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Research Article
Reproductive Biology of Female Bilih Fish (*Mystacoleucus padangensis* Bleeker 1852) in Naborsahan River Toba Lake, North Sumatera, Indonesia

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Abstract
Background and Objective: The study of reproductive biology of bilih fish (*Mystacoleucus padangensis*) has been conducted for one year in Naborsahan River, Toba Lake, North Sumatra. The aim of this study was to examine the reproduction characteristics of female bilih fish. **Methodology:** Sampling of fish was carried out at six stations determined based on the river characteristics and bilih fish habitat using bag net and cast net. Gonadosomatic index value varied from 0.12-31.70% with an average ranging between 5.70-9.16%. **Results:** Observation of 432 mature ovarians showed that the fecundity of bilih fish ranging between 8683-17824 eggs. Directly-measured oocyte diameter ranged from 70.6-877.8 µm, while histological-measured oocyte diameter ranged from 60.2-747.3 µm. **Conclusion:** Based on morphological and histological examinations, the gonad developmental stages of female bilih fish (ovarians) can be divided into five stages: Stage 1 (Immature), Stage II (Pre mature), Stage III (Maturing and Mature), Stage IV (Pre spent) and Stage V (Spent). Bilih fish spawning patterns are partial and the spawning peak occurred in August-September and February-March.

Key words: Sexual maturity, oocyte diameter, fecundity, spawning

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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

Bilih fish (*Mystacoleucus padangensis*) is one of Cyprinidae member[1]. This species has a very limited distribution in Indonesia and is originally listed as endemic fish of Singkarak Lake, West Sumatra, Indonesia[1-5]. However, since 2003 it has been recorded as the fish which successfully introduced in Toba Lake, North Sumatra, Indonesia[3-5].

The development of fish reproductive organs can be identified by morphology and histology. Histological observation is the most accurate method and generate a lot of information[6] but takes the longest time and has a high cost. On the contrary, morphological identification of gonads is the simplest, easy and fastest method but has accuracy allegedly too uncertain and subjective please rephrase this sentence to make it much clear[7].

Bilih fish reproductive biology studies that have been reported in Lake Singkarak by Rachmatika[8], Syandri[9,10], Junaidi[11], Junaidi *et al.*[12], Patriono *et al.*[13], Purnomo and Sunarno[14] and in the Lake Toba by Kartamihardja and Purnomo[15], Kartamihardja[5], Kartamihardja and Sarnita[3,4], Barus[16], Umar and Kartamihardja[17]. They only reported fish gonad development bilih classification is based on morphology of the gonads. Histological techniques have been applied to fish bilih Lake Singkarak by Syandri[2] but only reported the condition of gonad developmental stages found at that time and did not provide classification of bilih fish gonad development in detail. Determination of gonadal development does also considered inappropriate because it is based on fish with a different family.

Ovarian histological patterns of Teleostei fish was described in accordance with the division of ovarian tissue into five[18], seven or eight[19] stages of maturity. This classification was based on dominancy of gametogenic cell type presence. The purpose of this study was to determine the reproductive biology and histology of ovarians bilih fish of Naborsahan River, Lake Toba, North Sumatra. The results can be used for the purposes of management of this species.

MATERIALS AND METHODS

This study was conducted in Naborsahan River Ajibata District Toba Samosir Regency, North Sumatra (is this is the Toba Lake; if so please say that) . Sampling of fish was carried out at six stations which were determined based on the river characteristics and bilih fish habitat (Fig. 1). Sampling was carried out every month from April, 2013 to May, 2014. Sample analysis were done in Integrated Laboratory of Aquatic Resource Management Department, Faculty of Agriculture and Anatomical Pathology Laboratory, Faculty of Medicine, North Sumatra University.

The fish were caught using bag net and stocking net. Fish samples obtained were used to directly measure the fish total length with a accuracy of 1 mm, body weight and gonad weight with a accuracy of 0.01 g. The weight of the gonads were determined after the observation of morphometric characters of fish. After the weight of gonads were recorded, ovarians were directly inserted into Buffere Neutral Formaldehyde (BNF) solution. The ovarian developmental in stage III and IV were divided into two parts, one part was inserted into the BNF solution for histological slides and the other part was put into gilsen solution to count its fecundity and measure its egg diameter.

