

## Reproductive aspects of naleh fish (*Barbonymus* sp.): A native species from Nagan river, Aceh Province, Indonesia

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**Abstract.** Efizon D, Batubara AS, Muchlisin ZA, Elvyra R, Rizal S, Siti-azizah MM. 2021. Reproductive aspects of naleh fish (*Barbonymus* sp.): A native species from Nagan river, Aceh Province, Indonesia. *Biodiversitas* 22: 2682-2690. Naleh fish (*Barbonymus* sp.) is a native species in Indonesia and reproductive biology is key information to planning a better conservation strategy for this species. Hence, the present study aimed to examine the spawning season, sex ratio, and fecundity of the naleh fish harvested from Nagan Raya District, Aceh Province, Indonesia. Sampling was conducted from January to December 2016 at three sampling locations that were determined purposively based on the recommendations of local fishermen. The fish samples were caught using casting nets and gillnets at two-week intervals. A total of 761 fish samples, comprising 135 females and 626 males, with males predominating at a ratio of 1:4.6 (female: male). The gonadosomatic index (GSI) average of the females ranged from 0.58% to 4.28%, while 1.24% to 4.18% for males. The male fish first matured at 73 mm, whereas the female reached maturity at a size of 85 mm. The total fecundity ranged from 656-5725 eggs with an average of 2663.2 eggs, whereas the relative fecundity from 35.69-254.65 eggs gram<sup>-1</sup> body weight with an average of 136.88 eggs gram<sup>-1</sup> body weight. Our finding able to demonstrate that reproductive properties of *Barbonymus* sp. allow us to better estimate their spawning season either females and males, which can be useful for conservation planning and also their fecundity data was important for aquaculture development in the future.

**Keywords:** *Barbonymus*, fecundity, naleh, reproductive, spawning season

### INTRODUCTION

*Barbonymus* is a true freshwater fish distributed widely in Southeast Asia countries, including Indonesia, Malaysia, Thailand, Vietnam, and the Philippines (Kottelat 2001; Cheng et al. 2004; Satrawaha and Pilasamorn 2009). Ten species of *Barbonymus* have been described worldwide (Yang et al. 2012; Zheng et al. 2016; Froese and Pauly 2018). This organism is a commercial freshwater fish and shows promise for aquaculture (Muchlisin 2013) and has been introduced to several countries, such as India, Pakistan, Bangladesh, and Spain (Mondol et al. 2005; Gante et al. 2008; Leunda 2010; Mollah et al. 2011; Hossain et al. 2016). In Aceh Province, Indonesia, two species of *Barbonymus* have been recorded, namely, *Barbonymus schwanenfeldii* (locally known as Lampam) and *Barbonymus* sp. (with a local name of naleh) (Batubara et al. 2018). Lampam fish is distributed within the eastern and central parts of Aceh Province, whereas the naleh fish is only found in the western and southern parts of the region (Muchlisin and Siti-Azizah 2009; Muchlisin et al. 2015). A previous study showed that naleh fish is

genetically distant from other *Barbonymus* species that have been deposited in the Genbank and is therefore presumed a cryptic species (Batubara et al. 2021, pers. comm).

The lampam fish *B. schwanenfeldii* has been intensively studied by researchers in various aspects, for example, bioecology (Gante et al. 2008; Dewantoro 2015; Huwoyon and Kusmini 2017; Sabarudin et al. 2017; Apendi et al. 2018; Nyanti et al. 2018), genetics (Kamarudin and Esa 2009; Kusmini et al. 2017a; Radona et al. 2017), fish nutrition and immunology (Eslamloo et al. 2012; Song et al. 2012; Eslamloo et al. 2013; Zhu et al. 2016; Eslamloo et al. 2017; Idris et al. 2017; Nyanti et al. 2017; Huwoyon et al. 2018), pathology (Székely et al. 2009; Abdullah et al. 2018), and population dynamics (Isa et al. 2012). However, the reports on naleh fish as cryptic species in Nagan river are very limited.

To date, only four studies have been reported about naleh fish, and they mainly focused on morphological variations (Batubara et al. 2018), length-weight relations and condition factors (Batubara et al. 2019a), population dynamics (Batubara et al. 2019b), and genetics (Batubara et

al. 2021, pers. comm); meanwhile, the biological aspects of naleh fish has never been studied. This fish has been intensively harvested and subjected to (Batubara et al. 2019b), and therefore, conservation action is extremely crucial. According to the studies of Cnaani and Sivan (2009) and Muchlisin et al. (2010), information on the reproductive biology of fish is useful for developing an artificial breeding technology. The data on reproductive biology are also crucial to plan for better fisheries management strategy (Chew and Zulkafli 2012; Hossein et al. 2017). Barbieri et al. (2011) stated that the data on reproductive aspects is an essential reference to determine the reproductive ability of fish and population dynamics. Hence, the present study aimed to analyze several reproductive biological aspects of the naleh fish as cryptic species in Nagan river, Aceh Province, Indonesia.

