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Fish reproductive cycles of Wader Cakul (*Puntius binotatus*) during the rainy season

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ABSTRACT

Spotted barb (*Puntius binotatus*) is one of tropical fish in Indonesia freshwater, which has high economic value. To maintain the stock in nature, information about the reproduction cycle in mid rainy season is required for conservation, so that we can control the catch of spotted barb. The result of the research showed that a variety of GSL, GSI and GI's male and female. Quantity of oocyte primer 423; 664; 480; 380; meanwhile that quantity of secondary oocyte 73; 172; 411; 230. Result of water quality was measured in the river as temperature 28-30 °C, pH 6.8-7 and Dissolved Oxygen (DO) 3-3.4 ppm. The gonad weight greatly influenced the rate of GSL, GSI, and GI in determining fish reproduction was interrelated and directly proportional. Increasing of GSL was affected by the increasing value of GSI, and similarly, the GI value, which is directly proportional with increasing of GSI and GSL. The reproductive cycle of gonadal maturity spotted barb increased during the mid rainy season.

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1. Introduction

Indonesia, located upon two sides of the equator, varies enormous climate. Variation climate January by wind, rainfall the and temperature (Jangkaru, 2003). Indonesia has diversity fish in tropical waters very high, where there are 8.500 of fish massive, and is 45 % of the kinds of fish in the world, and 1.300 type of them is the type bream (Kottelat & Whitten, 1996). According to Haryono (2006), the majority of the fish tropical lay eggs/spawn during the rainy season since they occur a stimulus because of the temperature factors in the environment, a chemical change of the water and the flow of water. For example, fish cork (*Channa striata*) who breeding naturally during

the rainy season, Motan fish (*Thynnichtys polylepis*) that move to the raining regions for mating during wet season, and Tombro fish (*Tor tambroides* Bikr), which does not happen in the rainy season but in last half of the rainy season in January – March (Bakhris *et al.*, 2017).

Wader cakul (*Puntius binotatus*) is one of fish in freshwaters Indonesia that possess potential business. Thay can be found in the lake, pond, reservoir, the river, and sewer flow clear. The result of the use of the fish, at present, well obtained from fishing activities by relying on the supply of nature and has been no attempt for culturing. The fishing activity is usually conducted on the rainy season. This effort to ensure the conservation of fish resources can be done with the regulation and management, supported by a lot of the information reproductive biology study covering gender and the level of maturity the gonads so that it can be applied in basic knowledge of biology reproduction (Effendie, 2002). The registration of phases of ripeness the gonads in biology fisheries needed to know how a comparison between fish mating and no mating fish. Based on it, reproduced fish and no reproduced could be identified (Effendie, 1997). This stud aimed to gather information about the fish reproductive cycles of wader cakul in the middle of the wet season and know the relationship between gonad somatic index (GSI), Gonad Somatic Level (GL) and Gonad Index (GI) in determining the reproduction cycle.

2. Material and methods

Study was conducted in the river Kaporan, Kraksaan, sub-district Probolinggo; (Regional Technical Implementation Unit) fish health laboratory and the environment, Probolinggo, the province of East Java; Anatomy Histology Lab, School of Medicine Laboratory, Fish Reproduction Laboratory, Seeding and Fish Breeding the Faculty of fisheries and Marine Science, Brawijaya University, the province of East Java.

Materials were used in this study such as digital scales the balance, sartorius scales, a ruler, a film bottle, sectioset, object-glass, cover glass, the microscope, microtome rotary, water bath, basketball, a brush small, the camera and a tray, wader cakul fish with 30 males and females in each, formaldehyde 10 %, acetone, xylol, paraffin, liquid alcohol 96 %, hematoxylin, a paper label and eosin.

The descriptive methods were used in this study to observe the results. According to Narbuko and Achmadi (2012), research descriptive is research to reveal and solve a problem based on data that have provided, analyzed and interpretated, and also comparative and correlative. The sample of fish was obtained from Kaporan river at 8 a.m, as many as 30 male and female fish in each every observation with the lapse of 2 week time period. Fish was observed morphologically with measured its weighed and length, and fish then was dissected by using sectioset to found its anatomy for GSI determination according to range in determining Bagenal (1978) study. After that, the gonads were weighed by using the balance sartorius scale to obtain the value of GSI and GI as primary parameter.