Observation of gonadal development level were done macroscopically and microscopically. Macroscopical or anatomical observations based on size, color and volume in the abdominal cavity. Microscopic observations were done through gonad histological slides. Gonads were prepared for the histological examination by fixing the gonads in BNF. They were dehydrated in graded alcohol series, exposed to xylol and embedded in paraffin wax. Sections from 5-6 μm thick were prepared from the middle parts of the gonads. The sections were stained with hematoxylin and eosin, then mounted in Canada balsam and photographed with an Trinokular Carl Zeiss Primostar digital camera. Five stages for oocyte development under study were identified by microscopic examination according to Brown-Peterson *et al.*[18]. Gonadosomatic index (IGS) of bilih fish was calculated based on the formula by Effendie[20], where, IGS = (gonad weight $\times 10^2$)/fish b.wt. and Fecundity was measured in mature ovarians fish (in morphology exist in GDS IV and V). Fecundity measurement used gravimetric method[20,21] where, fecundity = (gonad weight $\times$ number of half egg sample)/eggs weight of half gonad part. Fecundity relationship with fish size (length and weight) were determined using regression analysis[22]. Egg diameter measurement with an accuracy of 0.01 μm were done on mature female fish eggs (in morphology exist in GDS IV and V) and the entire female fish which were made its histological slides. The results of egg diameter measurement were created into its frequency distribution using Microsoft Excel 2010. The frequency distributions of fish eggs diameter that were measured directly (GDS IV and V) and those measured by histological slides were used to determine the spawning pattern.

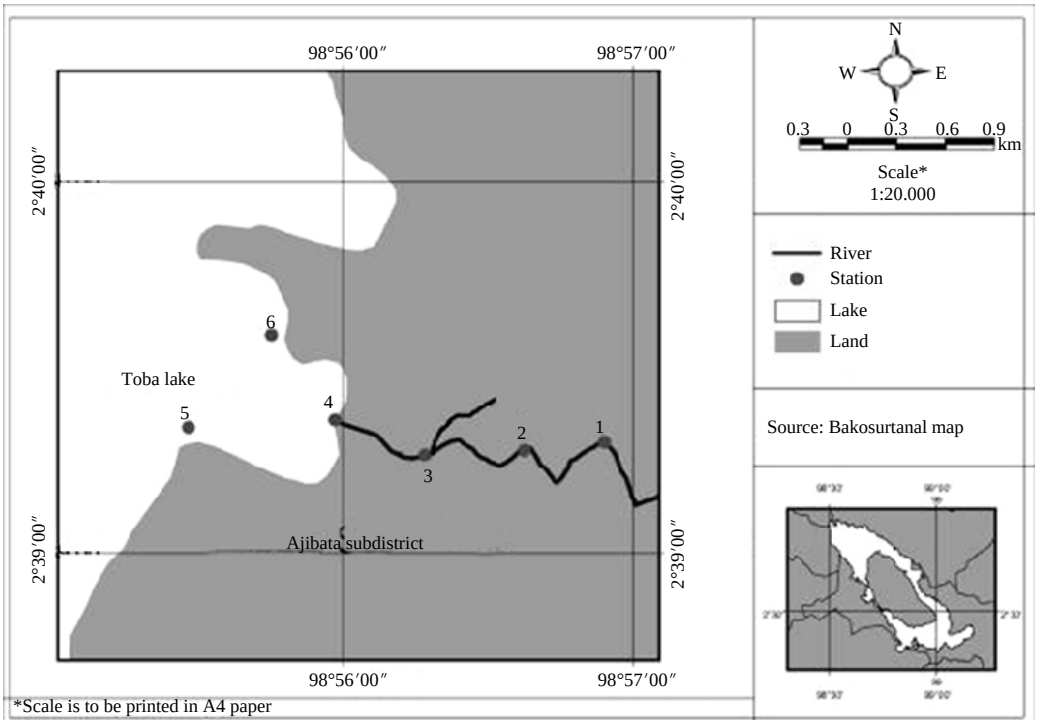

Fig. 1: Sampling station at Naborsahan River Toba Lake North Sumatera

RESULTS AND DISCUSSION

The total length of bilih fish ranged from 35-188 mm and the weight was 1.27-47.7 g. The value of GSI varied from 0.12-31.70% with average values ranging between 5.77 and 10.24% (Fig. 2). The GSI in this study showed a greater value than those of bilih fish in Lake Singkarak, which ranged between 7,24 and 11,1%[12]. The average value of the highest GSI in September 2013 (9.32%) and March 2014 (10.24%) but the highest number of gonadal mature females were discovered in March and September. This showed that the peak of spawning occurred in August-September and February-March (Fig. 2).

