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Research Article

Gonadosomatic Index, Oocyte Development and Fecundity of the Snakehead Fish (*Channa striata*) in Natural River of Mae La, Singburi Province, Thailand

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Abstract

Background and Objective: Reproductive cycle of snakehead fish (*Channa striata*) is necessary to inform management plans of the natural fish. This work aimed to study the gonadosomatic index (GSI), oocyte development and fecundity of *C. striata* in the Mae La River, Singburi province, Thailand from November, 2017-October, 2018. **Materials and Methods:** GSI was calculated from gonadal weight and body weight. Ovarian sections derived from standard histological methods were used to evaluate oocyte development, respectively. Fecundity was measured using the gravimetric method and the relationships of fecundity-total length, fecundity-body weight and fecundity-ovarian weight were evaluated. **Results:** The highest average GSI values for females and males were found in July (6.15±0.20 and 0.14±0.12, respectively). Ovarian histology revealed 4 stages of ovarian development: (1) immature, (2) maturing, (3) mature and (4) spent. Stages 1, 2, 3 and 4 were observed for 11, 10, 6 and 2 months, respectively. The highest percentages of immature, mature and spent oocytes were found in October (93.33%), July (89.56%) and August (33.33%), respectively. Fecundity ranged from 4,160-46,890. Fecundity varied depending on total length, body weight and ovarian weight, with correlation coefficients (R) of 0.986, 0.960 and 0.989, respectively. **Conclusion:** Oocyte maturation period of *C. striata* occurs from April to August. The highest gonads development was in July. Fecundity exhibited linear relationships with body length, body weight and ovarian weight.

Key words: Snakehead fish, reproductive biology, spawning, fecundity, Mae La River

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Mae La River is a natural river in Singburi province, Thailand with a width of 40-200 m and a length of 18 km, flowing through 3 districts, Inburi, Bangrachan and Muang. This river is a source of abundant fish. Snakehead fish caught from the Mae La River are known for having sweeter, softer and tastier flesh than snakehead fish from other water sources in Thailand. A nutrition analysis and taste test revealed that they have 2-fold more fat in their meat than snakehead fish from other water sources and from farms[1]. The reason that snakehead fish in the Mae La River are fat is due to the abundance of natural food sources. The water surface is covered with aquatic plants and weeds, making the water cool, suitable for fish habitat. The bottom of the river is a mud that has a lot of organic material. There is also an abundance of minerals that flow from the surrounding basins. These factors make fish in the Mae La River, especially snakehead fish, more delicious than others[1]. In addition, Singburi province has announced the snakehead fish as Thai geographical indication. The unique shape of snakehead fish from the Mae La River is known to people all over the country, with its chubby body, fan-shaped tail and pink fins. Therefore, snakehead fish from the Mae La River have been in high demand for a long time.

However, communities throughout the Mae La River basin are concerned about the declining abundance of fish and would like to conserve their population in this river. It has become more difficult to find snakehead fish in the Mae La River and the size of the fish that are found has decreased due to habitat degradation, especially in the areas of the river that serve as reproductive habitat and food sources. Human development along the river is leading to pollution in the river, which, coupled with use of the wrong type of fishing tools and fishing beyond the replenishment rate, has caused the abundance of larvae to decrease in the river. For areas with low water quality values, this may be due to the use of chemicals in agricultural areas or wastewater from homes on both sides of the Mae La River. These have negatively affected the amount of fish in the river[2].

Gonadosomatic index (GSI), oocyte stages, fecundity, fecundity-length relationships, fecundity-weight relationships are known as important parameters to represent details regarding reproductive status, ascertaining breeding period and facilitating the effective conservation and management of fish species[3-6]. A few studies were conducted to evaluate the reproductive biology and fecundity of *C. striata* in natural sites[7-9]. Therefore, present study intended to understand annual reproductive cycle of them to inform plans to increase the number of natural snakehead fish (*C. striata*) in the Mae La River, Singburi province, Thailand. This will help increase the potential production of snakehead fish to be sufficient for consumption as well as promotion of community income.

