Advances in Biological Sciences Research, volume 22
7th International Conference on Biological Science (ICBS 2021)

Reproductive Aspect and Embryonic Development of Wader Fish (*Rasbora lateristriata* Bleeker, 1854) from Purworejo, Central Java

Inayah Rizkia Lailiati[1] Devi Annisa Suci[1] Amalia Audina Rosa[1]

Veronica Aurelia Fernanda[1] Bambang Retnoaji[1,*]

[1]*Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[*]*Correspondening author. Email: bambang.retnoaji@ugm.ac.id*

ABSTRACT
Indonesian native fish "Wader pari" (*Rasbora lateristriata)* from Purworejo is one of the fish has high economic value, which is related to the community enthusiasts and high requests for the fish stock. The demand for fish availability and supply is increasing drastically, which caused fish population to decline and leads to possible population depletion in wild. Therefore, it is necessary to carry out intensive cultivation efforts to be able to maintain the population in the wild while at the same time meeting the demand for consumption needs. It is necessary to implement an intensive cultivation program, which is targeted to become one solution for the existing problem of fish threat. The development of Wader Purworejo cultivation is a constraint by the lack of biological information related to aspects of reproduction and embryo development of the fish. This study aims to determine the quality, eggs hatching rate and embryonic development stages of *R. lateristriata* from Purworejo. Eggs were collected and grouped randomly into a petri with a total of 100 eggs/petri dish. Egg quality was observed using a microscope focusing on the developmental progression of eggs from two cells to dome stage with 3 repetitions. Egg quality was determined based on the percentage of normal eggs. The hatching rate was calculated by comparing the number of eggs hatched with the total number of eggs at 24-36 hpf. The results showed that the percentage of normal eggs was 95.7% and hatching rate was 81.3%. Embryonic development from one cell stage to larvae hatching were about 24 hpf, which contained of stage of zygote stage, cleavage, blastula, gastrula, segmentation, pharyngula and hatching, respectively.

Keywords: *Egg quality, Embryonic development, Hatching rate, Wader pari.*

1. INTRODUCTION

The native Indonesian fish Wader (*Rasbora lateristriata*) is one of the potential fish that has high economic value, which is related to community interest and high demand for fish stocks. Recently, the demand for the availability and supply of fish has increased drastically, causing rapid depletion of the fish population in the wild. Therefore, it is necessary for intensive cultivation efforts to maintain the population in nature, as well as meet the demand for consumption needs [1]. The fish cultivation program must be supported with the continuous availability of high-quality fish larvae [2]. Wader fish from Purworejo is very potential to be cultivated. However, there is a constrained by the lack of biological information related to aspects of reproduction and embryo development of the fish.

Assessment of reproductive aspects which include egg quality and egg hatching rate as well as embryo development is a fundamental step that needs to be conducted to provide an overview of fish life cycle and can be utilized for fishery resources management and utilization. The development of eggs must be ensured that they are healthy and do not experience abnormalities that can hinder the growth process. Abnormal fish will have difficulty in getting food because of the impairment of fish movements [3]. Abnormal embryo development during the early embryonic developmental stage can lead

to decreased egg hatchability and cause fish embryo high mortality. In addition, information on the development of fish embryos plays an important role in increasing larval growth and maximizing larval survival [4]. Embryonic development is something that must be considered, this is related to the quality and quantity of seeds to be produced [5].

Research on embryonic development has been carried out on other wader fish species (*R. argyrotaenia*) [6] and *R. daniconius* [7], but has never been carried out on *R. lateristriata* from Purworejo. This research aims to study the aspects of reproduction and embryonic development of *R. lateristriata* from Purworejo.

2. METHOD

2.1. Place and time

This research was conducted at the Laboratory of Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada. The study was conducted from June to September 2021

2.2. Test Organisms and Its Maintenance

Wild type wader pari fish (*R. lateristriata*) were collected from Purworejo and were reared under laboratory conditions. Fish were kept in a fiber pond with close filtered circulation system at a temperature 28-29 °C and a pH of 7.0-7.5. The fish were fed at ad-libitum basis with commercial fish feed for 3 times a day, at 07.00-07.30, 12.00-12.30, and at 17.00-17.30, respectively.

2.3. Broodstock Fish Selection

The broodstock selection was carried out by examination of the gonads' maturity to determine the fish readiness for matting. Mature gonads were determined by massaging the abdomen to secrete white fluid (sperm) in mature males and egg granules in mature females.

2.4. Fish Mating and Embryo Care

Embryos were obtained by natural spawning with a ratio of 2 males and 1 female in the mating chamber, which contained 12 males and 6 females, respectively. Fish spawning were set at 16.00-17.00 and was kept at 01.00-05.00. Embryos were taken and stored on egg water medium at 28.5 °C. Egg water medium was prepared with a total volume of 1000 mL of water, added 1.5 mL of salt stock and 2 drops of methylene blue.