Stages of ovarians of *M. padangensis* described in five stages based on Brown-Peterson *et al.*[18] that has been accepted by many researchers as a standard procedure for determining fish gonadal development stages, i.e., Grier[23], Abaszadeh *et al.*[24], Zeyl *et al.*[25] and Dopeikar *et al.*[26].

Stage I (Immature), this stage could be found in bilih fish, which either ever or never spawn. Ovarian pairs were very small and stick close to the spine, often looked obvious and sometimes looked like a gray or transparent line and the blood vessels were unclear. Eggs could not be visible to the naked eye and the sex (male and female) could not be distinguished. In histological sections of ovarian oocytes, there were only oogonia and Primary Growth (PG) presented. There were no oocyte in atresia. Ovary wall was thin and there were little spaces between the oocytes (Fig. 3a).

Stage II (Pre mature) is an early stage of developing, emerging but not ready to spawn. Ovarian looked like reddish opaque tube occupying almost half of the body cavity. Real blood vessels were in the surface. Immature eggs, that resembled white spots have been seen with the naked eye. Primary growth, oocytes presented at this stage were alveolar Cortical Oocytes (CA), primary vitellogenic (Vg1) and intermediate vitellogenic (Vg2). There was no complex evidence of postovulatory follicles (POFs) or tertiary vitellogenic oocytes (Vg3). Some atretic follicles could be present. Early developing sub-stage: only PG and CA oocytes were present (Fig. 3b).

Stage III (Maturing and Mature), at this stage the fish was able to spawn and at the end of the developmental and physiological period, it was able to produce some eggs. The ovaries filled the body cavity. The ovarian color was yellowish gray. Core and hydration stages of migration could be distinguished. At this stage, the core migrated and begun to leave the central position and migrated towards the periphery. Yolk filled more than two-thirds of the cytoplasm.

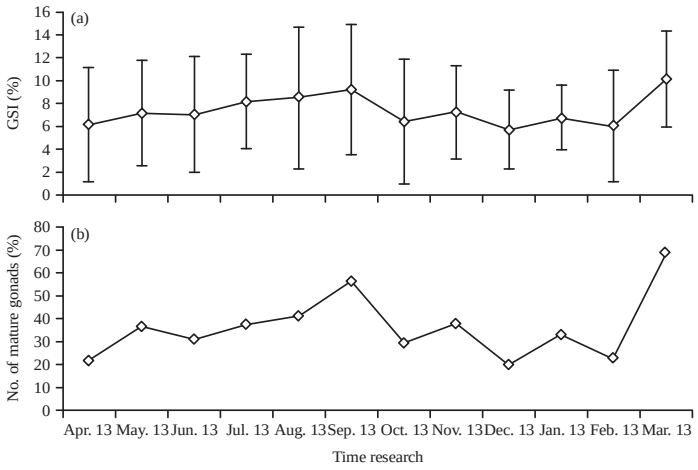

Fig. 2(a-b): (a) GSI and (b) Number of mature female Bilih fish gonads (*Mystacoleucus padangensis*) in Naborsahan River, Lake Toba, North Sumatra

The size of oocytes remained relatively stable. At hydration stage, yolk granules filled the whole cytoplasm. The core nucleus was in the oocyte circle polar (Fig. 3c).

Stage IV (Pre spent) is the stage of deterioration, spawning termination. The mushy ovarian and prominent blood vessels were characterstics of this stage. Oocytes presented at this stage were atretic and POFs were present. Some CA and/or vitellogenic (Vg1, Vg2, Vg3) oocytes were also present at this stage but with few numbers (Fig. 3d).

Stage V (Spent) is a regeneration stage, sexually mature or active reproducedtion. It is characterized by loose and red empty ovaries. Some rest of the eggs were in the resorption process (Fig. 3e). In the two final stages or after ovulation, the ovaries were small, red and granular with the rest oocytes scatters. These ovaries included POFs mature oocytes and mature eggs left unspawned. Ovarian imposed oocytes at different stages, with most of them were in oogonia and perinucleolar stage. It containes some atresia oocytes which were characterized by ooplasm and yellow degradation (Fig. 3e). Ovaries of Bilih fish is kind of asynchronous-group with a capacity for some ovulation in the reproductive seasons. This developing oocytes distribution pattern showed that Bilih fish is partial spawner, spawning may take place 2-3 times during their reproductive period.