MATERIALS AND METHODS

Sampling site and study period: A total of 600 snakehead fish were collected through monthly efforts with lure fishing and fyke nets between November, 2017 and October, 2018 from 4 sites in the Mae La River: (1) Mae La park, Mae La subdistrict, Bang Rachan district (14°56'43.414"N 100°19'58.801"E), (2) Klongmai village, Thapya subdistrict, In Buri district (14°57'15.241"N 100°19'44.28"E), (3) Karong floodgate, Huaychan subdistrict, In Buri district (14°58'41.092"N 100°18'25.608"E) and (4) Karong temple, Huaychan subdistrict, In Buri district (14°59'28.027"N 100°17'50.84"E) (Fig. 1).

Gonadosomatic index: The total length and weight of the fish were recorded to the nearest millimeter and gram, respectively. After dissection, the gonads were removed, dried thoroughly with blotting paper, weighed with a digital balance and recorded. The gonadosomatic index (GSI) was expressed as the percentage of body weight represented by the gonads. The following Eq. was used to calculate the GSI value:

$$\text{GSI} = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

Histological study of ovary: The ovaries were fixed in Bouin's solution, embedded in paraffin, sectioned at 6 µm and stained with Mayer's hematoxylin and eosin. The maturity stage of each ovary was determined according to the most advanced stage of oocytes present in the ovary.

Fecundity study: In the present study, the maturity stages of the ovary beyond the tertiary yolk globule stage were employed for fecundity estimation. *Channa striata* is a group-synchronous type spawner because the oocyte sizes vary during the reproductive season[9]. Therefore, 3 sub-samples from middle, interior and posterior part of either the left or right ovary were selected for fecundity estimation. After liberation from the ovarian tissue, the oocytes were thoroughly washed and spread on blotting paper to air dry. The gravimetric method was used for the fecundity estimation of each female. The relative fecundity was also calculated as:

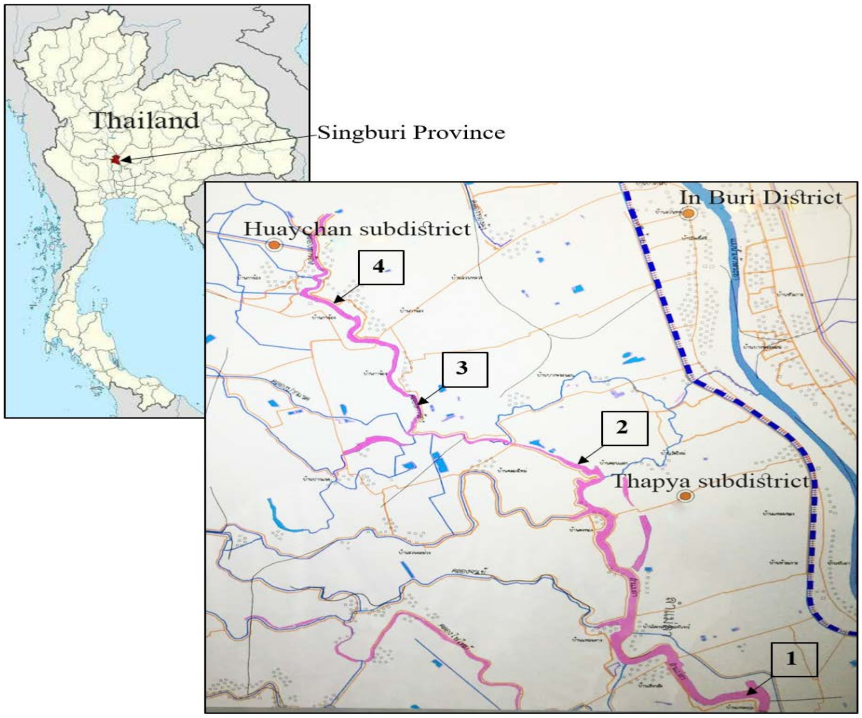

Fig. 1: Map of Thailand and sampling sites of *C. striata* in Mae La river (1-4)

$$\text{Relative fecundity} = \frac{\text{Number of oocytes}}{\text{Body weight}}$$

$$\text{Relative fecundity} = \frac{\text{Number of oocytes}}{\text{Ovarian weight}}$$

Fifty oocytes from each individual female were also measured the oocytes diameter.

