Full Paper

REPRODUCTIVE BIOLOGY OF THE YELLOW RASBORA (*Rasbora lateristriata*) INHABITAT OF THE NGRANCAH RIVER, KULON PROGO REGENCY

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Abstract

The purpose of this research was to identify the reproductive biology in the yellow rasbora (*Rasbora lateristriata*) in habitat of the Ngrancah River. To identify spawning events and embryo development, direct observation was employed during the spawning season in their natural environment, and then fertilized egg was incubated insitu. Spawning seasons underwent correspond to the end of rainy season and early dry season which characterized by clean freshly water and low temperature. The result showed that during spawning season, the broodfish migrated from Sermo Reservoir upward to the main river to find out the spawning site. In the early morning between 03.00 and 05.00 AM, the broodstock moved to the spawning site, then making aggregation and both female and male released their gametes in the shallower place. The aggregations were consisting around of 1 female and 3 males. The fertilized eggs would cleavage, and then embryo developed and yolk sac larval hatched within 23 hours at 27^{o}C. The knowledge about spawning events of yellow rasbora may be used for improved management tools in the future.

Key words: Spawning behavior, yellow rasbora, Ngrancah River

Introduction

Information on the timing and location of spawning in commercially harvested fish species is frequently needed to resolve biological questions associated with the management of the fishery, to explain observed changes in the juvenile and adult populations and to plan research programs. Identifying and describing reproductive biology has been challenging to fishery scientists because of the fairly inaccessible environment of fishes. This leaves a gap in knowledge for proper management of many fish species.

The fish family cyprinidae occur principally in Tropical South East Asian Countries (Nelson 2006) and the genus rasbora is widely distributed in Sumatra, Borneo, Java, Bali and Lombok (Kottelat *et al*. 1993), and of great economic importance in Central Java (Djumanto 2000). In Yogyakarta special region, investigations on the biology of rasbora of this genus was scarce (Triyatmo, *et al*. 2007). In addition, despite of the limited data information on *Rasbora lateristriata*, there are very few biological studies on *R. lateristriata* (Setyobudi 2000). This species has recently become important to the riparian communities of the Sermo Reservoir area of Kulon Progo regency principally because of its affordability, tasteful flesh and relative abundance in comparison with other genus in the river.

Based on the information of local fishermen suggested the spawning period for yellow rasbora was from early May to late July on spawning site concentrated along the riversides at depth less than 30 cm. Besides that, it was believed in the spawning also

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occurs in conjunction with presence of air cool during weather changing from wet to dry monsoon. The spawning period for female yellow rasbora was characterized by a gradual shedding of the ripe ova and females spawn over an extended period as only a portion of all the eggs were ripe at any one time.

Identifying reproductive biology in yellow rasbora is particularly challenging because it occupies a rather, inhospitable, remote environment, and short period spawning season. Because of the limited data regarding yellow rasbora spawning, considerable debate and speculation exists concerning their reproductive biology. So far, information on reproductive biology of *Rasbora lateristriata* in Ngrancah River, the upper part of Sermo Reservoir was sparse. Observation reproductive in yellow rasbora could contribute to improve knowledge for proper management. This paper, therefore, make some reproductive biology observations on the species in the Ngrancah River.

The aim of the study described here was to provide information on the reproductive biology of this species, in particular to describe the reproductive development, to confirm the timing and duration of annual spawning, sex ratio, and fecundity during spawning migrations and the embryo development.

Materials and methods

Description of study site

The Ngrancah River (7º48'34"S and 110º06'38"E; Fig 1) is the main river that water run through Sermo Reservoir. It runs through hilly of Hargo wilis village goes into Sermo Reservoir, and then flows out from the dam to the Ngrancah River bellow the dam.

The study site was characterized by clean freshwater with fast flowing and gravel type riverbed. It located between reservoir and sediment check dam erosion controlled approximately 500 m above the reservoir. Water elevated around 2.0 m during the rainy season and 0.5m in the dry season. Two climatic seasons, namely wet and dry season, was prevail in this area. The wet season runs from October to April while the dry season runs from May to September, however there is variation in the seasons.

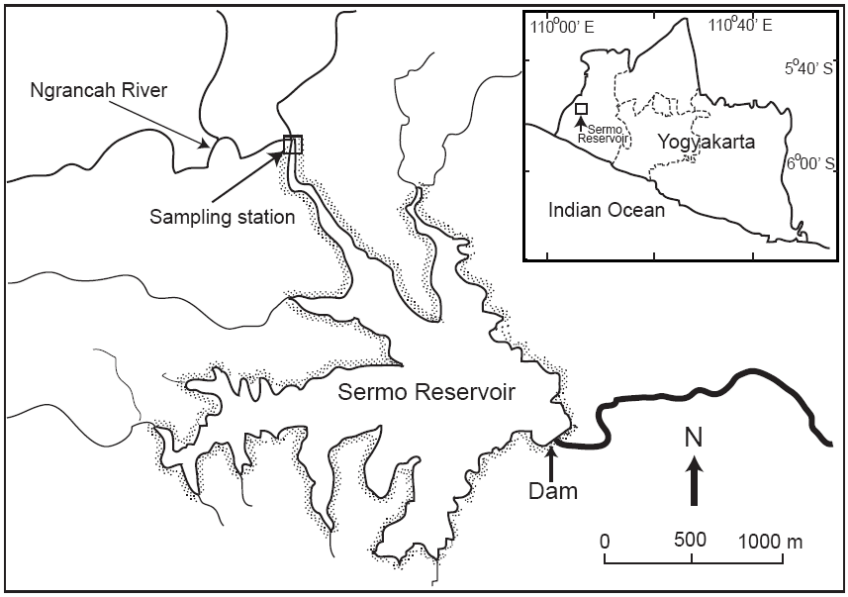

Fig. 1. A map showing sampling station located in Ngrancah River.