Based on Gonadal Development Stages (GDS) of bilih fish caught, they were in GDS I to GDS V. The percentage of gonadal mature females (GDS III) could be found in every month of sampling. The percentage of gonadal mature females (the highest GDS III) could be found in March, 2014, followed by in September, 2013 and the lowest percentage of GDS III and IV was found in December, 2013 (Fig. 4). The results in Fig. 4 showed that the number of female fish in GDS III almost always dominated in the number of fish caught during the study. The results of these observations showed that bilih fish spawn every month. This is in line with the statement by Rahardjo *et al.*[27] that the fish in the tropical environment generally spawn throughout the year. Beside spawn every month, bilih fish also spawn every day. Bilih fish perform spawning every day starting at 16.00-24.00 Western Indonesian time with the peak of spawning occurs at 19.00-22.00 Western Indonesian time, which is indicated by the number of bilih fish spawn reaches more than 90%[28]. This is in line with the results of the preliminary study performed for a month by sampling once a week, it could always found female fish with GDS III and IV. This is also indicated by the presence of fishing every day with a net bag carried by local fishermen as a trap for bilih fish that migrate from the lake to the river for spawning. Kartamihardja and Sarnita[3,4] stated that bilih fish in Singkarak Lake migrate from the lake to the river for spawning. Migratory habits for spawning also occurs in *Puntius bimaculatus* that grouped in a family with bilih fish (Cyprinidae) and in other Cyprinidae species in Sri Lanka[29].

Observation of 432 mature ovarians showed that the fecundity of bilih fish ranging between 8683-17824 eggs. This fecundity differed with fecundity of bilih fish in Toba Lake in 2009 ranging from 4568-15812 eggs with an average of 10897 eggs and in 2010 its fecundity ranging from 5956-16422 eggs with an average of 11286 eggs. Fecundity of bilih fish in 2010 was still higher than the fecundity of bilih fish in 2009. Similarly, the fecundity in 2005 ranged from 3654-14561 eggs with an average of 7580 eggs[15]. Compared with fecundity of bilih fish in Singakarak Lake, average ranges between 1.495-3.397 eggs[13] and average ranges between