The arithmetical relationship among the length-weight, fecundity-gonad weight and fecundity-body weight of *C. striata* were measured by using followings Eq:

$$\text{Fecundity-body length: } F = a+b\times BL$$

$$\text{Fecundity-body weight: } F = a+b\times BW$$

$$\text{Fecundity-ovarian weight: } F = a+b\times OW$$

where, F is the fecundity, BL is the body length, BW is the body weight, OW is the ovarian weight, a is the Intercept and b is the regression co-efficient.

Statistical analysis: Regression analysis were performed by using SPSS 22 software subjected to Pearson Chi-square (χ^2) test.

RESULTS

Gonadosomatic index (GSI): Results showed monthly changes in GSI from November, 2017 to October, 2018. The average GSI of both female and male snakehead fish was the highest in July 2018, at 6.15±0.20 and 0.14±0.12, respectively (Fig. 2). This result reveals that reproductive organ development reaches its peak in July. The GSI value is expected to increase as an individual reaches the reproductive season. In this study, GSI values represented the same pattern of reproductive cycle in both female and male fish. The female and male GSI values increased steadily from April until the highest value in July, then decreased, with the lowest value in October. The GSI is highest during the peak reproductive period and immediately decreases after the rapid birth of the spawning fish.

Ovarian histology and oocyte development: Macroscopic observation of the ovaries revealed different maturity stages of the fish. The oocyte development observed in *C. striata* throughout this study period was divided into 4 stages. The ovarian histology of *C. striata* showed group synchronous oocyte development. The ovaries presented 7 different oocyte stages (Table 1): (1) Chromatin nucleolar stage (Fig. 3), (2) Early perinucleolar stage (Fig. 3, 4), (3) Late perinucleolar stage (Fig. 3, 4), (4) Yolk vesicle stage (Fig. 3, 5),

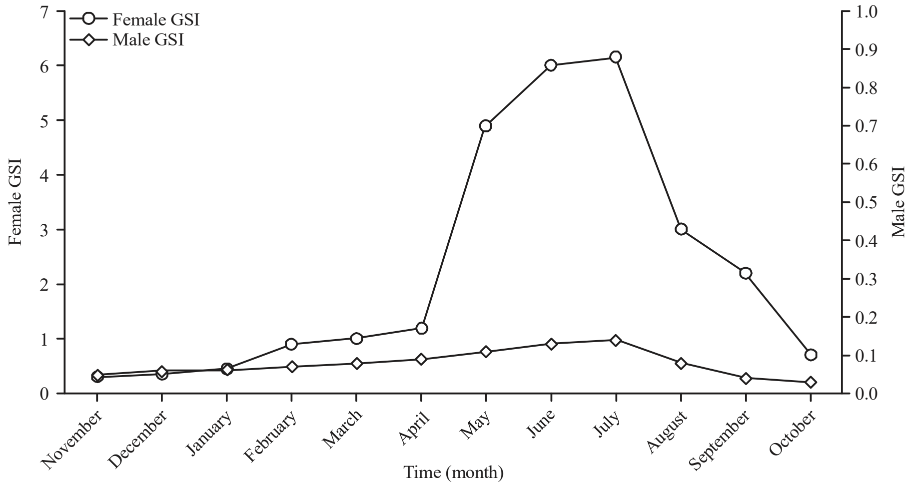

Fig. 2: Gonadosomatic index (GSI) of female and male *C. striata* between November, 2017-October, 2018

Table 1: Developmental stages of *C. striata* oocyte, according to histological characteristics

Stages of oocytes	Histological characteristics
Chromatin nucleolar stage	Oocyte contains spherical and large nuclei, there is one large nucleus
Early perinucleolar stage	Oocyte is a square shape, nuclear and cytoplasmic volume are increase, nucleolus numbers are increased but smaller size
Late perinucleolar stage	Oocyte with larger size is both square and oval shape, nucleolus size is increased, cytoplasm is less haematoxylin stained
Yolk vesicle stage	Oocyte contains small yolk vesicles that is not unstained, these yolk vesicles appear firstly around the cytoplasm and then gradually spread more into middle of cell, nucleolus appear around nucleus or inside nucleus
Early yolk granule stage	Oocyte contains a numbers of small yolk granules stained in light pink color, granules appear firstly around the cytoplasm and then gradually spread more into middle of cell
Late yolk granule stage	Yolk granules are increase in size and number, they are stained in dark pink color
Spent stage	There is empty in follicles, oogonia and perinucleolar oocytes are observed in ovaries