2.5. Egg Development

A total of 100 eggs were randomly chosen and placed onto a petri dish containing egg water medium. Egg quality was observed based on the egg development phase from cleavage to dome stage with a microscope. The period of egg hatchability was observed with a microscope at 24-36 hours post-fertilization (hpf). Hatching rate was calculated base the number of eggs hatched out of total 100 eggs.

2.6. Time-Lapse Imaging

Time-lapse was carried out to obtain data in the early stages of fish embryo development. Series images of embryos were recorded on a light microscope (DM750, Leica Microsystems) equipped with camera (ICC50E, Leica Microsystems). The time-lapse imaging was processed using SkyStudioPro and positioned in the center of the glass-bottom dish. After the time-lapse is performed, the embryos are carefully removed and preserved for further examination or morphological determination

2.7. Data Analysis

Images are selected, added, and merged using the FluoView software (Olympus Life Science). The embryogenesis data obtained were analyzed descriptively and presented in the form of time series images.

3. RESULT AND DISCUSSION

3.1. Egg Quality

The fish egg quality was assessed through cell morphology at the division stage during embryogenesis [8]. Fertilized eggs that developed into normal embryos were defined as good quality eggs [9]. The results showed that most eggs were succesfully developed into normal embryos (Table 1)

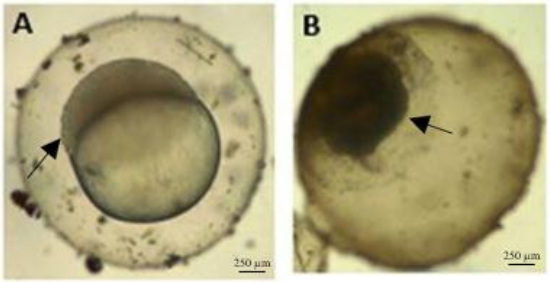

Figure 1. The phenotype of *R.lateristriata* embryo during an early stage of development, (arrow in A : Normal egg in dome phase, arrow in B : Abnormal egg show coagulation). Scale bar = 250 µm.

The phenotype of normal and abnormal eggs could be observed after two cell stage of embryonic development (Figure 1). Normal egg shows the cleavage of the blastodisk to form a dome stage. Abnormal egg cell shows the presence of coagulation and degradation of embryoblast and egg yolk. Embryonic abnormalities are

caused by several factors, including natural environmental pressures, anthropogenic pollution, breeding errors, genetics, parasites and handling [10-11].

3.2. Hatching Rate

The hatching rate is the ability of eggs to develop during the embryonic period until egg hatching [12]. The results showed that the hatcing rate of *R. lateristriata* is categorized as high (Table 1).

Table 1. Egg quality and hatching rate of *R.lateristriata*

Egg Percentage (%)		Hatching Rate (%)
Normal	Abnormal	
95.7 ± 1.53	4.3 ± 1.53	81.3 ± 4.73

The hatching rate is determined by sperm fertilization, unless there are other influences from environmental factors [13]. Fertilized eggs will develop into embryos and hatch into larvae, while unfertilized eggs will die and rot [14]. Dead eggs become white and dull, while healthy eggs are transparent or clear [15]. Environmental factors that affect egg hatchability are the temperature, dissolved oxygen and water quality [16].

3.3. Embryonic Development

The development of embryos into larvae (embryogenesis) starts from cleavage phase (cell division), morula, blastula, gastrula, organogenesis until hatching [17]. The process of embryogenesis in *R. lateristriata* is similar to those on *R. daniconius* [6]. However, the length of each stage until hatching time is different. *R. lateristriata* needs a shorter period to form 15 somites, 10 hours after fertilization, in contrast to *R. daniconius*, which needs approximately 12 hours to form 6 somites [18]. The developmental period is strongly influenced by the temperature of the water and use energy from yolk sac [19].

The gastrulation phase of *R. lateristriata* was ended at 4 hpf. The gastrulation phase is the initial phase of cell differentiation, that formed of three layers of ectoderm, mesoderm and endoderm. In the gastrulation phase, morphogenetic movements of involution, convergence and elongation of the epiblast, hypoblast and embryonic axis occur, and was end with epiboly [20]. In the early phase of gastrulation, the blastoderm begins to cover half of the yolk and continues to move towards the vegetal pole (50% epiboly) (Figure 2A) and thickening occurs on one side of the lateral equatorial plane of the yolk forming a ring-shaped circle called the germ ring (Figure 2B) [20]. This phase is continued with 75% epiboly (Figure 2C) and ends when the blastoderm appears to be pressing against the vegetal pole and then covers almost the entire yolk and germ ring thickened (90% epiboly) (Figure 2D) [19].