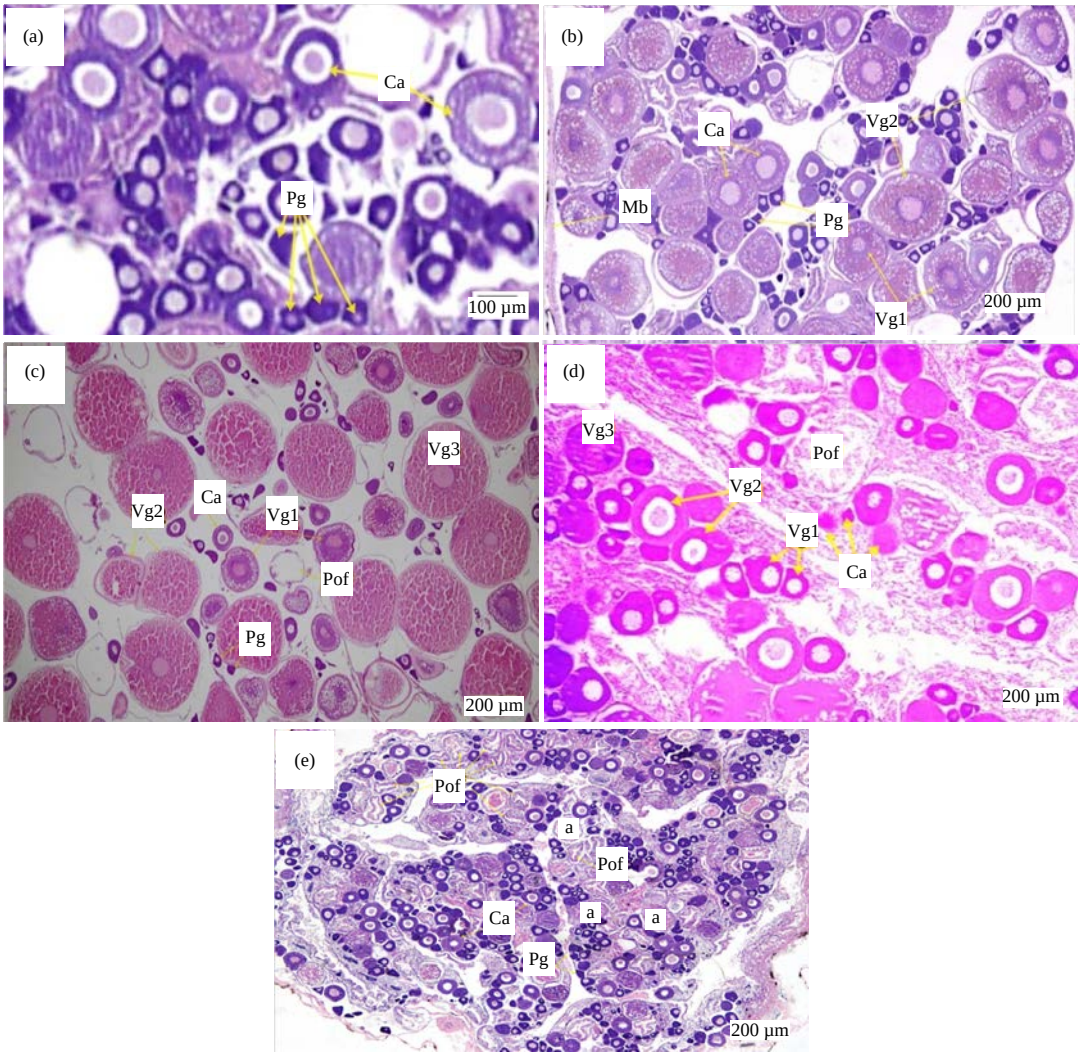

Fig. 3(a-e): Histology ovarium bilih fish (*Mystacoleucus padangensis* Bleeker 1852) in Naborsahan River, Lake Toba, North Sumatra, (a) Stage 1 (Immature), (b) Stage II (Pre mature), (c) Stage III (Mature and Maturing), (d) Stage IV (Pre spent), (e) Stage V (spent), Ca: Oosit alveolar kortikal, Pg: Pertumbuhan oosit primer, Mb: Muscle bundle), Vg1: Oosit vitelogenesis primer, Vg2: Oosit vitelogenesis sekunder), Vg3: Oosit vitelogenesis tersier), GVBD: Germinal vesicle breakdown, Pof: Postovulatory folikel, a: Oosit atresi

2155-5000 eggs[17], fecundity of bilih fish in Toba Lake is quite greater. Bilih fish fecundity ranges between 6907-9355 eggs per individual with body weight[28] ranging from 85.0-110.0 g. Fecundity differences may due to size differences (length and weight) of fish samples and it is also caused by other environmental factors including food. Rahardjo *et al.*[27] stated that the fecundity of fish can be affected by several factors such as environmental conditions and food.

Equation of relationship between fecundity with the length and weight of bilih fish shown in Fig. 5 showed a positive correlation. This indicated that the increasing of length and weight of female bilih fish in mature phase will be followed by an increasing in the fecundity rate. Thus the fish length and weight can be used to estimate the fecundity of bilih fish (Fig. 5). Fecundity of a fish species can be expected through the length and weight data, fecundity is generally more closely correlated with the weight data than the length data[29].

Fecundity of bilih fish in Toba Lake has a relationship with fish total length that following the logarithmic equation: F = 0.3369*L4.0924, (R^2 = 0965) for 2009 and for 2010 the equation was F = 1.9577*L3.3885, (R^2 = 0.878), while

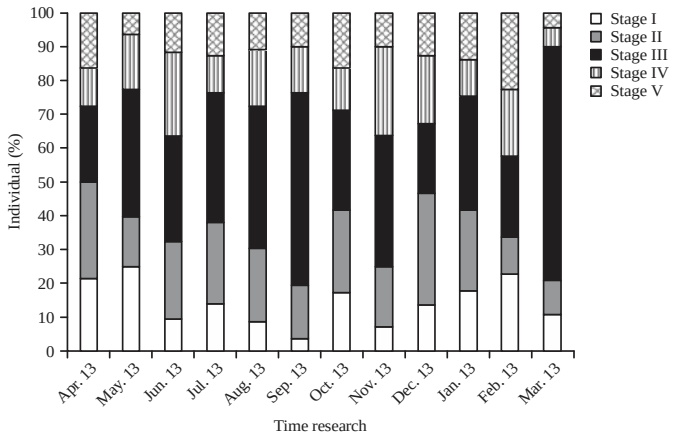

Fig. 4: Percentage gonadal development stage of bilih fish (*Mystacoleucus padangensis*) in Naborsahan River, Lake Toba, North Sumatra