Table 2: Developmental stages of *C. striata* ovary, according to histological characteristics

Stages of ovaries	Histological characteristics
1 (immature)	This ovarian stage contains oogonia, chromatin nucleolar oocytes, early perinucleolar oocytes and perinucleolar oocytes with large nuclei
2 (maturing)	This ovarian stage contains early perinucleolar oocytes, late perinucleolar oocytes and oocytes with yolk vesicles in some part of cytoplasm
3 (mature)	This ovarian stage contains oocytes that their cytoplasm is filled with yolk granules
4 (spent)	This ovarian stage contains empty follicles oogonia and perinucleolar oocytes

Table 3: Fecundity of *C. striata* with different body length

Body length	Mean of body length (cm)	Mean of body weight (g)	Mean of ovarian weight (g)	Mean of fecundity	Oocyte no./ body weight	Oocyte no./ ovarian weight
30.5-34.5	33.76	310.66	12.49	4,160.00	13.39	333.07
34.6-37.5	35.47	433.59	19.56	8,250.58	19.03	421.81
37.6-40.5	38.63	820.26	29.56	16,807.50	20.49	568.59
40.6-44.5	42.55	950.22	32.30	19,875.50	20.91	615.34
44.6-48.7	46.97	1,100.61	38.69	25,000.30	18.06	513.71
48.8-52.8	50.47	1,250.00	46.00	38,056.90	30.45	827.32
52.9-56.9	54.93	1,400.50	56.81	46,890.00	33.48	825.39

(5) Early yolk granule stage (Fig. 3, 6), (6) Late yolk granule stage (Fig. 3, 7) and (7) Spent stage (Fig. 7).

Based on the number of oocytes of each stage in the ovaries, ovaries can be divided into 4 phases (Table 2): 1 (immature), 2 (maturing), 3 (mature) and 4 (spent) (phase after spawning). Maturing ovaries were observed for 10 months, mature ovaries for 6 months and ovaries in the spent phase for only 2 months. The highest percentages of ovaries in the maturing and spent stages were observed in July (89.56%) and August (33.33%), respectively (Fig. 8).

Fecundity: The *C. striata* oocytes were amber colored, rounded and non-adhesive. The diameter of the oocytes ranged from 0.35-1.34 mm. During the present investigation, the fecundity of *C. striata* varied from 4,160 (for a fish with a total length of 33.76 cm and body weight of 310.66 g) to 46,890 (for a fish with total length 54.93 cm and body weight 1,400.50 g) (Table 3).

Fecundity was linearly correlated with body length (BL), body weight (BW) and ovarian weight (OW), expressed as the following equations (Table 4):

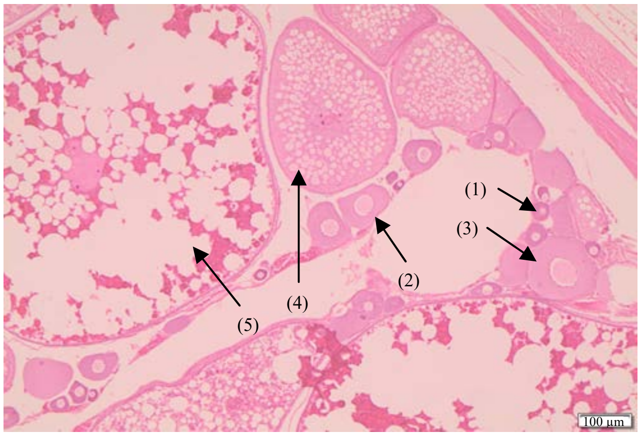

Fig. 3: Photomicrographs of oocyte development at different stages in *C. striata* ovary
(1) Chromatin nucleolar stage, (2) Early perinucleolar stage, (3) Late perinucleolar stage, (4) Yolk vesicle stage, (5) Late yolk granule stage