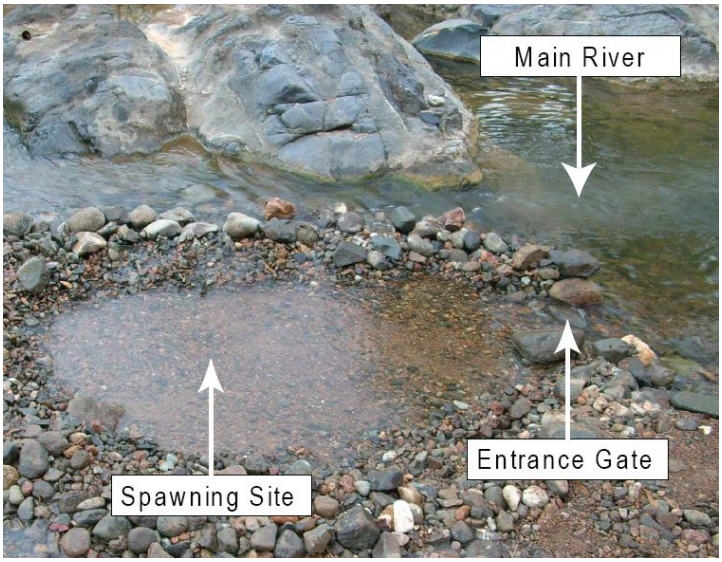

Fig. 2. Spawning site for yellow rasbora with an entrance gate facing to the main river constructed in the riverside.

Spawning sit
To promote the brood fish to spawn, the spawning site was provided and constructed in the riversides around 500 m above the reservoir. The place for spawning site was selected that have clean water flows smoothly, fairly shallow, available enough of sand and pebble, and no garbage around them. A day before observation of spawning, spawning site was constructed from the sand, pebble and small stone which it was arranged in oval shape around 2x3 m in size located at the depthless than 30 cm. The spawning site has one entrance gate (Fig. 2).

Fish sample
Fish were sampled from spawning site after mating were completely finish. We caught female fish a total of 109, and male fish a total of 230 from spawning site during the period from July to August 2007. Information recorded for each specimen included measurements for total length (TL) to the nearest 0.1 mm, body weight and gonad weight (W) to the nearest 0.01 g. The eggs numbers contain in each ovary were later recorded. The sex of each specimen was recorded and ova microscopically staged. Gonadosomatic indices (GSI) were calculated as the percentage of gonad weight to total body weight. Only ovaries contain with oocyte were used in the calculation of average fecundity for spent female, in order to minimize the influence of spawning on fecundity.

Spawning time
To determine the spawning time of both males and females, a series of sampling surveys were conducted two hourly from 18:00 to 06:00 h for periods of 3 consecutive days during the peak reproductive period in 2007. Surveys were conducted from 4–30th July 2007. During each survey, the observers looked at with naked eye the spawning site and noted the spawning activities.

Fecundity and clutch size
We estimated clutch size (the number of eggs deposited in one spawning) and fecundity from fish collected in the spawning site only. Fecundity is defined as a total number eggs in the ovary prior

to spawn, while spawning fecundity is defined as the number of eggs deposited in one continual spawning period in spawning site. Hence, the fecundity was calculated as number of eggs expelled during spawning and plus the remaining eggs in the ovaries of individual female fish.

To estimate sex ratios and the eggs numbers expelled during spawning, we caught all brood fish and collected all fertilized eggs in the spawning site after spawning was taken place. Brood fish in the spawning site were catch by fish trap made of bamboo in the early morning at 05.00 AM. Fish trap was installed on the entrance gate, and then the brood fish were suddenly shocked with flashlight hence they enter to the fish trap. All fish samples were collected and determined their sex by squashed gently in their belly part, and then preserved in 70% ethanol.

The remaining eggs in the ovaries were counted. The ovaries were removed from body cavity and stored in 70% ethanol. Ovary mass was recorded and all the eggs from both ovaries emptied into a plastic bottle of water and shaken gently to ensure uniform mixing. Then the eggs were transferred to a Petri dish where all ova were counted using a hand tally counter. Ova diameter measured along their median axis with a calibrated eyepiece micrometer fitted on a dissecting microscope at 50 magnifications. Data used to estimate the sex ratios were obtained from brood fish trapped within the spawning site.

Eggs Collection and incubation
The fertilized eggs were settling down at the bottom of spawning site and collected in the early morning around 05.00 AM. The eggs were scraped using scoop net from sandy bottom carefully and then put into pail to separate from sand and other debris. The eggs were distributed into incubation chamber which made of tea filter. They were a total of 24 incubation chambers and each incubation chamber was contained around 20 fertilized eggs. Incubation chamber were put and arranged in such a manner and tied with plastic rope in the basket. Small holes were made on around and at the bottom of the basket to allow water flow across. They were put at river part beside the spawning area which has flow and strong enough to move the eggs gently around the bottom of the incubation chamber.