The calculation of GI followed Cayré and Laloë (1986):

$$GI = \frac{GW}{\mathrm{L\^{}3}}\ x\ 10\^{}3$$

Note: GSI = Gonad Somatic Index (%)
GI = Gonad Index
GW = Gonad Weight (gram)
BW = Body Weight (gram)
L^3 = Body Lenght (cm)

Supporting research parameter included histology and water quality observation. The make slide of gonads histology was begun with the fixation process or organ preservation soaked in formalin 10 % during 18-24 hours and given a paper label as a sign. *Embedding process*, gonads were introduced to acetone for as many as 1 hours for 4x repetition (*dehydration*), xylol for 0.5 hours as many as 4x repetition (*clearing*). After that, gonads was put into a beaker glass containing 55 °C of paraffin liquid for 1 hour with 3x repetition (*impregnation*) in an incubator. The liquid paraffin was poured into mold and gonads were immersed into it and set a few minutes to build the block. *Sectioning process*, paraffin block, was cut and sliced by microtome rotary with a circumference of 4 µm.

Staining process, an object-glass, contained a wedge of the gonads (slide), was put into xylol for 15 minutes three times repetition, alcohol 96 %, 15 minutes three times repetition, washed running water for 15 minutes. The slide was soaked to hematoxylin for 15 minutes, washed running water for 15 minutes, dipped in alcohol acid for one dip, dyed with running water for 15 minutes, immersed into lithium carbonate for 2 – 20 seconds. The next step the slide was washed with running water for 15 minutes, was immersed into with eosin for 10 minutes, was immersed into alcohol 96 % for 15 minutes as many as three times repetition, and was immersed into xylol for 15 minutes as many as three times. *Mounting process*, the slide was added glue then covered with a cover glass. *The labeling*, the slide was labeled to ease distinguished each samples. The documentation of histology gonads slide was photographed under a microscope for observation microanatomy of Wader Cakul gonads. The water quality observation included temperature, pH, and DO.

3. Results and Discussion

3.1. Morphologically.

Morphologically, Wader Cakul has the form of the compressed body with silvery brown color. The kind of its scales is crossed by etched cycloid scales (*linea lateralis*). The barbell or tentacle is situated at the end of his mouth (Figure 1). These fishes have five fins ultimately. The dorsal fin was composed of 1 the fingers hard and soft eight the fingers harden (D.I.viii), a tail fin consisting of 20 the fingers soft harden (C.xx), anal fins composed of 8 the fingers soft harden (A.viii), the stomach fins consisting of 16 the fingers soft tighten (V.xvi) and pectoral fins consisting of 22 the fingers soft tighten (P.xxii).

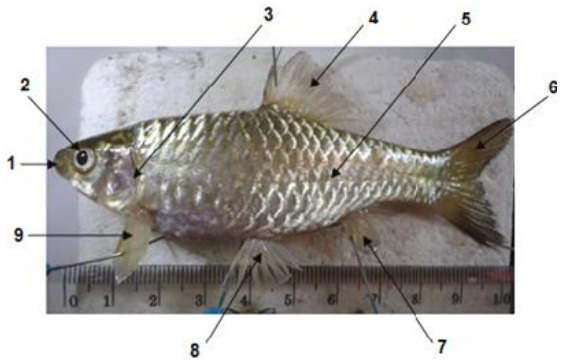

Figure 1. Morphology of Wader Cakul

Note:

1. Mouth	4. Dorsal fin	7. Anal fin
2. Eyes	5. Linea lateralis	8. Ventral fin
3. Operculum	6. Caudal fin	9. Pectoral fin

Wader cakul fish is more readily observable about his sexuality when it starts to mature. On a male fish can be seen on his body a leaner, while a female has more belly in its ventral part

(Figure 2). Based on anatomy part, wader cakul fish has organs like a fish teleostei in general. They have the testicles organs on a male and the ovary in a female as a reproduction organ.

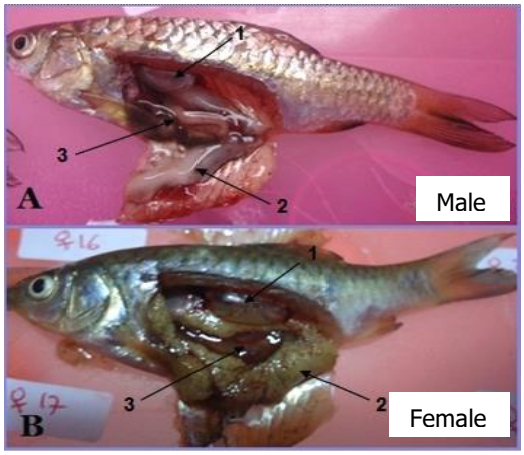

Figure 1. Anatomy of Wader Cakul Fish

Note:
1. Swim bubble
2. Reproduction organ
A. Testis
B. Ovarium
3. Digestive organ

