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Spawning period and fecundity of *Neolissochilus soroides* (Duncker, 1904) (Pisces, Teleostei, Cyprinidae) from a small Malaysian stream

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Abstract: Spawning and fecundity aspects of a cyprinid fish, *Neolissochilus soroides* (Duncker, 1904), from the Gombak River of Peninsular Malaysia were studied from November 2009 to October 2010. Results show that the fish reproduced year-round, but 2 peaks of fecundity were observed, coinciding with high rainfall distribution during November and April. The gonadosomatic index for both sexes was comparatively higher in the wet seasons. The high numbers of ripening and ripe oocytes observed during the wet seasons and the oocyte diameter distribution indicate that the species is a total spawner, with batches of ripe oocytes that are released over an extended time period. Absolute fecundity is highly related to ovary weight rather than body weight and total length.

Key words: Gonadosomatic index, oocyte development, Gombak River

1. Introduction

Cyprinid fishes of the genus *Neolissochilus* Rainboth, 1985 are naturally found throughout tropical and subtropical areas of southern and southeastern Asia (Rainboth, 1985). Twenty-five nominal species of the genus inhabit the freshwater habitats throughout their distributional range (Rainboth, 1991; Vidthayanon and Kottelat, 2003; Roberts and Khaironizam, 2008; Khaironizam, 2010). In Peninsular Malaysia, only 2 species, *Neolissochilus soroides* (Duncker, 1904) and *N. hendersoni* (Herre, 1940), are reported to occur in the river systems (Roberts and Khaironizam, 2008). Both species are confined to the montane streams, which are physically structured by variably deep pools and riffles with slow to fast flowing water; a complex mixture of debris, sand, bedrock, gravel, cobble, and boulder substrata; and crystal-clear waters. *N. soroides* has a distribution range through Cambodia (Rainboth, 1996), Thailand (Vidthayanon and Kottelat, 2003), Peninsular Malaysia (Khaironizam, 2010), Java and Sumatra (Kottelat et al., 1993), and Anambas Island in the South China Sea (Tan and Lim, 2004), while *N. hendersoni*, is endemic to the northern part of Peninsular Malaysia (Khaironizam, 2010).

Although some studies on the reproductive biology of *Neolissochilus* have been reported from India and Nepal (Ahmad, 1948; Swar and Craig, 2002), information regarding the reproductive biology of *N. soroides* is poorly reported. As the species has high market demand in the aquarium trade and is one of the popular freshwater game fishes in Malaysia (Khaironizam, 2010), information on the reproductive biology would be important for conserving its stock. To that effect, this paper describes for the first time aspects of spawning, gonadosomatic index, size of oocytes, and fecundity of *N. soroides* from a small tropical stream of the Gombak River, Peninsular Malaysia.

2. Materials and methods

Seven study sites were chose in the upper part of the watershed of the Gombak River, which is a tributary of the Klang drainage in Peninsular Malaysia (Figure 1). Detailed descriptions of the Gombak River were provided by Roberts and Khaironizam (2008). Sampling was conducted once a month from November 2009 until October 2010. A total of 389 *N. soroides* were sampled from November 2009 until October 2010 (Table 1) using battery-powered backpack electrofishing gear (Smith-Root, Model 12). In the field, the collected fish were weighed (total body weight, BW) using a Mettler Toledo digital balance to the nearest 0.1 g and were measured (total body length, TL) using vernier calipers to the nearest 1 mm. Due to the absence of sexual dimorphism, the sex of fish was determined by dissecting the abdominal area to observe the gonads.

After dissection, the condition of the gonads was noted, and their maturity stages were classified according to Swar

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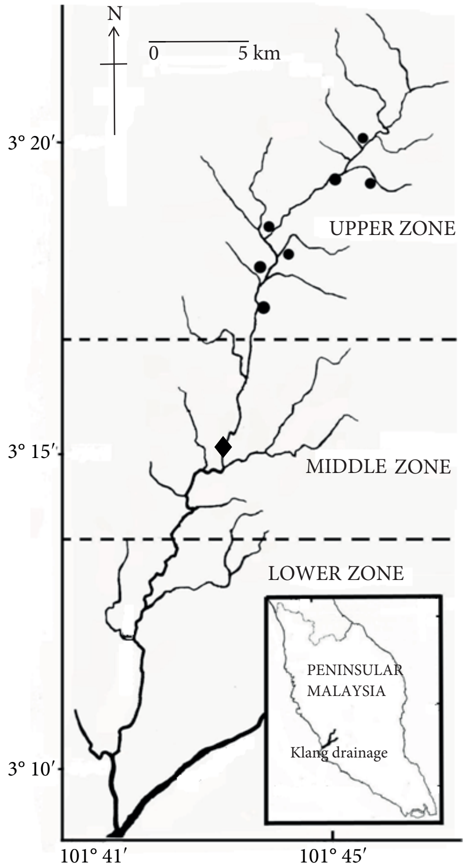