To observe embryonic development, every hour starting from 06.00 AM onward, one incubation chamber containing egg was taken out and all developing eggs were collected and preserved in 70% ethanol. The eggs were observed under binocular microscope and the highest percentage of embryo development was as the stage of embryonic development. Under binocular microscope extended with camera lucida, the developmental stage of embryo was drawn on a millimeter paper block then transfer onto paper trace.

Results

Spawning time
Adult fish settled in reservoir waters for feeding and grazing of plankton. They scattered to entire of reservoir water. Their gonads grow up to ripe during settle in reservoir water.

Once the rainy season was ended, broodfish migrated from the reservoir area to the river up and, spread along the river and congregated at the bottom part of the study site. They swam around the pebble while fed on plankton throughout the daylight and interact to one another before moving to the spawning site. The fish were not spawned when the water was turbid and too many of disturbance.

On the spawning time, the brood fish were pooled at the area closed to the spawning site in the evening. The brood fish were not moved to the spawning site yet until 10.00 PM. At the midnight, a few fish came into the spawning site, however the mating were not occurred yet. At 02.00 AM onward, the number of brood fish in the spawning site were

gradually increased, and then mating have taken place between 03.00 and 05.00 AM at the shallowest water. During mating, some female and male fish were assembled making a heap to ensure the fertility success of egg. The fish were concentrated at the upperparts of the spawning site during mating where the freshwater supply was overflowed, and then they distributed to the center part of spawning site after mating. The spawning activities run about one to two hours and were ended before sun rise. The fertilized eggs then settled down at the bottom between sands and gravels. No parental care was given to the eggs. The events of brood fish enter to the spawning site run with average every three days (Table 1), and more frequent in the peak spawning season.

The number of females entering the spawning site varied from 2 to 39, and 3 to 49 for males. However, mating would take place if there were some females engage, and suddenly stopped if there was disturbance from predators or fishermen. The fish were observed to spawn only on 7th, 16th, 17th, 21th, and 26th July, respectively, while other dates didn't spawn. Therefore occurring mating was about 0.5 for every entering fish to the spawning site.

Size at sexual maturity

The minimum TL spawning fish was 3.8 cm for males and 5.5 cm for females, and males were mature earlier than females (Fig.3). The length dominant was 7-9 cm for male fish, while 8-10 cm for female fish. The percentage of mature male suddenly increased at 7.0 cm TL then sharply decreased, while female fish increased markedly between total lengths of 7.0 to 9.0 cm, and then gradually decreased afterward.

The sex ratio of the spawning fish was biased from 1:1 (male : female = 230 : 109, χ^2-test= 71.11, n=10, $p < 0.05$, significant). During spawning and gamete releasing, each female fish would accompany by 1 to 2 males fish to ensure fertility success of egg. Male and female fish matured at the different length, females fish matured at a larger length than males.

Table1. Numbers of broodfish entered to the spawning site in July 2007

July, Date	4	7	16	17	21	22	23	24	26	30	Total
Female	2	12	23	39	8	5	5	5	8	2	109
Male	14	49	33	32	24	16	12	10	37	3	230

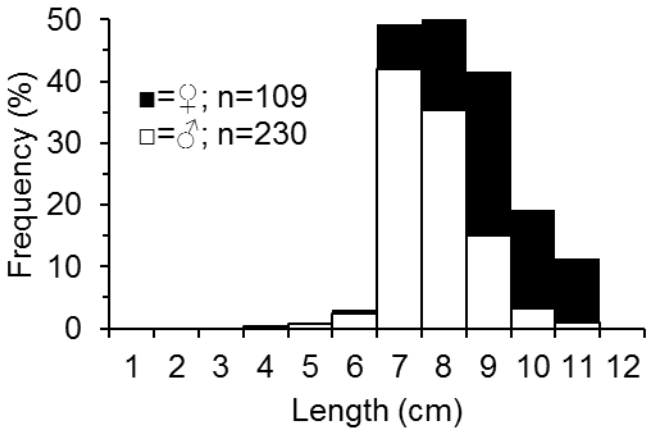

Fig. 3. Length frequency distribution of male and female *Rasbora lateristriata* trapped in spawning site. Closed and open bars indicate males and females, respectively. Data represent pooled samples from 10 collections at one spawning site. Abscissa values are 10 mm length class midpoints.

Fecundity

Ovaries from the 30 females before spawning contained of three size classes of oocytes, (1) *previtellogenic oocytes* of about 0.30–0.50 mm in diameter, (2) *maturing oocytes* of 0.50–0.75 mm and (3) *mature ova* between 0.75–0.90 mm. The broodstock were gathering in the main river before entering spawning site through entrance gate. The brood fish got into and out from the spawning site through entrance gate. There were found around 26% of ovaries didn't contain egg for population of female fish in the main river. Fecundity ranged from 1.750-5.182 with an average 3.466 eggs per fish. The relative fecundity before spawning was around 66 eggs per gram of body weight.

If brood fish trapped in the spawning site have already spawned, thus the fertilized eggs found in the spawning site owned to those female as spawning fecundity. On the first and second of eggs collecting in the spawning site, we found fertilized eggs at amount 528 from 4 females fish and 792 from 10 females fish, respectively. Therefore the spawning fecundity of individual female were 211 eggs.

After spawning, around 23.3% of ovaries were empty, while the remaining ovaries still contain eggs three size classes of oocytes (Table 2). The oocytes composed of small size 43.7%, medium size 22.8%, and large size 33.5%, respectively. The ovulated eggs were still found in ovaries of the spent fish. Those eggs expel easily by giving squeeze from stomach to genital pore and were greenish in color.