## MATERIALS AND METHODS

### Time and site

Sampling was conducted from January to December 2016 at the Nagan river, Nagan Raya District, Aceh Province, Indonesia. The samples were processed and analyzed in the Laboratory of Ichthyology, Faculty of Marine and Fisheries, Universitas Syiah Kuala in Banda Aceh, Indonesia.

### Sampling procedure

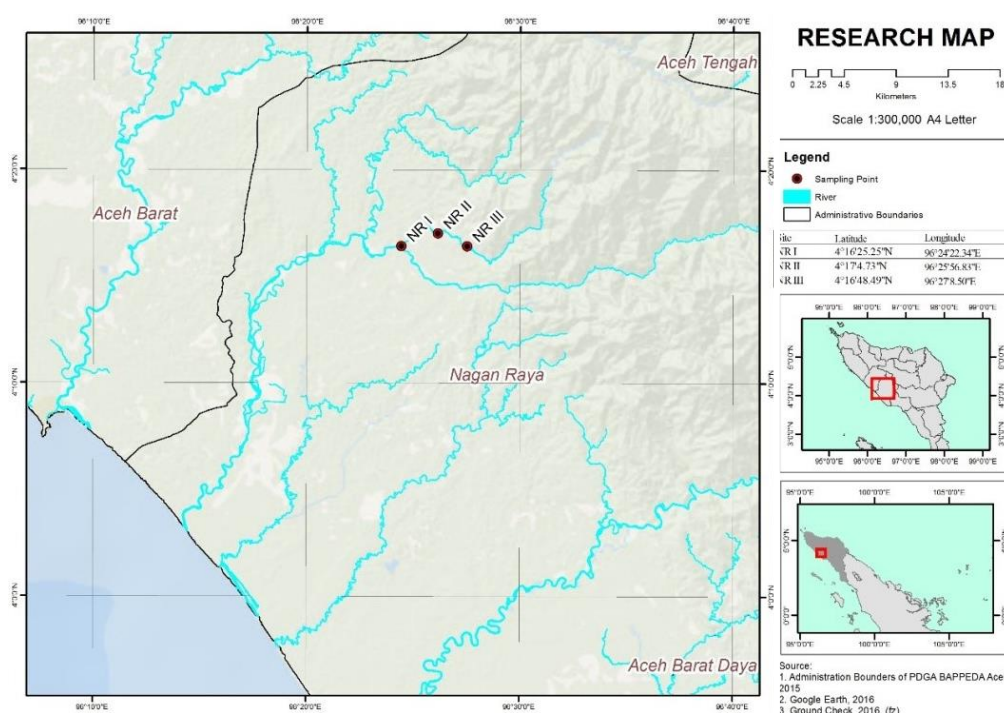
Sampling was performed twice per month (two-week interval) for 12 months at three sampling sites along Nagan river. The sampling sites were determined purposively based on information on locations where naleh fish are often caught as mentioned by local fishermen: (i) Babah

Krueng Village (4°16'25.25"N; 96°24'22.34"E); (ii) Blang Mesjid Village (4°17'4.73"N; 96°25'56.83"E); and (iii) Pante Ara Village (4°16'48.49"N; 96°27'8.50"E) (Figure 1).

The fish were sampled using gillnets, casting nets, and handline hooks from 08.00 AM to 16.00 PM. The sampled fish were washed, and the total body weight (g) and total length (mm) were measured using a digital balance (Toledo, AB-204, standard error = 0.01 g) and digital calipers (Mitutoyo, CD-6CS, standard error = 0.01 mm), respectively. The representative fish were photographed for documentation, preserved in 10% formalin, and then transported to the laboratory in Universitas Syiah Kuala, Banda Aceh for further analysis. The research was followed for the care and use of animals during the research (Section of Animal Care and Use in Research. Chapter 6, Article 11-13) (<https://lppm.unsyiah.ac.id/files/Etika-Penelitian-Unsyiah.pdf>).

