently, these findings indicated that the female and male *L. hoevenii* can be sexually mature as early as in 350 g and 180 g, respectively. Unfortunately, histological examination was not conducted on these gonad specimens to determine its developmental stage. Therefore, further examination is necessary to elucidate this hypothesis. Nevertheless, it was interesting to notice that the maximum fecundity per kg of BW in this study can reach to > 100,000 eggs. Such finding has clearly provided add-on knowledge on the maximum fecundity of *L. hoevenii*. Food availability and its composition of nutrients are commonly known to be able to influence fish age at first maturity, and also their reproductive performances, including fecundity (Luquet and Watanabe, 1986; Al Hafedh *et al*., 1999). As the farmed *L. hoevenii* are provided with a more balanced and nutritious diet compared to wild *L. hoevenii*, this is perhaps the explanation for the smaller potentially mature *L. hoevenii* and the higher fecundity of females observed in this study.

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 (Bhattacharya'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 Bhattacharya's (1967) method, while 2-5 normal modes were also found following the normality probability plot analysis. Such findings were similar to thosereported 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 Honj *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.

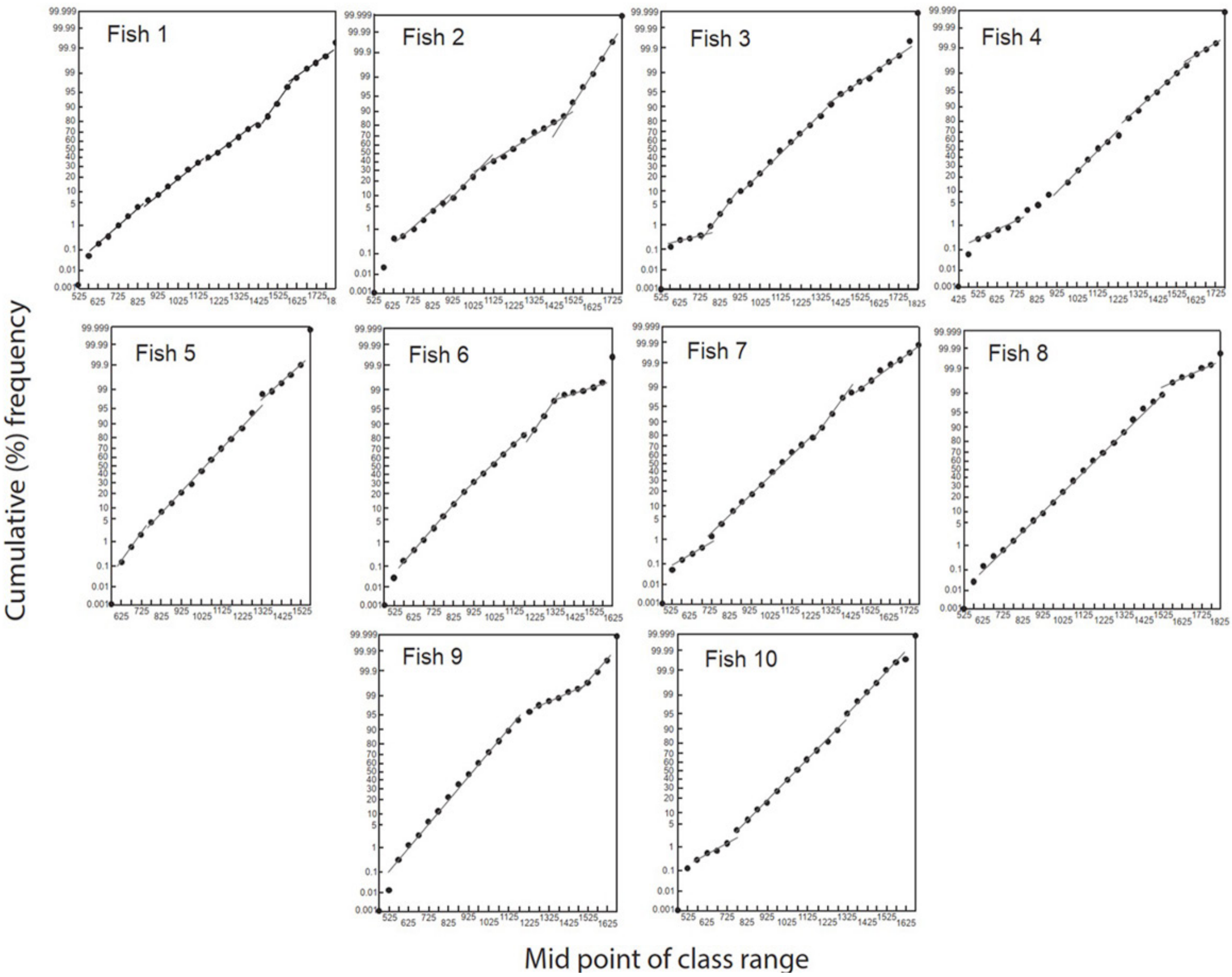

Figure 4. Normal probability plot of the cumulative % frequency distribution of *L. hoevenii* egg diameter

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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