Gonadosomatic for female spent indices varied significantly across length and body weight for populations. Minimum observed GSI was 1.37%, while a maximum was 35.65%. There was a weak relationship between GSI and oocyte number in ovaries (*y=20.71 x – 178*.4; r^2=39.1).

At ovulation, the ovary contains post-ovulatory follicles, previtellogenic oocytes, and atretic follicles with dark pigmentation. Individual female fish deposited only part of an ovulated oocyte, while the rest oocyte will be expelled in the next spawning. Vitelogenic oocyte will undergo development to ovulatory oocyte, and with the previous ovulated oocyte, they will be deposited in each spawning chance until all oocytes were released from ovaries. If there was no spawning chance, then ovulated oocyte developed to become atretic follicles and will be absorbed again.

Females with postovulatory follicles and vitellogenic oocytes in the ovary (post spawning stage) occurred frequently during the spawning season. In these ovaries, vitellogenic oocytes develop normally, while the postovulatory follicles continue to disappear from the ovaries until the developing oocytes attain the migratory nucleus stage, when they form an advanced cohort. These findings indicate that *R. lateristriata* a multiple spawner. However, the spawning interval in this fish was remains unclear. During the spawning periods, spawning occurred between 3:00 and 5:00 h and the fertilization rate was>90%.

Length weight relationship

Scatter plots diagram (Fig. 4) indicate exponential relationship of length and weight for both male and female. A pooled relationship for each sex, computed from samples collected in spawning site, revealed the following length-weight relation group $W = 0.006L^{3.025}$ (r^2=0.942) for male, and $W = 0.004L^{3.131}$ (r^2=0.929) for female. The t-test indicate b=3 ($p<0.05$), both growth rate in length and weight was proportional. The length weight relationship of *R. lateristriata* was conform to the general iso-metric formula $W = aL^b$.

Table 2. Total length, body weight, gonad weight, gonad index and the remaining egg from 30 females of *Rasbora lateristriata* after spawning collected in July 2007 at the spawning site in Ngrancah River

Length (cm)	Weight (g)	Gonad weight (g)	GSI (%)	Oocyte			Total
				Small	Medium	Large	
5.43	0.86	0.26	30.23	0	0	182	182
6.44	1.79	0.32	17.87	0	0	0	0
6.58	1.88	0.51	27.13	0	0	11	11
6.60	3.59	1.28	35.65	233	189	458	880
7.00	3.30	0.44	13.33	15	4	5	24
7.34	3.37	0.30	8.90	0	0	0	0
7.36	3.29	1.01	30.70	104	83	224	411
7.47	3.03	0.55	18.15	0	0	0	0
7.54	2.65	0.26	9.81	0	0	0	0
7.57	3.07	0.63	20.52	0	0	0	0
7.57	3.07	0.63	20.52	49	38	29	116
7.64	2.19	0.30	13.70	0	0	0	0
8.22	4.84	1.31	27.07	204	78	118	400
8.32	4.71	1.53	32.48	102	170	415	687
8.44	6.01	1.05	17.47	165	44	34	243
8.54	4.19	0.93	22.19	74	18	3	95
8.89	5.25	1.20	22.86	29	48	56	133
9.29	7.61	1.95	25.62	82	57	43	182
9.50	8.40	1.64	19.52	208	54	58	320
9.66	6.99	1.32	18.88	75	34	39	148
9.83	5.94	2.04	34.34	145	211	333	689
9.85	5.57	1.02	18.31	0	24	18	42
10.12	8.33	1.81	21.73	140	88	134	362
10.23	7.29	1.40	19.20	400	91	23	514
10.50	7.31	0.10	1.37	0	0	0	0
10.71	6.08	0.50	8.22	0	0	243	243
10.74	10.85	2.43	22.40	229	269	353	851
11.39	9.96	2.56	25.70	146	108	86	340
11.53	11.59	2.61	22.52	101	55	201	357
11.54	12.62	2.61	20.68	118	87	200	405
Average		1.51	23.84	142	76	114	332
Standard Dev.		2.21	16.40	140	72	98	252

Remarks: Oocyte size indicate small (<0.49 mm), medium (0.50-0.75 mm), large (>0.76 mm), respectively

Fertilization

Ovulated eggs were 0.72 mm in diameter, dark-yellowish, spherical and contained no oil droplet. The average number of eggs per female spent was 221. Unfertilized eggs were dark-white without embryo, whereas newly fertilized egg was colorless, spherical 1.07 mm with embryo 0.87 mm of diameter start to develop. *R. lateristriata* has no adhesive material and scatter to demersal between sands and gravels.

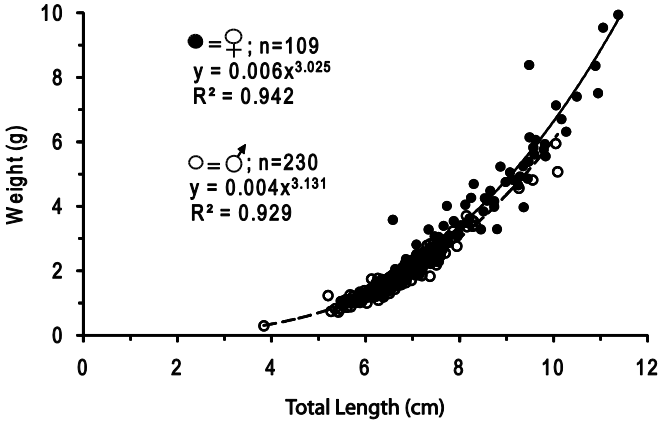

Fig. 4. Length weight relationship of spawning fish collected in spawning site. Closed and open circles indicate males and females, respectively.