Fig. 4: Oocyte development of *C. striata*
(1) Early perinucleolar stage, (2) Late perinucleolar stage

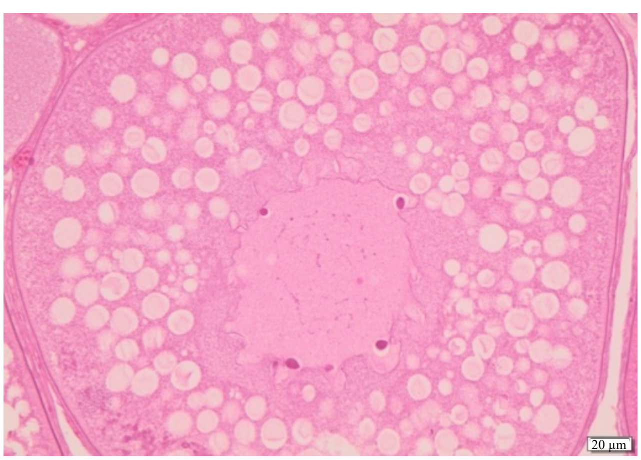

Fig. 5: Oocyte development of *C. striata* at yolk vesicle stage

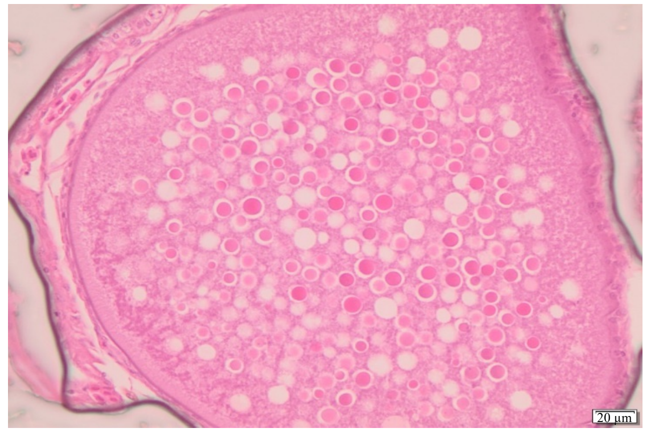

Fig. 6: Oocyte development of *C. striata* at yolk granule stage

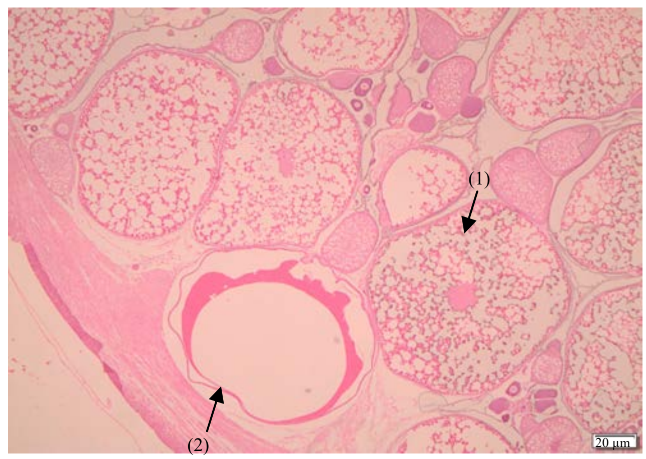

Fig. 7: Oocyte development of *C. striata*
(1) Late yolk granule stage, (2) Spent stage

- Y = (-60363.369)+1920.690 (BL)
- Y = (-9878.480)+36.418 (BW)
- Y = (-11060.686)+1004.486 (OW)

The fecundity increases when the breed size increases. The strongest correlation was the relationship between fecundity and ovarian weight (R = 0.989).