Figure 1. Map of sampling localities of *N. soroides* (●) and the Drainage and Irrigation Department of Malaysia station (♦) in the Gombak River. Inset map shows general localities of Klang drainage in Peninsular Malaysia.

and Craig (2002). The gonads were then weighed (testes weight, TsW and ovary weight, OvW) to the nearest 0.1 g, and the gonadosomatic index (GSI) for each individual fish was calculated as the gonad weight relative to the body weight and expressed as a percentage (De Vlaming et al., 1982).

The left and right branches of the ovaries were removed from the visceral cavity and placed in 5% formalin for 2 weeks to allow sufficient hardening. The ovaries were then opened to loosen the oocytes from the ovarian tissues. The oocyte diameter was measured by an optical micrometer mounted in a compound microscope. The oocytes were counted and classified according to the diameter and characteristics of the oocytes (Ganias et al., 2004). The oocyte classification was based on the modified methods of Swar and Craig (2002). The absolute fecundity (F) was counted only for the yolky oocytes of the ovaries at developing and gravid stages, which could be laid during the reproductive season (Bagenal, 1968). The least-squares linear regressions between F and TL, BW, and OvW were calculated according to the method of Bagenal (1968). The GSI for each fish was calculated as the weight of the gonads relative to the body weight and was expressed as a percentage (De Vlaming et al., 1982). The monthly data of GSI and size of oocytes were compared according to wet and dry seasons based on the rainfall data. In order to determine the differences of the gonad weight and GSI value of different maturity stages of the male and female fish, one-way ANOVA and group t-tests for independent samples were used. Area rainfall data during the study period of November 2009 to October 2010 were obtained from the nearest Drainage and Irrigation Department of Malaysia station, which is about 7 km from the study area (Figure 1).

3. Results

The study areas experience rainfall throughout the year (Figure 2). However, the heavy rains or wet season was from November 2009 to April 2010 (which coincided with the northeast monsoons) and the dry season was from May 2010 to October 2010 (which coincided with the southwest monsoons). During the study period, 2 peaks of rainfall were observed; the first was in November and the second was in April, with total monthly rainfalls of 436.5 mm and 405 mm, respectively (Figure 2).

The mean gonad weight and GSI of females and males of *N. soroides* in the area increased gradually from maturing virgin stage to the peak value at the gravid stages, and decreased to the lowest value at the spent stage. For females and males at the gravid stage, the means of gonad weight (2.5 ± 1.7 and 0.8 ± 0.3, respectively) and GSI values (6.88 ± 0.25 and 4.99 ± 0.25, respectively) were significantly higher compared to other maturity stages (Table 2). The GSI values for female and male fish also were found to be highly correlated with the monthly rainfall (female GSI at developing stage, $r^2 = 0.67$, $F_{(1,10)} = 8.163$, $P = 0.017$; female GSI at gravid stage, $r^2 = 0.54$, $F_{(1,10)} = 11.547$, $P = 0.007$; male GSI at developing stage, $r^2 = 0.83$, $F_{(1,10)} = 48.623$, $P = 0.0001$; male GSI at gravid stage, $r^2 = 0.71$, $F_{(1,10)} = 24.212$, $P = 0.0001$).

Highest GSI values were recorded at the end of the dry season (October) and throughout the wet season (November to April), and the peak values were observed in December 2009 and April 2010 (Figure 2). Similar fluctuation patterns of the GSI values were also observed for ovaries and testes at the developing stages, and 2 peaks of the mean GSI values for both ovaries and testes were observed in November 2009 and April 2010. The results also show that during the wet season, GSI values of both females and males with gonads at developing and gravid stages were significantly higher compared with GSI values in the dry season (Table 3).