Fertilization rate, estimated by the number of unfertilized eggs to the total incubated egg, was about 92%.

Description of early ontogeny

These descriptions based on the samples preserved in 70% ethanol, observation was done under compound microscope, and traced back using camera lucida.

Egg and embryo development

Following the modification of life history model of Balon (1975, 1990), early ontogeny was divided into two periods, i.e. *embryo*, which includes a series of three developmental phases: cleavage-egg, embryo and internal organ development, and *yolk-sac larva.* The *cleavage-egg* phase began with activating of egg and ended with appearing of embryonic shield. Description of *embryo* phase was based on appearing more prominent morphological. The yolk sac larvae phase includes the time from hatching to yolk egg absorbed.

A series of chronological states was defined as a number of hours (h) after activation.

Phase: fertilization and activation.

We estimated the fertilization and activation occurred at around 03.00 h. The eggshell diameter was about 0.77 mm. Temperature ranged from 25^{o}C to 26^{o}C during egg cleavage and embryo development, and then increase between 27^{o}C to 28^{o}C until hatching.

Phase: cleavage egg.

Cleavage was started less than 1 h after activation and formation of the perivitelline space. About 2 h after activation, the blastodisc consisted of 32 cells, and then 64 cells after 3 h. The eggshell diameter was about 1.09 mm and blastodisc at this stage was about 0.85 mm, filling the central portion of the perivitelline space. At 4 h, the blastodisc consisted of 128 cells. At 5 h, the germ ring was visible, although at the animal pole the blastodisc remained high and was very convex. The germ ring covered up to 15% of the yolk.

Phase: embryo.

6h: The germ ring covered up to 50% of the yolk. The embryo spanned the entire perivitelline space. The eggshell diameter was about 1.11 mm and blastodisc at these stages was about 0.77 mm.

7h: The germ ring covered up to 75% of the yolk. The eggshell diameter was about 1.15 mm and blastodisc at these stages was about 0.77 mm.

8h: The end of blastula stage and the early stage of gastrula, and the thickness blastodisc increased. The eggshell was about 1.14 mm and blastodisc at this stage was 0.75 mm.

Phase: organ development

9h: Embryonic shield was visible, and possible to distinguish the beginning of lateral outgrowths on the head that represent the future optic vesicles. It covered to yolk egg, and enlargement of anterior part indicated organ development begun. Germ ring was disappeared. Embryonic shield as narrow space and transparent was located along surface dorsal area of yolk sac. The diameter of egg shell was 1.05 mm and the yolk was 0.72 mm.

10h: Embryonic shield further developed, and covered up to 40% of yolk. The eggshell diameter was 1.16 mm and yolk was 0.7 mm. On the anterior side appeared candidate of the head along 0.25 mm, whereas the posterior side was the body become visible along 1.47 mm.

11h: Embryonic shield developed continually, and covered up to 70% of yolk. The eggshell diameter was 1.07 mm and yolk was 0.67 mm. Optic vesicle was appeared clearer at length 0.2 mm.

13h: Four somites were visible and myomeres started to build up near to the tail. Caudal were attached to the yolk on the ventral side.

15h: Twenty two somites were visible and yolk was decrease to smaller size. Somites developed from posterior to anterior. Optic vesicle was visible, and optic lens was fully developed. The eggshell diameter was 1.17 mm and yolk was 0.67 mm. Notochord was started to develop from posterior part.

17h: Fourteen myomeres were visible. Tail-bud were detached from the yolk forming a free caudal. The length of the embryo was twice over of the yolk diameter (0.60 mm).

19h: Twenty myomeres were present, and stick on the axial skeleton. The length of the embryo was 2.1mm. The yolk diameter was 0.52 mm.

21h: Twenty four myomeres were present. The length of optics lens was 0.17 mm. The length of the embryo was 1.95 mm, and yolk diameter was 0.50 mm.

Phase: yolk sac larva

23h: *R. lateristriata hatched* after 23 hours of incubation at a preflexion state, and the length of notochord was 2.40 to 2.65 mm. Newly hatched embryos had a small yolk as endogenous nourishment of larva.

25h: The length of larva was 2.82 mm, the yolk diameter was 0.45 mm, and eye was 0.12 mm. There was pigmentation of the eyes. Many capillaries appeared on the ventral portion of the caudal. No fin rays were developed yet. A common finfold was visible extending without indentations from the back of the head to the tail.

Discussion

Spawning behavior

In this study, *R. lateristriata* mainly spawned during May to July coincided with the end of rainy season, thus clean and freshly of water was available and with periods of low water temperature. It would seem likely, therefore, that low temperature and clear water were the primary causes for this fish to spawn. By ending of rainy season, turbidity and water volume of Sermo Reservoir gradually decreased. These results would stimulate the adult fish, and as part of their spawning behavior, migrate to the mainstream locate above the reservoir. On the other hand, the fishermen could catch the *R. lateristriata* easily in the main river than in the Sermo Reservoir. As the result, it would lead to increase in catchability of *R. lateristriata* due to fish more condensed in small area during in the mainstream and trapped easily in the spawning site.

Gravid female and adult male of *R. lateristriata* would enter to the spawning site in the early morning, then they both mate and release gamet in the shallowest place. Spawning site is usually the places that have running water, and gravel or sandy bottom to ensure oxygenated. By mating in the narrower place, it will increase the successful of egg fertilization and less of predator for their progeny.