3.2. Gonad Somatic Level (GSL).

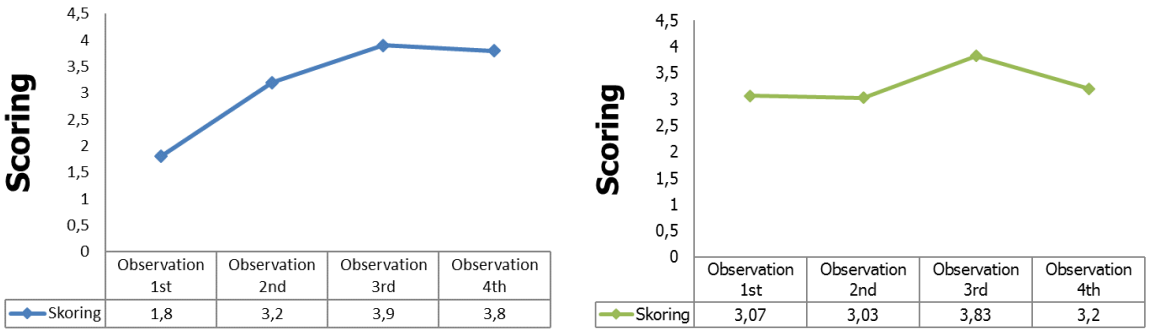

Figure 2. GSL score of male and female wader cakul fish

The Figure 2 showed GSI of wader cakul male and female fish increasing that could be identified in the observation 3rd (the first week of January) by 3.9 and 3.83 respectively (Figure 3). They were in GSI IV (second development stage) which means that fish is on the mature condition. According to Makmur *et al.* (2017), the reproduction organ of fish could be called mature when it have reached to GSI IV.

3.3. Gonad Somatic Index (GSI).

Figure 4 showed the GSI of wader cakul fish varying at any time observation. The highest score of male GSI reach 3.29 %, while female GSI reach 11.46 % happened on the 3rd observation. This is in accordance with time on the condition dominant of GSI where the observation the first week of january. Wader cakul male and female fish have an average point GSI that is directly proportional to value GSL. According to Kurniati *et al.* (2017), the increase of GSI influence the increasing of GSL.

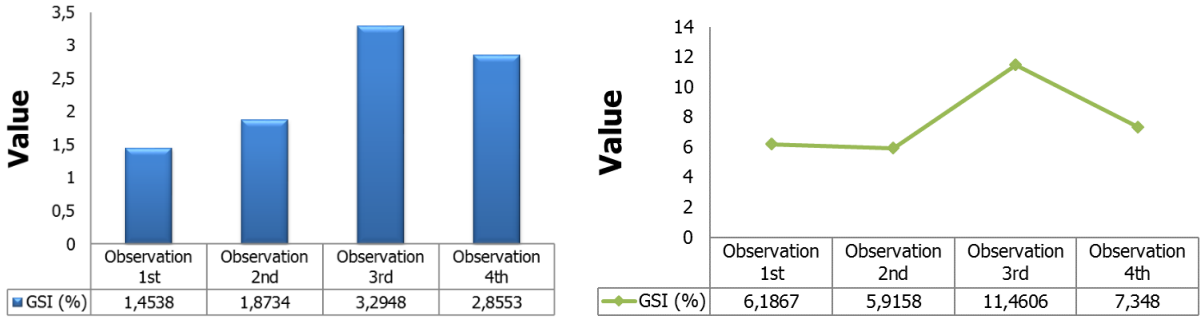

Figure 4. GSI of wader cakul male and female fish

3.4. Gonad Index (GI).

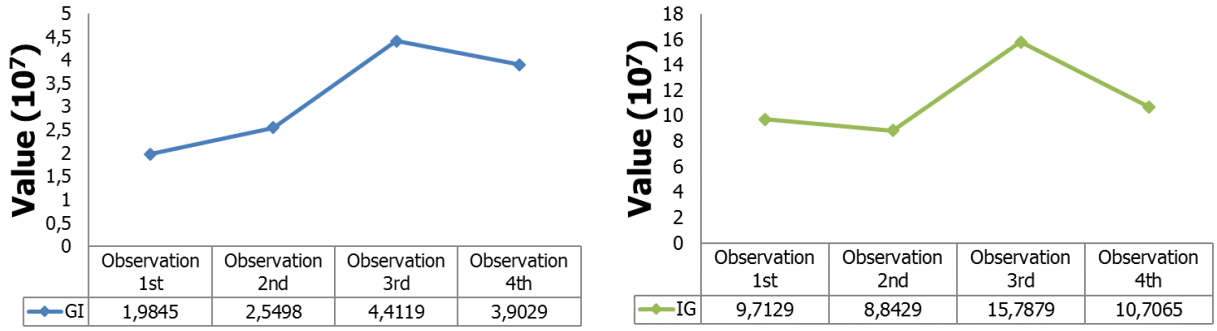

Figure 5. GI of wader cakul male and female fish

Figure 5 showed the highest GI alue of male dan female fish found at third observation by 4,47 x 10^7 and 15,68 x 10^7 where the value was in line with the increasing value of GSL and GSI. According to Effendie (2002), the range of GI between 1-10 could be determined the level of maturity the gonads on maturing process and the range of 10-20 have mature.