For the first time, the size and the characteristics of oocytes of *N. soroides* were reported, and 5 classes of oocyte

Table 1. The sex, sample size, and total length range of *N. soroides* sampled from the Gombak River from November 2009 to October 2010.

Month	Sex	n	Total length range (cm)
Nov	F	17	11.1–20.1
	M	23	8.4–15.2
Dec	F	16	10.6–20.9
	M	21	9.1–15.5
Jan	F	13	10.0–19.2
	M	24	8.2–13.1
Feb	F	12	10.5–13.0
	M	24	9.0–13.9
Mar	F	14	10.2–19.7
	M	28	8.9–16.0
Apr	F	17	10.3–22.1
	M	20	9.2–15.2
May	F	7	10.0–13.2
	M	19	9.2–12.5
Jun	F	6	11.5–15.0
	M	15	9.2–12.6
Jul	F	6	10.7–14.2
	M	17	9.2–13.3
Aug	F	7	10.7–17.2
	M	17	9.1–13.3
Sep	F	12	10.9–19.8
	M	19	8.5–15.2
Oct	F	12	10.0–19.2
	M	23	8.1–19.2
	Total	389	8.1–22.1

Abbreviations: F = females; M = males; n = number of fish with mature gonads.

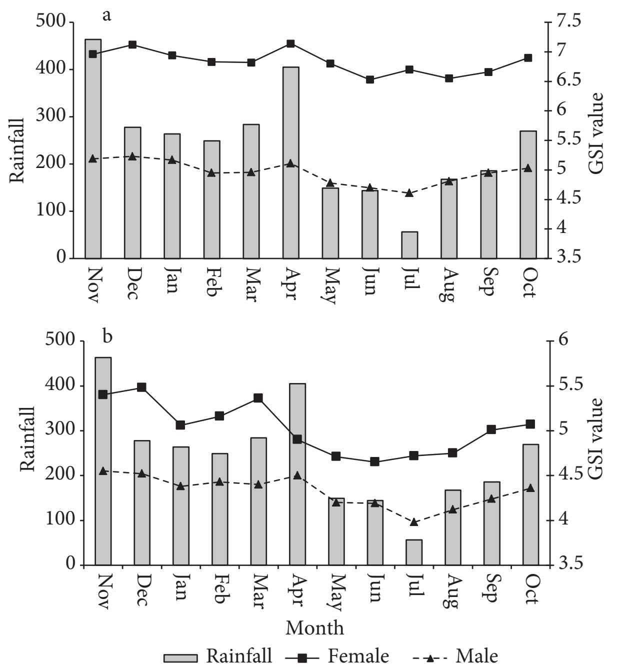

Figure 2. Monthly rainfalls and gonadosomatic index (GSI) at a) developing and b) gravid stages of *N. soroides* in the Gombak River from November 2009 until October 2010.

development were observed (Table 4). The immature oocytes (usually with a diameter of less than 5 µm) were not counted due to their fragility and the difficulty of handling them during the counting process.

The frequency distribution of oocyte sizes was continuous in all maturation stages (Figure 3) in *N. soroides*. A unimodal distributional pattern of oocytes was observed in the maturing virgin and maturing ovaries, and a bimodal distributional pattern of oocytes was observed in the developing and gravid ovaries (Figure 3). The monthly oocyte size frequency of the combined developing and gravid stages shows a bimodal pattern throughout the year (Figure 4), and this pattern was most notable in November, December, January, March, April, September, and October. The total number of ripening and ripe oocytes during these months was significantly higher than in the dry season (ANOVA: $F_{(1,10)} = 6.961$; $P < 0.05$).

Absolute fecundity of 139 of the developing and gravid stage female individuals of *N. soroides* ranged from 803 to 6218 oocytes per fish (Table 5). The regression analyses between F and TL, BW, and OvW showed a positive linear regression ($Log_{10} F = -0.12 + 1.61 Log_{10} TL$, $r^2 = 0.48$, $F_{(1,137)} = 69.386$, $P = 0.000$; $Log_{10} F = 2.49 + 0.58 Log_{10} BW$, $r^2 = 0.53$, $F_{(1,137)} = 85.824$, $P = 0.000$; and $Log_{10} F = 3.18 +$

Table 2. Mean and standard deviation of gonad weight and gonadosomatic index (GSI) of *Neolissochilus soroides* from the Gombak River.