R. lateristriata have a short life cycle in Ngrancah River, the stock consisting mainly of two to three age groups. Success of the stock depends mainly on the I-age group, the dominant mature group. However, should any severe mortality occur, prohibition of fishing for one season would ensure a good return from the spawning group. Thus the stock would recover rapidly since individuals of this stock have a fast growth rate and reach sexual maturity within one or two years.

Reproductive strategy

R. lateristriata was reproductively active for two or three months at the end of the rainy season while other species such as *Chana striata* and other local fish species had prolonged breeding periods coincident with high rainfall. The presence of plenty larva in the main river suggests that *R. lateristriata* may have bred at once of low rains falls. This result confirm that occurrence of larva could point out recent spawning or improved survivorship of juveniles.

Numbers of larva and juveniles correlated with adult numbers, the latter inversely varying with discharge rates of Sermo Reservoir, and suggests (1) the flood–drought cycle affected adult and juvenile densities similarly, or (2) fluctuating discharge rates generated population cycles which influenced reproductive activity via behavioral or other mechanisms. Gametogenesis in tropical fish may be influenced by photoperiod, temperature, water quality and nutrition (Lam 1983) and other reasons. Endogenous rhythms may be involved in sexual cycling in tropical fishes (Lam 1983) and take gonadal development to final maturity until spawning is triggered by sudden environmental fluctuations.

Reproductive activity of *R. lateristriata* coincided with the onset of the rains ended each year despite its interannual variability, and spawning must have been cued to the stimuli of low rainfall in the watershed. Proximal factors triggering spawning in tropical fishes may be associated with annual flooding (Welcomme 1979, Lam 1983) and may include changes in water chemistry, water temperature, flow rates, food supply and availability of spawning sites. In this study the most likely physicochemical stimuli for causing fish to spawn were the decreasing of flow rates, increased water quality changes associated with dilution and reduced temperatures of water and also siltation.

It is suggested that for multiple spawners such as *R. lateristriata* changes in environmental conditions at the end of the rainy season initiate spawning behavior and so long as conditions remain favorable and constant, repeated gonadal maturation and spawning could occur. Ultimate factors selecting for reproductive timing in the seasonally breeding species include selecting habitat in the end of rainy season lead to decrease predation pressures. Physiological mechanisms to respond to external cues are unlikely to change except under very strong and contrary selection pressures. This suggests that (1) the conditions of intermittency in this stream are insufficiently different from those of reservoir environments, (2) there has been insufficient time for the evolution of adaptations to intermittency, and perhaps more importantly, (3) the reproductive style of these species is opportunistic.

Fecundity and spawning patterns

Spawning site selection, mate choice and sperm limitation may influence

spawning success as well as disturbance via presenting of predator and fishermen (Nakatsuru & Kramer 1982). Ideally, numbers of eggs collected should be compared with eggs obtained by stripping or dissection (Burt *et al*. 1988). There are also drawbacks of using oocyte size distribution analysis.

In species with relatively short breeding seasons such as *R. lateristriata*, ovum maturation, as determined from oocyte size distribution analysis, was relatively synchronized. However, it could not be determined whether all the eggs were deposited at one time or in a number of sessions. Evidence from this study was described as spawning more than once per season since there were ovulated eggs remain in ovaries of spent female, and a lot of mature eggs which would develop soon to advanced stages. Ovaries of *R. lateristriata* showed a continuous distribution from reserved oocytes to mature ova and laboratory observations of ovaries confirmed small brood spawning. However, the total number of clutches deposited, total spawning period and total egg production was still unclear. For *Corynopoma riisei,* clutch size correlated with female size (Alkins, 2000).

Total egg production for large females exceeded that predicted from ovarian fecundity. It is tentatively concluded that small females may mature one ovarian complement of eggs and deposit this in several clutches over relatively short periods. However in larger females, it is tentatively suggested that repeated ovarian cycling or continuous maturation of eggs could result in many clutches over a prolonged period. Based on the ratio of ovarian fecundity (3466) to spawning fecundity (211) for *R. lateristrita* the equivalent of up to 16 ovarian cycles may occur, and spawning take place almost continuously over periods of three to five months depending on the size of the female and following environmental conditions. Considering the mean clutch size to ovarian fecundity ratio for *R. lateristrita* the eggs in a full ovary could be deposited in 6 to 16 clutches. Duration of spawning periods estimated with those deduced from GSI data, and continuous maturation of ova (Table 2), and spawning fecundity, it estimated more than one session of breeding per season.

Based on the spawning fecundity, the *R. lateristrita* experienced multiple spawning. However, the spawning interval was still unknown whether several days or weeks. In this study, the multiple spawning styles appear to be a direct consequence of fish size. Multiple spawning is crucial importance to small fish for a variety of reasons (Nikolsky 1963, Cambray & Bruton 1984, Burt *et al*. 1988). Notably it allows for considerable increases in fecundity not otherwise possible due to size and nutritional constraints (Wootton 1984).

Reduction in egg size could enhance fecundity but a lower limit is set by the increasing disadvantage suffered by smaller and less provisioned young. Repeat spawning has been shown to increase fecundity of many small fishes, for example gobies (Miller 1979), sticklebacks (Wootton 1984), darters (Gale & Deutsch 1985), tropical zebra fish (Hisaoka & Firlit 1962), and lemon tetra (Burt *et al*. 1988). The relatively large size of *Astyanax* could potentially allow for synchronous maturation and spawning of a large number of eggs in a season (Alkins 2000). That capacity may be reduced in *R. lateristrita* because of its smaller size and relatively large size of oocytes compare to body size. Small brood spawning could be directly related to small size acting as a constraint to increase ovarian fecundities.