DISCUSSION

This investigation of the reproductive cycle of the snakehead fish (*C. striata*) consisted of the GSI study along with the study of gonad histology. Changes in gonad histology indicate the spawning season of *C. striata*. The fertility index of snakehead fish with an average body weight of 895.12±10.51 g was assessed between November, 2017

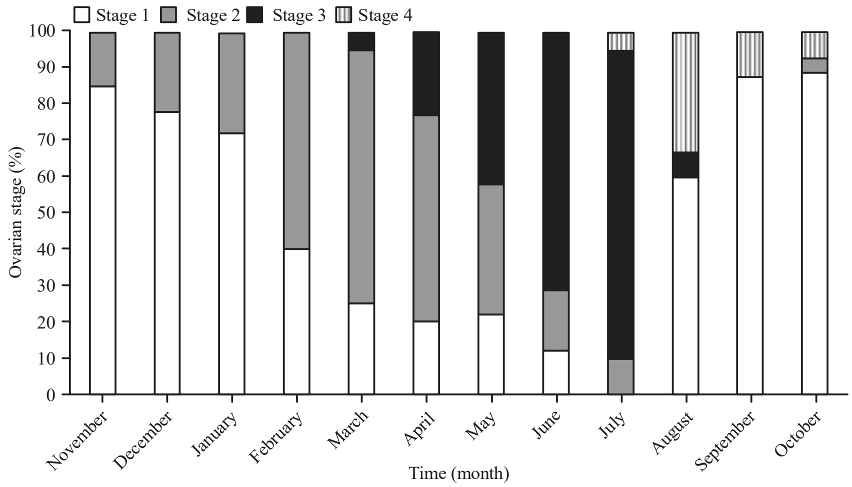

Fig. 8: Stages percentage of ovarian development during November, 2015-October, 2016
1: Immature stage, 2: Maturing stage, 3: Mature stage, 4: Spent stage

Table 4: Correlation coefficient, regression equation of relationships between fecundity and body length, body weight or ovarian weight

Relationships	Correlation coefficient	Regression equation
Fecundity (Y) and body length (x)	0.986	Y = (-60363.369)+1920.690x
Fecundity (Y) and body weight (x)	0.960	Y = (-9878.480)+36.418x
Fecundity (Y) and ovarian weight (x)	0.989	Y = (-11060.686)+1004.486x

and October, 2018. It was found that the reproductive indexes of female and male snakehead fish ranged from 0.30-6.15 and 0.03-0.14, respectively. The female GSI was close to GSI values previously reported[8] for *C. striata* of 0.01-4.83 and[9] 0.67-5.10 and for *C. punctatus*[10] of 0.194-5.64.

The period of high GSI and fecundity values demonstrated an appropriate period for breeding stock selection. The reproductive cycle of *C. striata* in this study indicates that snakehead fish in a natural river have a spawning season during May-July. This information is useful for planning in fishing control in order to enhance breeding and the number of young fish in the Mae La River. Based on this study, snakehead fish ovaries with mature oocytes corresponded to a high GSI in May, June and July, with mean values of 4.9±0.19, 6.0±0.22 and 6.15±0.27, respectively. This implies that snakehead fish have a spawning season for 3 months (May-July).

Ovarian development of the snakehead fish showed 4 developmental stages. This differs from the synchronous oocyte development in species such as the African catfish (*Clarias gariepinus*), in which there is only one oocyte stage in the ovaries, causing the fish to have a long spawning period[11]. Ovaries in the immature, maturing and mature stages were observed in 11, 10 and 6 months of the study period, respectively, while ovaries in the spent stage were observed in only 2 months. A similar finding was reported for *Channa striata* in Bangladesh[12]. The highest percentages of immature and mature ovaries were recorded in October, 2018 (93.33%) and in July, 2018 (89.56%), respectively. In August 2018, the highest percentage of spent ovaries was observed (33.33%), which was a small percentage of occurrences. This implied that oocytes in the ovaries developed rapidly from the perinuclear stage to the yolk granule stage. This phenomenon was different from those reported for *Epinephelus marginatus*[13], *Caulolatilus princeps*[14] and *Esox Lucius* L.[15], which exhibit slow oocyte development.