	Sexes	Gonad developmental stage						ANOVA
		Maturing virgin	Maturing	Developing	Gravid	Spawning	Spent	
Gonad weight (g)	Female	$0.4 \pm 0.3^{a,x}$	$0.6 \pm 0.3^{a,x}$	$1.4 \pm 0.9^{b,x}$	$2.5 \pm 1.7^{c,x}$	$0.3 \pm 0.1^{a,x}$	$0.1 \pm 0.01^{a,x}$	$F_{(5,133)} = 20.335$ *
	Male	$0.2 \pm 0.1^{a,y}$	$0.3 \pm 0.2^{b,y}$	$0.6 \pm 0.3^{c,y}$	$0.8 \pm 0.3^{d,y}$	-	$0.2 \pm 0.04^{ab,y}$	$F_{(4,245)} = 67.840$ *
	t-test	$t_{(0.05,61)} = 5.462$ *	$t_{(0.05,73)} = 4.331$ *	$t_{(0.05,86)} = 6.819$ *	$t_{(0.05,133)} = 9.336$ *	-	$t_{(0.05,16)} = 2.453$ *	
Gonadosomatic index (GSI)	Female	$1.7 \pm 0.5^{a,x}$	$3.0 \pm 0.3^{b,x}$	$5.1 \pm 0.3^{c,x}$	$6.8 \pm 0.3^{d,x}$	$1.5 \pm 0.3^{a,x}$	$1.1 \pm 0.4^{e,x}$	$F_{(5,133)} = 1156.387$ *
	Male	$1.5 \pm 0.3^{a,y}$	$2.5 \pm 0.2^{b,y}$	$4.3 \pm 0.2^{c,y}$	$5.0 \pm 0.3^{d,y}$	-	$1.3 \pm 0.6^{e,x}$	$F_{(4,245)} = 1820.687$ *
	t-test	$t_{(0.05,61)} = 2.480$ *	$t_{(0.05,73)} = 6.584$ *	$t_{(0.05,86)} = 12.750$ *	$t_{(0.05,133)} = 37.499$ *	-	$t_{(0.05,16)} = 0.816^{NS}$	

Statistical significance: *, significantly different ($P < 0.05$); NS, not significantly different ($P \geq 0.05$); different superscripts (a, b, c, d, or e) in a row show that the means of the compared maturity stages are significantly different ($P < 0.05$) while different superscripts (x or y) in a column show that the means of the compared sexes are significantly different ($P < 0.05$).

Table 3. Mean and standard deviation of gonadosomatic index (GSI) of *Neolissochilus soroides* from the Gombak River in the wet and dry seasons.

Number	Wet season	Dry season	ANOVA
Ovary at developing stage	5.28 ± 0.19	4.80 ± 0.25	$F_{(1,28)}$ = 37.623 *
Ovary at gravid stage	6.97 ± 0.22	6.53 ± 0.21	$F_{(1,46)}$ = 47.468 *
Testes at developing stage	4.45 ± 0.14	4.16 ± 0.23	$F_{(1,57)}$ = 37.177 *
Testes at gravid stage	5.10 ± 0.21	4.86 ± 0.22	$F_{(1,85)}$ = 25.435 *

*: significantly different (P < 0.05).

0.61 Log_{10} OvW, r^2 = 0.94, $F_{(1,137)}$ = 1222.716, P = 0.000, respectively). The high regression coefficient value (r^2 value) for ovary weight reveals that fecundity was highly correlated with ovary weight compared with total length and body weight.

4. Discussion

Bagenal (1968) reported that most tropical fish reproduce throughout the year. Wootton (1979) also noted that tropical fish that spawned uniformly throughout the year generally showed smaller variations in the GSI value than those that spawned in the short season or only in the wet or dry season. This was also observed for the studied species. The variations of the mean GSI value are lower compared with other tropical cyprinids (De Silva et al., 1985). The results also reveal that the females at the mature stages yielded much higher GSI values compared with the males, which suggests that the gonads of females in the same maturity stage are heavier compared with those of males (Table 2).