### Gonad maturity stage and gonadosomatic index (GSI) analyses

The fish samples were anesthetized and overdosed using oil clove, weighed, and measured (total length, mm). Then, the fish were sacrificed by abdominal section. The gonads were removed and cleaned using tissue paper. The gonads were weighed and observed for various characteristics (morphology, color, surface texture, and the presence of milt or oocytes). The fish were grouped into five stages of gonad maturity following the work of Haryono et al. (2015). The classification was developed by Haryono et al. (2015) for *B. balleroides*, a relative of naleh fish. The GSI was calculated based on the work of Muchlisin et al. (2010) as follows:  $GSI (\%) = \text{gonad weight (g)} / \text{Total body weight (g)} \times 100$ .



**Figure 1.** Map of Nagan Raya District, Aceh Province, Indonesia showing the sampling sites (black dots)

### Sex dimorphism, sex ratio, and fecundity analyses

Sex dimorphism was observed to distinguish the morphological and coloration differences between male and female fish. The sex ratio was calculated based on the work of Adenike (2013) and Muchlisin et al. (2010) as follows: Sex ratio = Total male fish/Total female fish. The relative and total fecundities were examined using gravimetry following the work of Muchlisin et al. (2011) and Muchlisin (2014) as follows:  $TF = n \cdot (W_t/W_s)$ ;  $RF = TF/BW$ , where TF is the total fecundity (oocytes per spawning season), RF is the relative fecundity (oocytes  $g^{-1}$  bodyweight),  $n$  is the total oocyte in a subsample of the gonad,  $W_t$  is the total weight of the gonad (g),  $W_s$  is the total weight of the subsample of the gonad (g), and BW is the total body weight of fish (g).

### Histological analysis

Histological samples were prepared based on the work of Muchlisin et al. (2010) as follows: the gonad samples were preserved with 10% formalin for 5 days. After one week, the gonad samples were washed with tap water and dehydrated in an increasing alcohol series (70%-96% alcohol) for 30 min. Then, the gonad samples were cleared using xylene, embedded in paraffin, and then sectioned at 4-5  $\mu m$  thickness using a microtome (SLEE Modelo CUT 4062). The sections were stretched in a 40°C water bath prepared with distilled water. The sections were mounted on an object-glass slide and then dried for 24 h at 37°C followed by one h drying at 60°C on a stove. The sections were stained with eosin for 15 min, dehydrated using 70% alcohol, and dried at 40°C for 25 s. The samples were observed under a stereomicroscope (Olympus CX23, Japan).

### Ethics approval and consent to participate

The authors confirm that the ethical policies of the journal have adhered to, and the study is in compliance with Syiah Kuala University Research and Ethics Guidelines, Section of Animal Care and Use in Research.

Chapter 6, Article 11-13 (<https://lppm.unsyiah.ac.id/files/Etika-Penelitian-Unsyiah.pdf>).

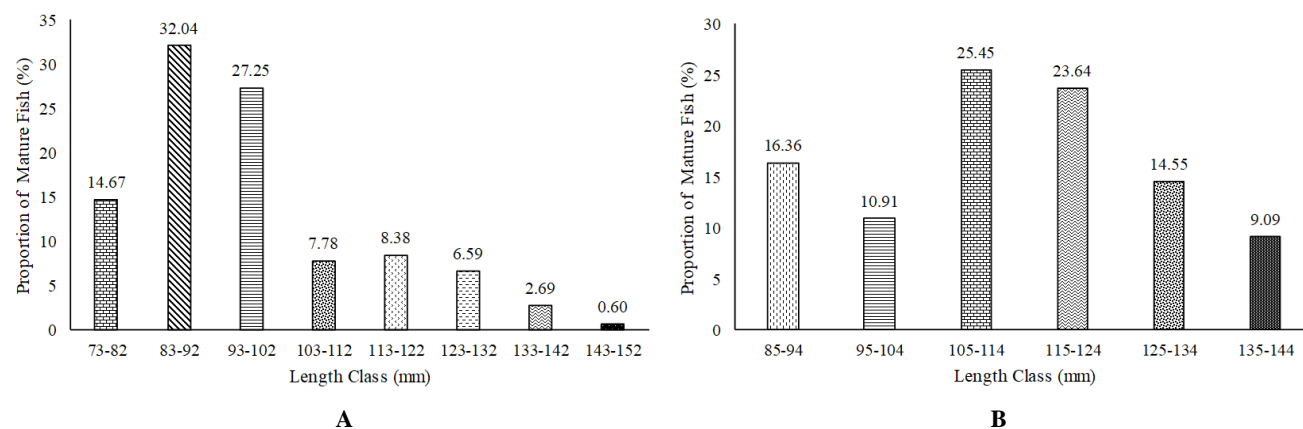
### Data analysis

The data are presented in tables and figures and were analyzed descriptively by comparison with relevant references and reports. In addition, we conducted statistical analysis (regression) on several research parameters including total length between fecundity and body weight between fecundity.