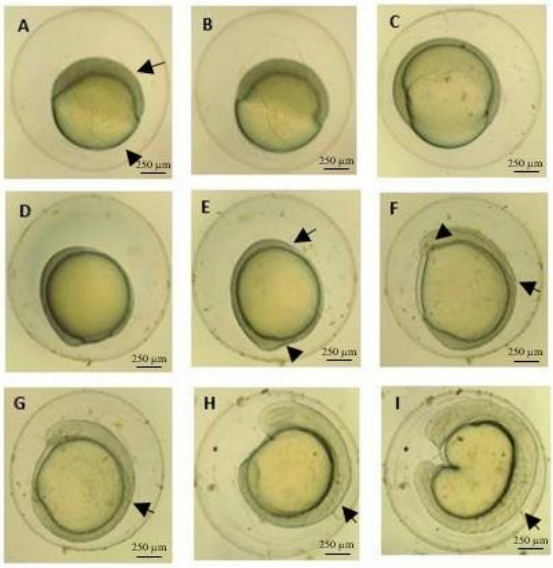

Figure 2. Embryo development of *R. lateristriata*. A: epiboly 50% (the arrow show blastoderm and the arrowhead show yolk), B: germ ring, C: epiboly 75%, D: epiboly 90%, E: bud (the arrow shows the head bud and the arrowhead shows the tail bud), F: 3-somit (the arrow shows 3-somit and the arrowhead show optic primordium), G: the arrow shows 6-somit, H: the arrow shows 10-somit, I: the arrow shows 15-somit. Scale bar = 250 µm.

The embryo's shape was started to be seen at 5 hours 38 minutes after fertilization. The embryo will start to stand out in the blastoderm, and it can be determined which is the top or the side marked by formation of a head bud at the animal pole and a tailbud at the vegetal pole and the epiboly had been closed by 100% (Figure 2E) [20]. The organogenesis phase begins when the head and tail can be distinguished, the eye sockets begin to form and followed by the formation of somites [19]. Somites are mesoderm blocks located on both sides of the neural tube in vertebrate embryonic development, which develop from anterior to posterior [22].

The formation of 3, 6, 10 and 15-somites in *R. lateristriata* occurred at 6, 7, 9 and 10 hpf, respectively (Figure 2F-I). The formation of the initial 6 somites appears to form more rapidly than later somites at a rate of about 3 somites per hour and 2 somites per hour thereafter at standard temperatures [22]. The formation of segmented somites from presomite (paraxial) mesoderm is influenced by the segmentation clock generated by cyclic genes. These cyclic genes consist of Notch and Wnts signals that are expressed in the presomitic mesoderm. The limit for expression of somite patterning genes was determined by retinoic acid (RA) and fibroblast growth factor 8 (FGF8). RA is expressed in a rostrocaudal gradient whereas FGF8 is expressed in a caudorostral gradient. RA increases somite patterning genes while FGF 8 suppresses RA activity and inhibits maturation of pre-somitic mesoderm into somites,

resulting in the formation of somites with the same size [23].

R.lateristriata from Purworejo has good egg quality at the percentage of normal eggs of 95.7% and also showed a high hatching rate of 81.3%. Embryo development until the formation of 15 somites were occured at 10 hpf.

AUTHOR CONTRIBUTIONS

All author contributed equally to this work

ACKNOWLEDGMENTS

We gratefully acknowledge to Universitas Gadjah Mada (UGM) that support this study by research grant program "Rekognisi Tugas Akhir (RTA)" 2021 (Grant number: 3143/UN1.P.III/DIT-LIT/PT/2021).

REFERENCES

[1] Zulfadhli, W. Nastiti, R. Bambang, Perkembangan Ovarium Ikan Wader Pari *Rasbora lateristriata* Blekeer, 1854: Pendekatan Histologi, Jurnal Perikanan Tropis, vol. 3(1), 2016, pp. 32-39. DOI: 10.35308/JPT.V3I1.34.

[2] E. Goyard, C. Goarant, D. Ansquer, P. Brun, S. de Decker, R. Dufour, C. Galinie, J.M. Peignon, D. Pham, E. Vourey, Y. Harache, J. Patrois, Cross breeding of different domesticated lines as a simple way for genetic improvement in small aquaculture industries: heterosis and inbreeding effects on growth and survival rates of the Pacific blue shrimp Penaeus (Litopenaeus) styrostris, Aquaculture, vol. 278, 2008, pp. 43–50. DOI: 10.1016/j.aquaculture.2008.03.018.

[3] M.G.H.K. Kordi, Budi Daya Perairan, Citra Aditya Bhakti, 2009.

[4] S. Puvaneswari, K. Marimuthu, R. Karuppasamy, M.A. Haniffa, Early embryonic and larval development of Indian Catfish, *Heteropneustes fossilis*, Eurasian Journal of Biosciences, vol. 3(3), 2009, pp. 84-96. DOI: 10.5053/ejobios.2009.3.0.12.