Larval development

With respect to the 23 hours period of incubation, this refers to the number of hour-degree needed for fertilized eggs of *R. lateristriata* to hatch. Incubation period of *R. lateristriata* was longer

compare with other cyprinids such as *Puntius gonionotus* that hatch within 14 hours of incubation in the same water temperature (Djumanto 1993). This caused by size of the mature egg and yolk of *R. lateristriata* was rather bigger than silver barb. In the same temperature, the bigger size of fertilized egg will develop slowly and yolk absorption needs hours-degree longer, as the consequence will hatch latter. However, the bigger size of yolk sac larva hatching will increase to survivorship. In addition, the bigger larva will grow up faster and better escape from predator than the smaller ones; therefore a chance to survive is better. On the other hand, the fecundity was fewer compared to other fish with smaller size of oocytes.

One interesting feature of embryo incubation is short cleavage phase followed by embryo forming and internal organ development hence allows predicting of hatching time. Perhaps, it will be very helpful for fish farmer and researchers to breed the wild fish in captive breeding programs. Thus, the extinctions of vulnerable species could be anticipated, and increasing of population could be done easily.

Combining fewer eggs per clutch, however with a larger volume of yolk, relatively short embryonic development and the hidden of yolk sac larva in remote area, all predisposes for further parental protection. By eliminating the most vulnerable period and avoiding predation, this species have a survival advantage for their progeny. Fewer eggs per clutch are as practice for female to reduce mortality during early life history. By fewer numbers of larvae, accordingly the proportion of food for individual larva and a chance for getting food will increase.

Also, combination of low fecundity and complex reproductive behavior, with its restricted distribution, makes *R. lateristriata* potentially vulnerable to commercial collecting. It is possible to breed and raise this species in captivity, and efforts should be made to reduce the capture of wild specimens.

Relevancy of spawning mode to conservation efforts

Information on spawning behavior is useful for protecting these species (*R. lateristriata*), however it does little to predict imperilment, and since range limit is the overwhelming cause of endangerment. However, for some species, spawning mode, coupled with phylogenetic information, may be used to predict imperilment. Knowledge of spawning modes can also be used in recovery plans, particularly of species whose range size is declining. Physical habitat loss or degradation is almost certainly responsible for the extirpation of many fishes, and often the habitat may have become unsuitable because of the requirements of fish for spawning. For example, many species that broadcast their eggs aggregate in large numbers to spawn over suitable substrate (Ito & Yanagisawa 2003). Alteration of habitat may reduce the size of spawning areas or fragment populations so forming large spawning aggregations is difficult.

Dams are responsible for fragmenting populations of riverine fishes, and are a major cause of decline for some species (Moyle & Williams 1990). Dams also alter flow patterns in rivers, and may therefore be responsible for loss of breeding habitat for some fishes. Reproduction in silver barb (Djumanto 1993), which also broadcast eggs, is directly related to flow regimes in large rivers. Low flows affect spawning habitat availability and may allow silt to settle over eggs. Siltation may cause egg or brood mortality in several ways. Loss of range by species such as *R. lateristriata* that need clean gravel areas for spawning can be attributed to increase siltation. Species that need clean substrate for spawning, such as broadcasters, are positively affected by siltation in streams.

Information on spawning used by fishes with various spawning modes, such as the lithophilic spawners, is important for protection efforts. Lithophilic spawning fishes use habitat structure (sand and gravel) as spawning substrates. Although the sand and gravel used vary, obviously habitat structure is important to these species.

Any conservation effort aimed at habitat protection must ensure that both the spawning and typical habitat are included in the plans. The spawning substrate used and even spawning mode may be plastic for some species, but few through studies of spawning behavior in yellow rasbora exist. Our understanding of flexibility in spawning mode and brood survival under different conditions is therefore minimal. It's probably that some species have a greater degree of flexibility in spawning characteristics than others.

For some species, however, both spawning mode and substrate may be compulsory. Such complex ecological relationships should be taken into account when developing protection plans for different species. For nest associates, conservation plans must focus on the fish community as a whole, and not just the species of concern. The hosts of nest associates are considered keystone species in many stream communities (Vives 1993). Nest associates benefit from the parental care of hosts, which improves brood survivorship. It is not known if nest associates would spawn without hosts, and whether brood survivorship would be enough to keep populations under these circumstances. In some situations hosts benefit from nesting associations as well, this also highlights the importance of keeping community structure as conservation measure (Johnston 1994).

Introduced fishes may cause the decline of native species due to competition or predation. Introducing nonindigenous species may also affect the reproductive success of native fishes by hybridization. When species of nest associates are introduced into new drainages and contact native species, hybrids are also formed (Johnston *et al*. 1995). The frequency of formation of these hybrids may be greater when closely related species come into contact, and the effect on populates native species must be carefully monitored. In cases where populations of fishes are critically imperiled, captive breeding programs should be established so that field populations can be supplemented with laboratory-raised animals. Knowledge of the spawning modes of these species is necessary to the success of these programs.