## RESULTS AND DISCUSSION

### GSI and proportion of mature fish

Compare to the males, the average GSI of female naleh fish was higher in January and April. The proportion of mature female fish was also higher during this month. The female GSI decreased during February and March, increased in April, and then decreased gradually from May to October (Table 1). The proportion of mature female fish also increased with the increase in GSI. Meanwhile, the high GSI values of male fish were observed in February, May, and June (Table 2). Matured fish were noted throughout the year, but higher proportions of matured females were recorded in January and April, whereas higher proportions of matured males were noted in February, May, and June. These findings indicated the asynchronous gonad maturation between male and female naleh fish where when the female GSI value was high, the male GSI value was low and vice versa. However, the synchronous gonad maturity was recorded in May and June. Based on the total length of fish samples, most of the matured female and male naleh fish reached sizes of 105-125 and 83-103 mm, respectively. The females matured first at the size of 85 mm, whereas males achieved maturity at a size of 73 mm (Figure 2).



**Figure 2.** Proportion of mature fish based on length class for 12 months sampling: A. Male (n = 334 fish); B. Female (n = 55 fish)

**Table 1.** Gonad development stage and GSI of Female naleh fish

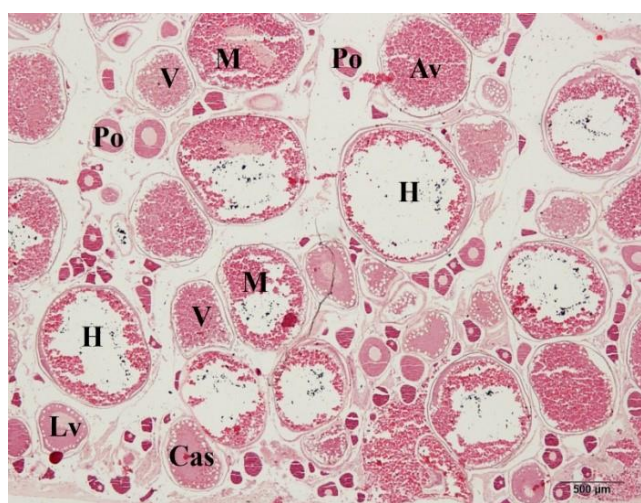
Month	Total sample	Total fish based on gonad development stage (%)					GSI (%)	Gonadal development (%)		Total (%)
		I	II	III	IV	V		Immature	Mature	
January	13	7.69	30.77	7.69	<b>53.85</b>	0.00	0.87-11.23( <b>4.02</b> ± 3.00)	38.46	<b>61.54</b>	100
February	11	36.36	<b>45.45</b>	9.09	9.09	0.00	0.56-9.19(2.12 ± 2.43)	<b>81.82</b>	18.18	100
March	6	0.00	16.67	16.67	<b>33.33</b>	<b>33.33</b>	0.87-3.77(1.97 ± 1.08)	50.00	<b>50.00</b>	100
April	8	0.00	25.00	12.50	<b>62.50</b>	0.00	0.90-9.23( <b>4.28</b> ± 3.09)	25.00	<b>75.00</b>	100
May	10	0.00	20.00	<b>30.00</b>	<b>30.00</b>	20.00	1.15-5.24(3.00 ± 1.29)	40.00	<b>60.00</b>	100
June	7	28.57	14.29	<b>57.14</b>	0.00	0.00	0.86-5.20(3.15 ± 1.93)	42.86	<b>57.14</b>	100
July	9	11.11	<b>44.44</b>	11.11	11.11	22.22	0.49-5.63(2.41 ± 1.54)	<b>77.78</b>	33.33	100
August	15	<b>53.33</b>	26.67	6.67	13.33	0.00	0.20-7.05(2.48 ± 2.17)	<b>80.00</b>	20.00	100
September	8	12.50	<b>37.50</b>	0.00	12.50	<b>37.50</b>	0.34-7.52(1.63 ± 2.41)	<b>87.50</b>	12.50	100
October	4	25.00	25.00	25.00	0.00	25.00	0.19-1.54(0.58 ± 0.63)	<b>75.00</b>	25.00	100
November	18	<b>44.44</b>	27.78	16.67	11.11	0.00	0.31-6.48(1.80 ± 1.79)	<b>72.22</b>	27.78	100
December	26	26.92	19.23	<b>42.31</b>	11.54	0.00	0.56-6.22(2.37 ± 1.66)	46.15	<b>53.85</b>	100