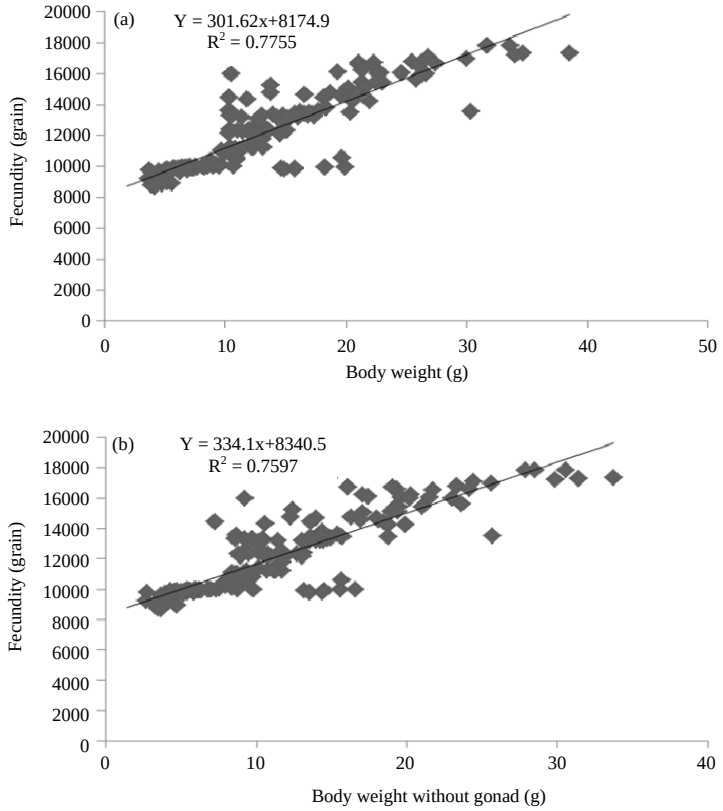

Fig. 5(a-b): Fecundity relationship with (a) Body weight and (b) Body weight without gonads ikan bilih (*Mystacoleucus padangensis*) in Naborsahan River, Lake Toba, North Sumatra

in Singakarak Lake followed the equation F = 0.03632*L2.6653 (R^2 = 0.82). This equation showed the relationship between the total length size with the number of eggs produced: The longer the fish size, the more the number of fish eggs produced[17].

Oocyte diameter which directly measured ranged from 70.6-877.8 µm and diameter histological oocytes ranged from 60.2-747.3 µm. The study results of bilih fish in 2009 and 2010 showed that the fish with mature gonads and ready to spawn has oocyte diameter size 0.33-0.76 and 0.54-0.87 µm.