An understanding of the spawning modes of species not only identifies habitat important to the spawning process but also highlights the importance of complex ecological relationships of fishes. This understanding helps in conservation efforts in the field, and is also critical to captive propagation, when necessary. Information on spawning behavior should be used in conjunction with information on ecology, habitat requirements and life history to predict and prevent the imperilment of more fishes. The lack of information for most species of imperiled should highlight the need for more research on the basic biology of the species if we wish to prevent their extinctions.

Conclusions and recommendations

This study represents an important first step for promoting spawning substrate of *R. lateristriata* to spawn in their habitat. Providing of spawning substrate followed by spawning success achieved in this study may represent a starting point for possible increasing population of this species in the wild environment. Spawning was fairly stimulated by temperature and water quality rather than other factors.

Spawning of *R. lateristriata* was taken place in the early morning at the shallower area of spawning site. The spawning fecundity was relatively low compare to other cyprinids. Fertilized egg would cleavage, and then embryo developed, and spawned within 23 hours incubation.

Life history or other ecological information essential in conserving rare species is often lacking or incomplete. This information is necessary to insure that habitat and community composition is appropriate at sites where reintroductions are planned. A proactive approach to protect or conserve potentially drastic extinction of fishes is required, because many fishes are currently on the brink of extinction. Species recovery programs are dependent on information integrated across many disciplines. New incentives need to be developed to attract professionals and students to more traditional research areas because systematic and field biology are so important in supporting to conserve rare fishes.

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References

Alkins, K.M. 2000. Reproductive timing of fishes in a tropical intermittent stream. Env. Biol. Fish. 57: 49–66.

Balon, E. K. 1975. Reproductive guilds of fishes: a proposal and definition. J. Fish. Res. Board Can. 32: 821–864.

Balon, E. K. 1990. Epigenesis of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes. Guelph Ichthyol. Rev. 1: 1–48.

Berkman, H. E. and C. F. Rabeni. 1987. Effect of siltation on stream fish communities. Env. Biol. Fish. 18: 285–294.

Burt, A., D.L. Kramer, K. Nakatsuru and C. Spry. 1988. The tempo of reproduction in *Hyphessobrycon pulchripinnis* (Characidae) with a discussion on the biology of 'multiple spawning' in fishes. Env. Biol. Fish. 22: 15–27.

Cambray, J.A. and M.N. Bruton. 1984. The reproductive strategy of a barb, *Barbus anoplus* (Pisces: Cyprinidae), colonizing a man-made lake in South Africa. J. Zool. Lond. 204: 143–168.

Djumanto, 2000. Growth parameters estimation of Nile Tilapia (*Oreochromis niloticus*) in Rawapening Lake Central Java. GMU Journal of Fisheries Science. II (2): 63-70.

Djumanto, 1993. An investigation into gonadal maturation and development of spawning method and breeding practices of the silver barb (*Puntius gonionotus*). Master thesis. AIT, Thailand.

Gale, W.F. and W.G. Deutsch. 1985. Fecundity and spawning frequency of captive tessellated darters fractional spawners. Trans. Amer. Fish. Soc. 114: 220–229.

Hisaoka, K.K. and C.F. Firlit. 1962. Ovarian cycle and egg production in the zebrafish, *Brachydanio rerio*. Copeia 1962: 788–792.

Ito, S. and Y. Yanagisawa. 2003. Mate choice and mating pattern in a stream goby of the genus *Rhinogobius. Env. Biol. Fish.* 66: 67–73.

Johnston, C. E. 1994. The benefit to some minnows of spawning in the nests of other species. Env. Biol. Fish. 40: 213–218.

Johnston, C. E., J. S. Ramsey, S. T. Sobaski & C. K. Swing. 1995. Introduced species of fishes in the southern Appalachians: consequences for conservation. J. Tenn. Acad. Sci. 70: 65–76.

Kottelat, M., A.J. Whitten, S.N. Kartikasari and S. Wirjoatmodjo. 1993. Freshwater Fishes of Western Indonesia and Sulawesi. Periplus-EMDI, Hongkong.

Lam, T.J. 1983. Environmental influences on gonadal activity in fish. pp. 65–116. *In*: W.S. Hoar, D.J. Randall & E.M. Donaldson (ed.) Fish Physiology, Volume 9(B), Academic Press, New York.

Miller, P.J. 1979. Adaptiveness and implications of small size in teleosts. Symp. Zool. Soc. Lond. 44: 263–306.

Moyle, P. B. & J. E. Williams. 1990. Biodiversity loss in the temperate zone: decline of the native fish fauna of California. Conservation Biology 4: 275–283.

Nakatsuru, K. and D.L. Kramer. 1982. Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). Science 216: 753–755.

Nelson, J. S. 2006. Fishes of the world, Fourth edition. John Wiley & Sons, Inc. 601 p.

Nikolsky, G.V. 1963. The ecology of fishes. Academic Press, London. 352 pp.

Setyobudi, E. 2000. Structure and potential reproduction of Nile Tilapia (*Oreochromis* sp.) population in Sermo Reservoir. Master thesis. GMU, Yogyakarta.

Triyatmo, B. Rustadi, Djumanto dan Susilo, B. P., 1997. Fisheries Study in Sermo Reservoir. Research report. 65 pp.

Vives, S. P. 1993. Choice of spawning substrate in red shiner with comments on crevice spawning in *Cyprinella*. Copeia 1993: 229–232.

Welcomme, R.L. 1979. Fisheries ecology of floodplain rivers. Longman, London. 317 pp.

Wootton, R.J. 1979. Energy costs of egg production and environmental determinants of fecundity in teleost fishes. Symp. Zool. Soc. Lond. 44: 133–159.