Noted: Bold value is the highest recorded in the respective month

**Table 2.** Gonad development stage and GSI of male naleh fish

Month	Total sample	Total fish based on gonad development stage (%)					GSI (%)	Gonadal development (%)		Total (%)
		I	II	III	IV	V		Immature	Mature	
January	70	15.71	25.71	27.14	<b>31.43</b>	0.00	0.31-5.95(2.25 ± 1.29)	41.43	<b>58.57</b>	100
February	54	3.70	11.11	27.78	<b>55.56</b>	1.85	0.68-8.47( <b>4.18</b> ± 1.95)	16.67	<b>83.33</b>	100
March	51	15.69	19.61	21.57	<b>37.25</b>	5.88	0.20-6.74(2.56 ± 1.66)	41.18	<b>58.82</b>	100
April	75	<b>40.00</b>	12.00	10.67	37.33	0.00	0.10-7.63(2.19 ± 1.98)	<b>52.00</b>	48.00	100
May	57	0.00	<b>38.60</b>	33.33	26.32	1.75	0.63-10.53( <b>3.01</b> ± 1.62)	40.35	<b>59.65</b>	100
June	52	13.46	15.38	19.23	<b>51.92</b>	0.00	0.44-5.54( <b>3.15</b> ± 1.40)	28.85	<b>71.15</b>	100
July	52	26.92	21.15	7.69	<b>38.46</b>	5.77	0.17-14.71(2.91 ± 2.74)	<b>53.85</b>	46.15	100
August	51	29.41	19.61	11.76	<b>39.22</b>	0.00	0.41-7.52(2.71 ± 1.92)	49.02	<b>50.98</b>	100
September	43	18.60	<b>39.53</b>	18.60	20.93	2.33	0.17-10.04(1.85 ± 1.68)	<b>60.47</b>	39.53	100
October	56	21.43	<b>26.79</b>	17.86	19.64	14.29	0.12-9.04(1.24 ± 1.57)	<b>62.50</b>	37.50	100
November	39	<b>38.46</b>	30.77	23.08	7.69	0.00	0.14-4.57(1.44 ± 1.00)	<b>69.23</b>	30.77	100
December	26	<b>38.46</b>	19.23	11.54	30.77	0.00	0.43-3.74(1.59 ± 0.88)	<b>57.69</b>	42.31	100

Note: Bold value is the highest recorded in the respective month



**Figure 3.** Asynchronous maturity of naleh gonad: primary oocytes (Po), lipid vesicles (Lv), cortical alveolus stage (Cas), vitellogenic (V); advanced vitellogenic (Av), migrating (M), and hydrated (H) stages

Histological analysis of female gonads (ovary) showed that the gonads consisted of oocytes of several sizes, indicating that the fish exhibited asynchronous gonad development (Figure 3). This study revealed seven stages of gonad development among the female naleh fish, namely: primary oocytes with an average size of  $\pm 50 \mu\text{m}$ , lipid vesicle stage (average oocyte size of  $\pm 275 \mu\text{m}$ ), cortical alveolus stage (average oocyte size of  $\pm 385 \mu\text{m}$ ), vitellogenic stage (average oocyte size of  $\pm 650 \mu\text{m}$ ), protein granule stage (average oocyte size of  $\pm 750 \mu\text{m}$ ), migrating stage with the oocyte size of  $\pm 1000 \mu\text{m}$ , and migrating stage with the oocyte size of  $\pm 1250 \mu\text{m}$  (Figure 4). No significant correlation was observed between GSI and rainfall in 2016 (Figure 5).