Based on the histological study and the amount of yolk accumulation in *C. striata* oocytes, it was found that oocytes at the yolk vesicle stage showed yolk vesicle forming from the oocyte edge forward near the nucleus. In this stage, yolk vesicles around the nuclear membrane were white in color and similar to the circumnuclear ring of granules around the nucleus reported in *Gadus morhua* L. oocytes[16].This appearance was similar to that of Balbiani bodies caused by the grouping of the ribonucleotides (ribonucleoprotein) around the nucleus. This pattern of yolk granule accumulation occurs similarly in other teleost fish[17], such as *Mollotus villosus villosus*[18] and *Xiphias gladius*[19]. After the establishment of yolk granules in the fish oocytes, the oocytes further develop into the mature stage. Therefore, the appearance of yolk granules could indicate spawning season.

In the fecundity study, the minimum number of oocytes (4,160) was observed in September and the maximum (46,890) in July. The oocyte diameter ranged from 0.35-1.34 mm. In Taiwan, Li *et al.*[20] reported the diameter of mature oocytes with a mean of 1.16±0.01 mm. In Malaysia, Yaakob and Ali[21] also noted approximated size of spawned oocytes as 1.39 mm. Fecundity was linearly correlated with body length, body weight and ovarian weight. Fecundity was more related to body length rather than body weight. It was also found that fecundity was strongly related to ovarian weight. Similar correlations have been reported for *C. striata*[9] as well as *Dorosoma cepedianum*[22] and *Lophiomus setigerus*[23].

The largest female fish, with a total length of 54.93 cm and body weight 1,400.50 g, exhibited a fecundity of 46,890. The smallest sized fish, with a total length of 33.76 cm and body weight of 310.66 g, exhibited a fecundity of 4,160. The number of oocytes produced by a female is dependent on different factors, such as size, age and condition types of the samples[24]. During the present investigation, the number of oocytes was directly proportional to the weight of the fish and fecundity increased progressively with the ovarian weight of fishes. Similar observations were also made by Roy *et al.*[25] and Musa and Bhuiyan[26]. In addition, the fecundity of the snakehead fish was previously reported to average 6000 oocytes per 100 g of broodstock[27] with an oocyte diameter between 1.2 and 1.5 mm[28-31].

Physical factors such as water temperature, water content, amount of light and pH of water are important factors controlling the reproductive cycles of fish species[32]. Moreover, biological factors including phytoplankton, aquatic plants and aquatic animals are also important determinants of fertility and young fish development. In this study, the maturing stage of germ cells began in April, 2018 and peaked in July, 2018. The maturing period was characterized by high water conductivity, which means that there is a high amount of organic matter in the water[2]. High organic matter in river water leads to increases in food sources, i.e., phytoplankton, aquatic plants and aquatic animals, that are suitable for young fish development.

The useful information from this study can be used for planning actions to increase the number of natural snakehead fish in the Mae La River by (1) enhancement of conservation consciousness along with the use of rules about fishing during the spawning season (May-August), (2) establishing the period of high GSI and fecundity (July) as an appropriate period for breed stock selection of snakehead fish in order to increase the number of lures in natural water sources and (3) enhancement of awareness about fish and resource conservation in the Mae La River to prevent various threats from affecting the ecological system and habitats of snakehead fish. At present, Singburi province is conducting the "Mae La Model" project to encourage farmers to undertake organic rice farming and to raise fish during the free period of rice cultivation.

CONCLUSION

This study of the reproductive biology of snakehead fish (*C. striata*) in the Mae La River, Singburi province between November, 2017 and October, 2018 demonstrated that the gamete maturation period occurs from April-August. The highest GSI values for both females and males were observed in July. The female fecundity ranged from 4,160-46,890 oocytes. Fecundity (Y) exhibited linear relationships with body length, body weight and ovarian weight. The findings in this study suggest that further expansion of the population size of *C. striata*, especially during its reproductive season from April-August, is recommended.

SIGNIFICANCE STATEMENT

This study discovers significant information regarding reproductive biology, gonadosomatic index (GSI), gamete maturation and fecundity, of snakehead fish (*Channa striata*) in the Mae La River, Singburi province, Thailand. These data are beneficial to understand the fecundity, spawning season and breeding season of the fish to inform plans to increase the number of natural snakehead fish in the Mae La River. This study will help increase the number of natural snakehead fish to be sufficient for consumption as well as promotion of community income.

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