[5] S. Olivia, G.H. Huwoyon, V.A. Prakoso, Perkembangan embrio dan sintasan larva ikan Nilem (*Osteochilus hasselti*) pada berbagai suhu air, Bulletin Litbang Budidaya Air Tawar, vol. 1(2), 2012, pp. 135–144.

[6] M. Zamroni, Nurhidayat, C. Sawung, Pemijahan dan perkembangan awal larva ikan Rasbora (*Rasbora argyrotaenia*), Prosiding Konferensi Akuakultur Indonesia, 2011, pp.213-222.

[7] S. Paul, S.K. Sarmah, Study of embryonic and larval development of *Rasbora daniconius*, Biological Farm-An International Journal, vol. 11(2), 2019, pp. 72-76. DOI: 10.13140/RG.2.2.25431.91047.

[8] D.A. Pavlov, N. Emel'yanova, Morphological criteria of egg quality in marine fishes: activation and cleavage of eggs of *Zebrasoma scopas* (Acanthuridae), Journal of Ichthyology, vol. 48(7), 2008, pp. 533–548. DOI: 10.1134/S0032945208070072.

[9] B. Julien, Egg quality in fish: present and future challenges, Animal Frontiers, vol. 5(1), 2015, pp. 66–72. DOI: 10.2527/af.2015-0010.

[10] E. Prochazka, Incidence of malformations in fish embryos/larvae (review). Smart Water Research Facility, Griffith University, 2009.

[11] A.M. Silverstone, L. Hammell, Spinal deformities in farmed Atlantic Salmon, Canadian Veterinary Journal, vol. 43(10), 2002, pp. 782-784.

[12] S. Marsela, V.M. Ati, R.S. Mauboy, Hatching rate and abnormality of Sangkuriang Catfish larvae (*Clarias gariepinus*) which in the induction of heat shock temperature, Jurnal Biotropikal Sains, vol. 15(3), 2018, pp. 1-13.

[13] U. Hasan, Daya tetas telur dan sintasan larva dari hasil penambahan madu pada bahan pengencer sperma ikan Lele Sangkuriang (*Clarias* sp), Jurnal Warta, vol. 54, 2017. DOI: 10.46576/wdw.v0i54.285

[14] I. Effendi I, Pengantar Akuakultur, Penebar Swadaya, 2004.

[15] E.D. Masithah, P. Usanti, M.A. Alamsjah, Penggunaan Ovaprim dalam Pemijahan Buatan untuk Meningkatkan Ovulasi Ikan Mas Punten (*Cyprinus carpio* L.), Lembaga Penelitian Universitas Airlangga, Surabaya, 2004, pp. 87.

[16] Ghufron, I.R. Eka, P. Eko, Pengaruh kejutan suhu panas terhadap waktu penetasan, daya tetas telur, abnormalitas dan kelangsungan hidup larva ikan Tengadak (*Barbonymus schwanenfeldii*), Jurnal Ruaya, vol. 6(2), 2018, pp. 21-29. DOI: 10.29406/rya.v6i02.1021

[17] Ardhardiansyah, U. Subhan, A. Yustiati, Embriogenesis dan karakteristik larva persilangan ikan Patin Siam (*Pangasius hypophthalmus*) jantan dengan ikan Baung (*Hemibagrus nemurus*) betina, Jurnal Perikanan dan Kelautan, vol. 7(2), 2017, pp. 17- 27.

[18] L.A. Fuiman, Special consideration of fish eggs and larvae, In: L.A. Fuiman and R.G. Werner (eds.), Fishery Science: The Unique Contributions of Early

Life Stages, Blackwell Publishing Company. Oxford, 2002, pp.1-32.

[19] M. Herjayanto, C. Odang, T.S. Dinar, Embriogenesis, perkembangan larva dan viabilitas reproduksi ikan Pelangi *Iriatherina Werneri* Meinken, 1974 pada kondisi laboratorium, Jurnal Akuatika Indonesia, vol. 2(1), 2017, pp. 1-10. DOI: 10.24198/jaki.v2i1.23389

[20] C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullmann, T.F. Schilling, Stages of embryonic development of the zebrafish, Developmental Dynamics, vol. 203, 1995, pp. 253-310. DOI: 10.1002/aja.1002030302.

[22] E. Hanneman, M. Westerfield, Early expression of acetylcholinesterase activity in functionally distinct neurons of the zebrafish, Journal of Comparative Neurology, vol. 284(3), 1989, pp. 350-361. DOI: 10.002/cne.902840303.

[23] T.W. Saddler, Langman's Medical Embryology, 10th Edition, Lippincott Williams & Wilkins, 2006.