#### Sex ratio, fecundity, and dimorphism

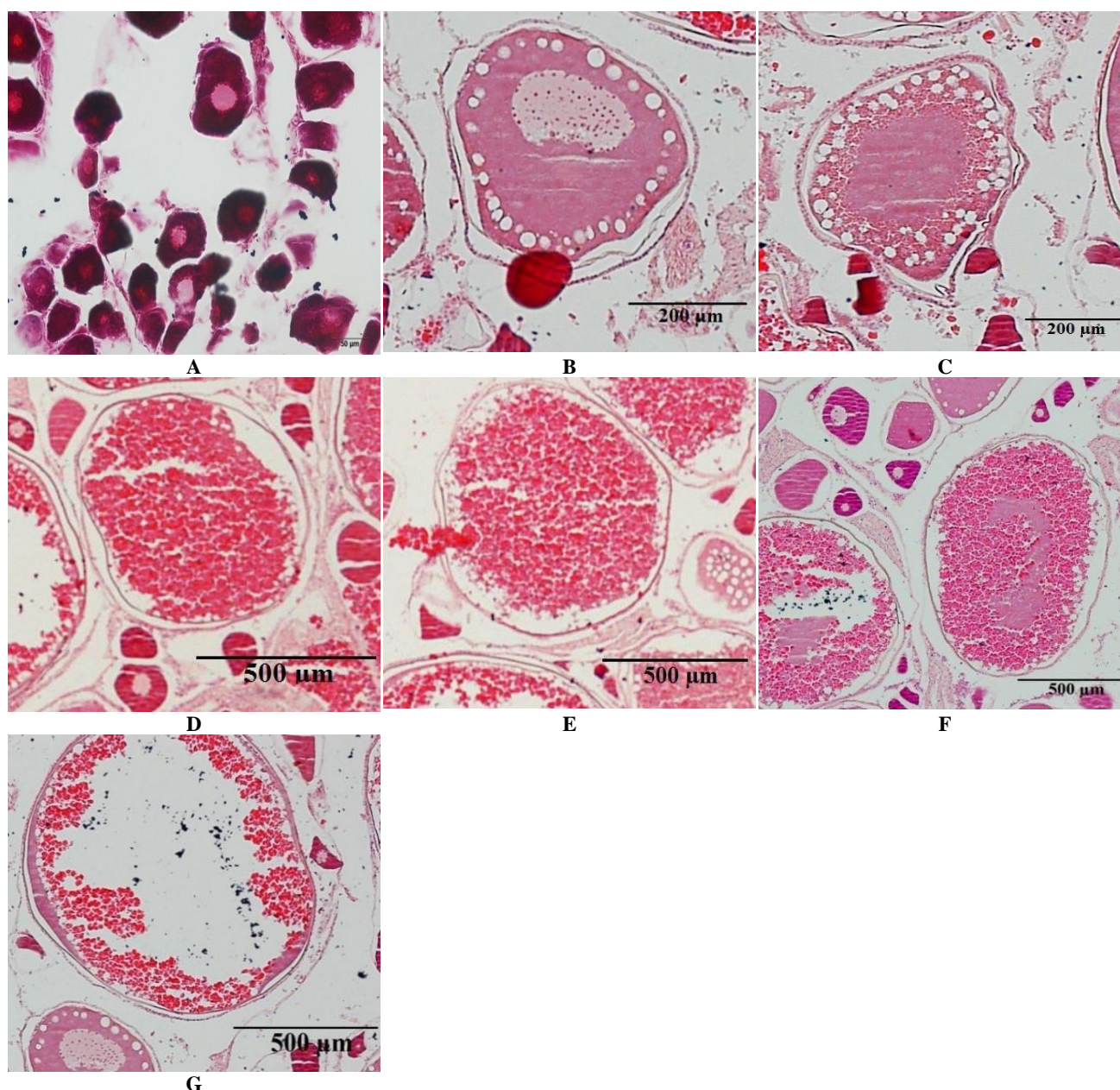
The results revealed that the male naleh fish was predominant throughout the year with the highest ratio in October, whereas the sex ratios of males and females were equal in December (Table 3). The total fecundity ranged between 656-5725 eggs with an average of 2663 eggs per



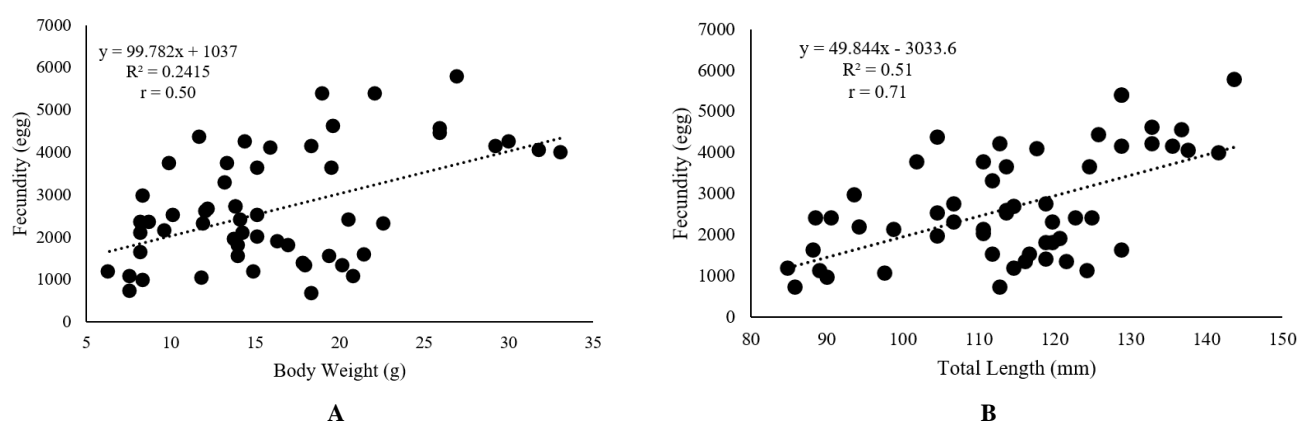
spawning season, whereas the relative fecundity ranged between 36-255 eggs  $\text{g}^{-1}$  bodyweight with an average of 137 eggs  $\text{g}^{-1}$  bodyweight. A strong relationship was noted between fecundity and body weight, that is, fecundity increased with the increase in body weight (Figure 6.A). A similar trend was also observed in the fish length, that is, the fecundity increased as the total length increased. For example, the fish with a length between 85 mm to 95 mm yielded 1110.8 eggs per fish, and it increased to 4552.5 eggs at lengths between 135 mm to 145 mm (Figure 6.B). Male and female fish showed sexual dimorphism, in which the male fish are smaller than females. The male fish showed a compressed body shape, whereas the females exhibited a unanimous body shape. However, the sex coloration between male and female was difficult to distinguish.