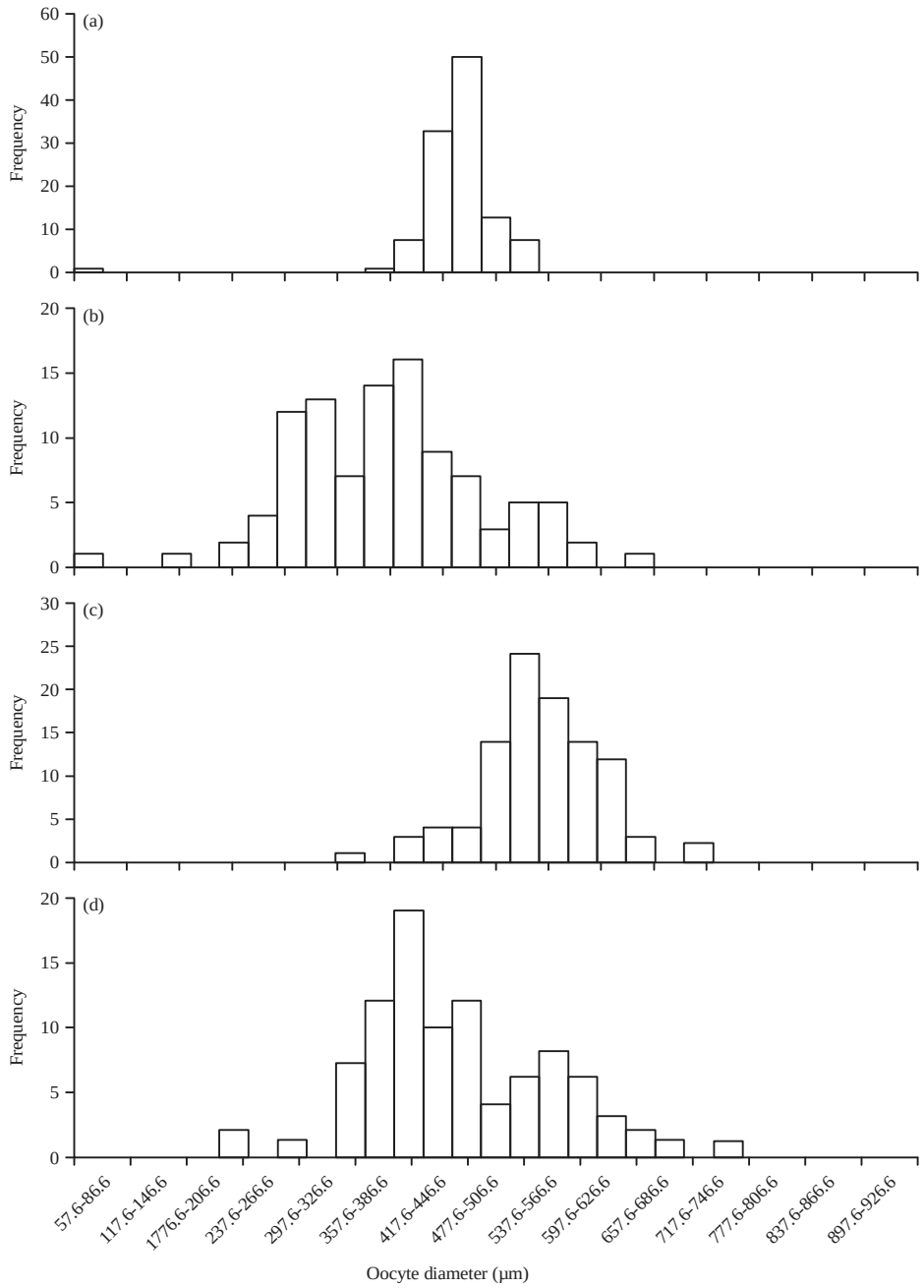

Fig. 6(a-d): Distribution of oocyte diameter measured directly from the ovary bilih fish (*Mystacoleucus padangensis*) in Naborsahan River, Lake Toba, North Sumatra, (a and b) Stage II and (c, d) Stage IV

The highest diameter[4] ranged from 0.44-0.65 and 0.55-0.76 µm. Compared to Singkarak Lake oocytes diameter of fish that are ready to spawn in Lake Toba is relatively larger[17].

Description of fish spawning type can be known through distribution frequency analysis of oocytes diameter size in the ovaries of the fish. Ovarian oocytes diameter size of bilih fish spread formed one to three deployment modes (Fig. 6 and 7). Oocyte diameter distribution of bilih fish in Singkarak Lake only formed two peaks while the oocyte diameter distribution of bilih fish in Toba Lake could form three peaks[4,15].

The results in Fig. 6 and 7 found one to three peaks. The ovaries of spawner fish recurrent at which have been matured, oocytes can usually be found at almost every developmental stages with different size ranges continuously, ranging from