De Vlaming (1983) suggested that the annual cyclical pattern of oocyte development can be determined by the oocyte size–frequency distribution or oocyte size batches. In the present study, the oocyte size–frequency distribution was found to have a bimodal distribution (Figure 3). Ovaries from the spawning and spent stages (postspawning stages) contained only oocytes of less than 12 µm. Thus, it is assumed that most of the oocytes larger than 12 µm are shed out of the ovaries in the current spawning season, and those of smaller size are probably regressed or held for the next spawning season. The occurrences of mixed size groups of oocytes in mature ovaries suggest that this species probably spawns more than once during the spawning seasons, releasing a fraction of the oocytes each time. However, in this study, we were unable to determine the number of oocyte batches that had been spawned in each spawning act. This was due to the difficulty of observing spawning acts in the river.

However, the monthly distribution of the size frequency of oocytes (Figure 4) reinforces a bimodal distributional pattern of oocyte size, and this occurred in every month of the study period, indicating protracted spawning periods. The high percentage of ripening and ripe oocytes or larger size oocytes during heavy rainfall periods (from late September or early October to late December or early January, and in the short periods of March and April) suggests that 2 peaks of spawning activity take place in a year and may precede the heavy rains.

Protracted multiple spawners usually take their spawning cues from extrinsic factors such as rainfall, water level, temperature, and photoperiod (De Vlaming, 1972; Hontela and Stacey, 1990; Gillet and Quétin, 2006). In tropical regions, such as in Peninsular Malaysia, temperatures, day length and photoperiod, and annual

Table 4. External macroscopic observations of the oocyte size and development characteristics of *N. soroides*.

	Classification	Size oocytes	Characteristic
I.	Immature	≤5 µm	Soft, irregular shape, transparent color, no yolk observed
II.	Maturing	6–10 µm	Soft, irregular shape, yolk grayish in color
III.	Developing	11–15 µm	Opaque, oval shape, yolk white to pale yellow in color
IV.	Ripening	16–20 µm	Spherical shape, opaque, yolk yellowish in color
V.	Ripe	21–25 µm	Spherical shape, opaque, yolk yellow to yellowish orange in color

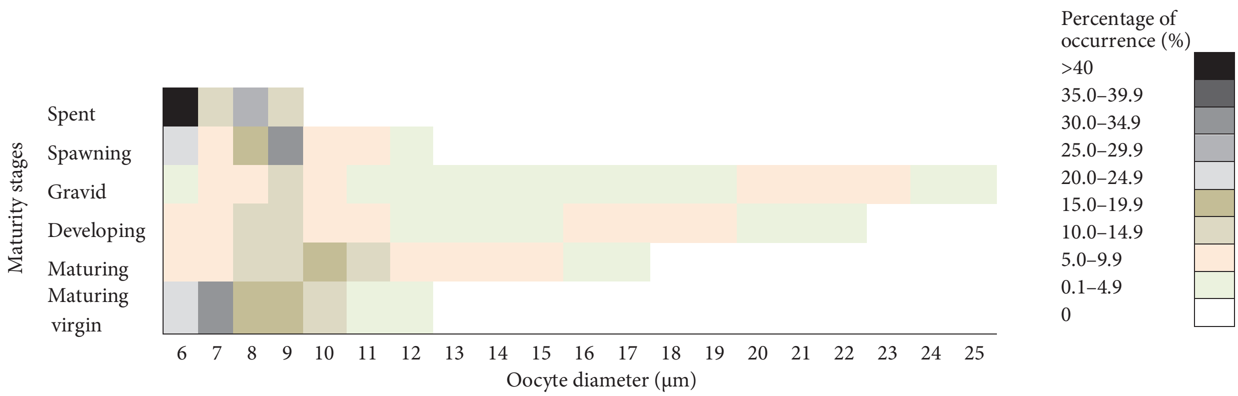

Figure 3. Diameter and size–frequency distribution of oocytes at different ovarian maturity stages of *N. soroides* in the Gombak River.

rainfall distribution may have little seasonal variation and probably had little effect on fish breeding activities (Hontela and Stacey, 1990; Gillet and Quétin, 2006). Thus, the lack of extreme dry or wet periods in the study areas may influence the extension of the fish breeding periods throughout the year (Lowe-McConnell, 1979; Wootton, 1979; De Silva et al., 1985).