**Table 3.** Sex ratio of *Barbonymus* sp. according to monthly sampling

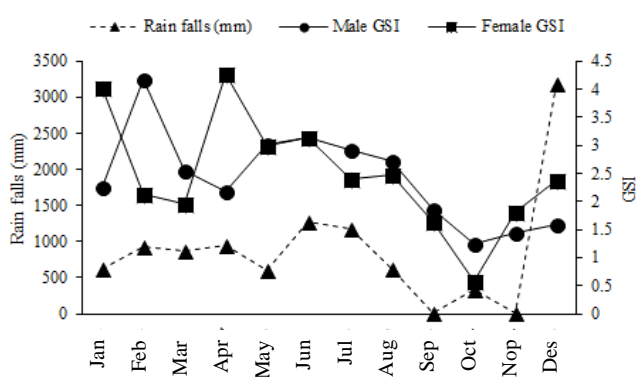
Month	Total individuals		Proportion (%)		Ratio (M: F)
	Male	Female	Male	Female	
January	70	13	84.34	15.66	5.4
February	54	11	83.08	16.92	4.9
March	51	6	89.47	10.53	8.5
April	75	8	90.36	9.64	9.4
May	57	10	85.07	14.93	5.7
June	52	7	88.14	11.86	7.4
July	52	9	85.25	14.75	5.8
August	51	15	77.27	22.73	3.4
September	43	8	84.31	15.69	5.4
October	56	4	93.33	6.67	14.0
November	39	18	68.42	31.58	2.2
December	26	26	50.00	50.00	1.0
Average	52.17	11.25	81.59	18.41	6.1



**Figure 4.** Gonad development of female fish based on oocyte size: A. Primary oocyte; B. Lipid vesicle; C. Cortical alveolus stage; D. Vitellogenic; E. Advanced vitellogenic; F. Migrating; G. Hydrated stages



**Figure 6.** A. correlation between body weight and fecundity and B. the correlation between total length and fecundity



**Figure 5.** Relationship between rainfall and average GSI of naleh fish in 2016

The study revealed that naleh fish *Barbonymus* sp. are multiple spawners, that is, they spawn over a year with the peak of spawning season in May and June or at the end of the rainy season. Histological analysis showed several sizes of oocytes in the ovary, indicating the asynchronous gonad development pattern. Asynchronous multiple spawners were also recorded for *B. schwanenfeldii* from Perak River, Malaysia (McAdam et al. 1999). However, a contrary finding was reported for the *B. gonionotus* from Rajshahi waters Bangladesh (Bhuiyan et al. 2006) and from Serayu river Central Java, Indonesia (Haryono et al. 2015), where this species thrives as a total spawner. According to the work of Colmenero et al. (2013), a gonad of total spawner fish had oocytes with a homogenous size, indicating the same level of development (synchronous); these fish can lay eggs as a whole during spawning, whereas multiple spawner fish feature different levels of oocyte development (asynchronous) in the gonad, which consists of several oocyte sizes that indicate different levels of maturity; thus, at the time of spawning, the eggs released are only those that have matured, whereas the unmatured ones will be released in the next (Muchlisin 2014). Haryono et al. (2015) reported that spawning patterns in fish are influenced by the availability of food sources, water

temperatures (seasons), and locations; therefore, fish of the same species but different locations possibly have different spawning patterns as detected in this study. However, no correlation was detected between rainfall and the spawning season of naleh fish. However, in general, the peak spawning season of freshwater fish in tropical region occurs during the rainy season as recorded in several species, including depik *Rashora tawarensis* in Lake Laut Tawar, Indonesia (Muchlisin et al. 2010, 2011), *Prochilodus brevis* in the Brazilian (Chellappa et al. 2009), and *R. lateristriata* in Ngrancah River, Indonesia (Sentosa et al. 2010).