3.5. Fish Gonad Histology.

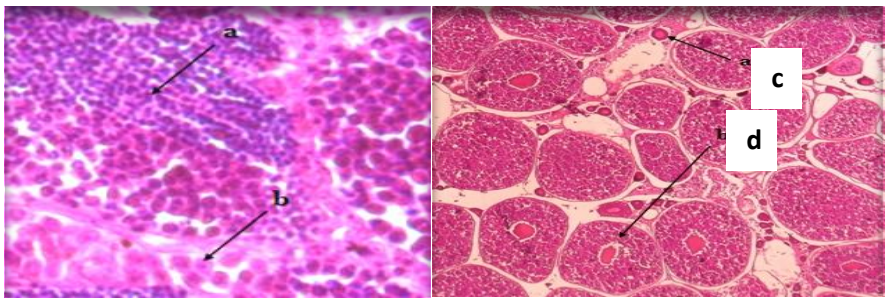

Figure 6. Male fish gonad histology with 1000X magnification. (a) primary spermatocyte, (b) secondary spermatocyte, Female fish gonad histology with 1000X magnification. (c) primary oocyte, (d) secondary oocyte

The core of both of them could see spermatocyte and oocyte. The difference of the primary oocyte / spermatocyte and secondary oocyte / spermatocyte are their size (Figure 6). The oocyte primary / spermatocyte primary were characterized by size smaller than the secondary oocyte / spermatocyte. The size of the oocyte was larger compared by a spermatocyte so that easier to calculate the number of the oocyte primary and secondary in a cross section of the gonads histology.

3.6. The number of primary oocyte and secondary oocyte.

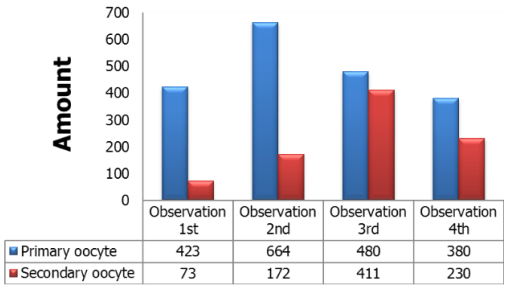

Figure 7. The number of primary oocyte and secondary oocyte

Figure 7 showed that the number of secondary oocyte on the third observation increased while in the number of oocyte primary were getting down. This showed that the increase oocyte become a determination of the spawning gonads.

3.7. Reproduction cyrcle of wader cakul fish.

Fish reproductive cycle is a process changes that happened to the reproductive system of fish (the gonads) to achieve new produced organisms as his offspring periodically. Reproduction or breeding in fish could be mean the new species born. According to Viette *et al.* (1997), reproductive cycle can be seen from third observation, including macroscopic aspects, GSI and histology. The macroscopic of gonads has three phase of reproduction cycle such as pre-reproduction, reproduction and post- reproduction. Based on the gonad conditions observed for about two months in the middle of the rainy season (in early december 2013 until the end of 2014), it was found the average of male wader GSL reached GSL II-IV with 1,4-3,3 % of GSI. While the average level of maturity the gonads level of wader female reached GSL III-IV with 5,9-11,5% of GSI. Our result showed that in the middle of the rainy season was the best of the development of wader cakul fish gonads. According to Faizah (2010), the amount of the GSI can be used as indicators the time of fish spawning.

Observations on shows the importance of reproductive know cycle fish wader cakul is as an effort to conservation knowing the spawning seen from ripeness gonadnya this knowledge of reproductive cycle fish can applied to the cultivation of intensive system.

3.8. Water quality.

The measurement result the quality of water for research could be seen in Table 1.

Table 1. Water quality during research

Parameter	Value	References	
		Nugroho (2013)	Sutisna & Sutarmanto (1995)
pH	6.8 - 7	6 – 8	6.7 – 8.6
Temperature (˚C)	28 – 30.2	24 – 34	25 – 30
DO (ppm)	3 – 3.4	2.1 – 8.3	5 – 6

The measurement result of the water quality is quite similar if compared with Nugroho (2013) study. The result of pH measurement result in the northern Probolinggo was obtained 6-8 in range. The measurement of DO was obtained the range of 2.1-8.3 ppm where the range still could be

accepted by a species of the cultivation of fresh water in nature (such as *Channa gachua*) and with temperature between 24 – 34 °C. According to Bakhris *et al.* (2017), the temperature with 29-30 °C temperature range is optimal for the biota waters in the tropics.

4. Conclusion.

Reproduction cycle of wader cakul in male and female increased during the middle of the rainy season. The highest peak happened on third observation. There were relation GSL, GSI and GI on the determination of reproductive cycle of wader cakul. Increased GSL affected by the increase in the GSI value. Same case with GI that was directly proportional with increasing of GSI and GSL.

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