The absolute fecundity of *N. soroides* can be considered to be low compared with other conspecies such as *N. hexagonolepis* (Swar and Craig, 2002). This result was probably related to the size of the fish sampled from the study areas. The maximum size of females and males sampled from the Gombak River was smaller compared with *N. hexagonolepis* from India and Nepal (Swar and Craig, 2002). Although the fish studied were low in fecundity, there is a linear relationship between fecundity and total length whereby larger mature fish would be expected to have more eggs, as indicated by Bagenal (1968).

Although the fecundity of this fish was significantly correlated with total length, body weight, and ovary weight, the regression coefficient from the fecundity and these body size relationship equations shows that fecundity is more related to ovary weight than to body weight and total body length. The increase of the ovary weight is due to the increase of the rapid accumulation of yolk protein or vitellogenin in the developing oocytes (Wallace and Selman, 1981). This result suggests that the increase of egg numbers in the ovaries may reflect the increase of ovary weight, which then corresponds to the GSI values (Jons and Miranda, 1997).

Thus, it can be concluded that *N. soroides* is a total spawner. Fractions of oocytes were released during repeated spawning acts, with nearly all oocytes shed out at the end of the gonadal maturity stage. Absolute fecundity of this species is highly related to ovary weight rather than body weight or total length. In the Gombak River, the spawning activity of the fish actively takes place during the rainy months or wet seasons.

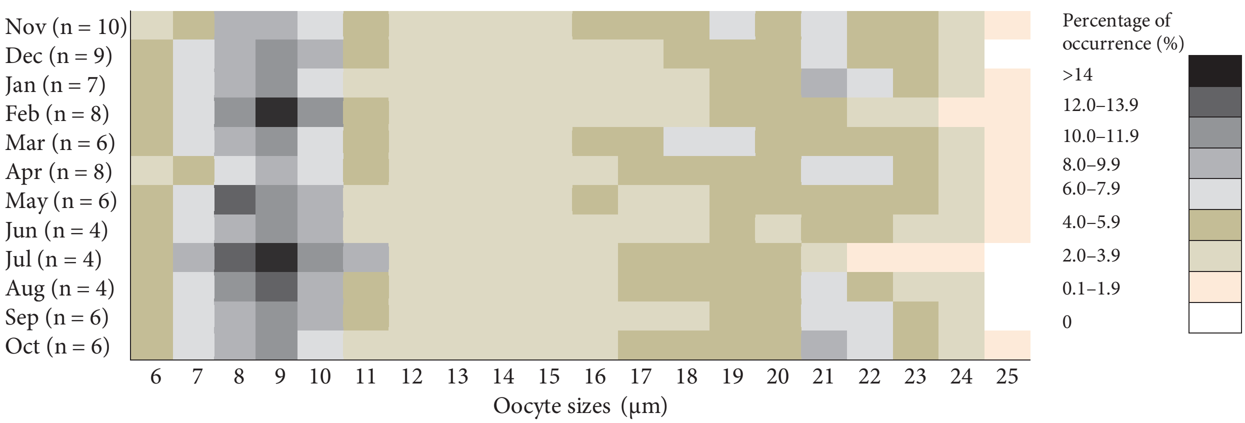

Figure 4. Monthly oocyte diameter (µm) and oocyte size–frequency distribution of the combined developing and gravid ovarian maturity stages in female *N. soroides* in the Gombak River from November 2009 until October 2010.

Table 5. Mean, standard deviation (SD), and range of absolute fecundity of the combined developing and gravid ovarian stages of *N. soroides* from Gombak River.

Total length class (mm)	n	Absolute fecundity	
		Mean ± SD	Range
10.0–10.9	3	1806 ± 734	1099–2565
11.0–11.9	19	1601 ± 519	803–2589
12.0–12.9	14	1954 ± 674	935–3288
13.0–13.9	13	2048 ± 648	1278–3268
14.0–14.9	7	2049 ± 600	1270–2987
15.0–15.9	5	2936 ± 1216	1474–4767
16.0–16.9	1	2691	2691
17.0–17.9	2	2767 ± 499	2414–3119
18.0–18.9	4	4623 ± 942	3265–5392
19.0–19.9	7	3351 ± 1015	2318–5378
20.0–20.9	2	4619 ± 371	4357–4881
21.0–21.9	0	-	-
22.0–22.9	1	6218	6218
Overall	78	2354 ± 1168	803–6218

n is number of ovaries at developing and gravid stages used in analysis.

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