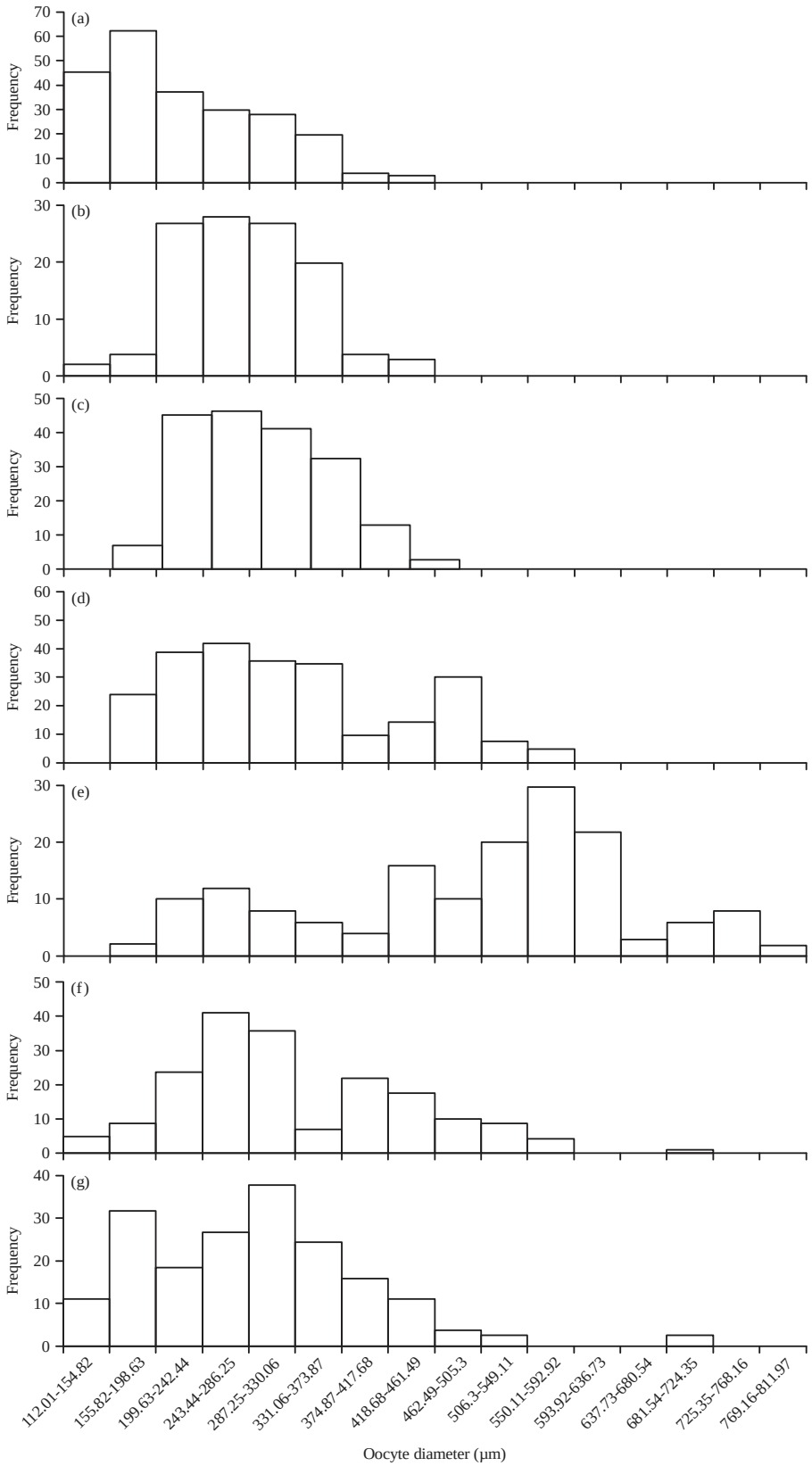

Fig. 7(a-g): Distribution of oocyte diameter measured directly from the histology ovary bilih fish (*Mystacoleucus padangensis*) in Naborsahan River, Lake Toba, North Sumatra, (a, b) Stage I, (c) Stage II, (d, e) Stag III, (f) Stage IV and (g) Stage V

oocytes that have not yet and had the yolk[30]. The results of this study differed from that statement. Figure 6 and 7 showed the existence of a peak showed that the oocytes cannot always be found at almost all developmental stages, with a wide range sizes continuously. The results of this study aborted that statement. This was also confirmed by the results shown in Fig. 3 which showed an oocytes size group in the ovary histological slide of bilih fish. The formation of two peaks of oocytes diameter will occur in bilih fish which has released a mature oocyte size group in its spawning so that remained two peaks. The formation of a peak oocytes diameter also occurred after the fish has twice oocytes size group in twice spawn so that only remained a single peak. This cycle continued throughout bilih fish did gonadal growth.

Hunter *et al.*[30] also stated that there is no great distance between oocyte maturity level except temporary distance between oocytes undergoing hydration with oocytes which have yolk and this kind of fish usually has a long reproductive period and spawn several times during a spawning season. The analysis result of distribution frequency of oocyte diameter size of bilih fish ovary (Fig. 6 and 7) found no great distance between maturity levels. Based on this result, it was known that bilih fish is a recurring spawner type which can spawn several times in a spawning season.

CONCLUSION

The gonad developmental stages of female bilih fish (ovaries) can be divided into five stages: Stage 1 (Immature), Stage II (Pre mature), Stage III (Mature and Maturing), Stage IV (Pre spent) and Stage V (spent). Fecundity or reproductive potential of bilih fish varied based on the fish length and weight. Bilih fish spawning patterns are partial, the spawning peak occurred in August-September and February-March. In order to manage bilih fish in Naborsahan River it is necessary to create the arrangements of bilih fish fishing activities that take place around the river.

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