The present study revealed that the *Barbonymus* sp. males first mature at 73 mm followed by the females at a size of 85 mm, with the total fecundity reaching 2663 eggs. These sizes are smaller than those of other *Barbonymus*, for example, Jasmine and Begum (2016) reported that the *B. gonionotus* females in Padma waters, Bangladesh first show mature gonads at 130 mm size, with the mean total fecundity reaching 58,660 eggs. Moreover, Bhuiyan et al. (2006) reported that the female *B. gonionotus* from Rajshahi waters, Bangladesh first mature at 159 mm in size with an average fecundity of 6964 eggs. Besides, female *B. balleroides* first mature at a size of 175 mm, with a total fecundity of 17,347 eggs (Haryono et al. 2015). The size of the first mature fish gonad is an important indication of overfishing, where overfished species tend to mature earlier or at smaller sizes. Therefore, based on this present study and data on population dynamics (Batubara et al. 2019b), the naleh fish in the Nagan Raya River has been overfished, and this previous finding is supported by the present study. A similar finding was observed with *Rashora tawarensis* (locally name of depik), another endemic species of fish in Aceh (Muchlisin et al. 2010).

The results showed that the fecundity of naleh fish was positively correlated with body weight and the total length, where the fecundity increased with increasing fish length and body weight. This finding coincides with that of Bhuiyan et al. (2006), who worked on *B. gonionotus*. A similar finding was also reported for *R. tawarensis* in Lake Laut Tawar (Muchlisin et al. 2010) and *Scaphirhynchus albus* and *Noturus placidus* (Bulger et al. 2002; Albers et

al. 2013). In general, the fecundity of naleh fish is lower than other *Barbonymus* species, for example, *B. gonionotus* in the Padma waters (Jasmine and Begum 2016) and Rajshahi waters Bangladesh (Bhuiyan et al. 2006), *B. schwanenfeldii* in Jambi waters (Kusmini et al. 2018) and the Iberian Peninsula (Gante et al. 2008), and *B. collingwoodii* in Opak River, Bantul, Jogjakarta (Yusuf 2013). However, the fecundity of naleh fish is higher than that of *B. balleroides* in Cirata Reservoir, Cianjur, West Java (Kusmini et al. 2017b). Bryan et al. (2007) stated that fecundity is not only influenced by internal factors (genetics, age, and size) but also by external factors, including feed sources and environmental conditions. Therefore, fish of the same species but different water habitats will possibly have varying fecundities due to environmental conditions and the availability of food sources.

The sex ratio of naleh fish shows that males were predominant with a ratio of 1:4 (female: male), whereas the mature fish sex ratio was 1:6 (female: male). The ideal sex ratio is when the numbers of males and females are balanced (Haryono et al. 2015; Jasmine and Begum 2016). Baroiller et al. (2008) indicated that the sex ratio of fish in nature is strongly influenced by the temperature and pH of the waters, for instance, in tilapia, warmer waters will produce more male fish. This condition is probably related to global warming, where the average earth temperature has been increasing over the years (Levitius et al. 2000; King et al. 2006). The low number of females will have an impact on the low number of new recruitments of progeny; on the other hand, the threat to the naleh fish population in the Nagan river has been increasing over years due to overfishing, environmental damage, and pollution (Batubara et al. 2019b). Besides, mature female fish are mainly targeted by local fishermen because the eggs are popularly consumed by the locals. This practice is probably another reason why the number of female fish is lower. Besides, based on the observations in the present study, in the dry season (or at the end of the rainy season), which is also the peak of the spawning season of this species, local fishermen use poison to catch fish. This condition gives pressure on the naleh fish population in the Nagan river.

In conclusion, naleh fish are asynchronous multiple spawners; they spawn throughout the year with the spawning season peak in May and June. The males are predominant in the population with the sex ratio male: female was 6.1. The male fish first mature at 73 mm, followed by females at 85 mm, with the average total and relative fecundities of 2663 eggs and 136.88 eggs gram<sup>-1</sup> body weight, respectively.

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