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Histopathological Analysis of Faunus Ater Ovotestis in Bale and Reuleueng Rivers, Aceh Besar Regency, Aceh Province Indonesia

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Abstract

BACKGROUND: Faunus ater is one of the macrozoobenthos that is often consumed by the community, especially in the Leupung and Lhoknga areas, Aceh Besar District. The presence of Pb and Zn is suspected to be able to damage the body cells of F. ater, especially the ovotestis organ. Ovotestis is an organ in mollusks in general that can produce egg cells and sperm cells simultaneously.

AIM: The purpose of this study was to analyze the level of damage to the Ovotestis of F. ater based on the state of the damaged Ovotestis cells.

METHODS: The method of this research method is F. ater that samples were taken from Bale and Reuleung River, each river is divided into three stations and at each station, three samples of F. ater are taken. Ovotestical histopathological analysis was carried out at the Histology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University. Preparation of ovotestis histology preparations using the paraffin method. Previously, F. ater was terminated and carcass surgery was performed. The level of damage to female gametes and male gametes was carried out descriptively by observing gonadal cells undergoing necrosis, hypertrophy, and lysis. Observation of the level of damage to the ovotestis tissue of F. ater was carried out using a cell damage scoring system, namely, the level of damage, the type of damage, and the scoring value.

RESULTS: The level of tissue damage to the ovotestis organ of F. ater was at level III with a score of 6. The highest percentage of damage occurred in Krueng Bale, namely, 19.027% for male gonads and 42.687% for female gonads. While the highest percentage of damage to ovotestis organ occurred in Krueng Reuleung 15,489% for male gonads and 40,695% for female gonads.

CONCLUSION: The result shows that there was damage to the gonads of F. ater in Krueng Bale and Krueng Reuleung based on the number of fully-formed oocytes/sperm, the number of incompletely formed oocytes/sperm, and the number of damaged oocytes/sperm.

Introduction

The use of the sea for human welfare is increasing in line with the increase in population. The increase in population and various activities in the coastal area causes this area to be often used as a final disposal site, leading to an increase in the amount of waste. These wastes, including those containing heavy metals in marine waters, can disrupt the biogeochemical balance, especially in the coastal zone [1].

Aceh Besar district, which is directly adjacent to the Malacca Strait in the north, is a coastal area so this area is very potential in the marine fisheries sector, especially in the Bale River area which is located in the Lhoknga sub-district and the Reuleueng river in Leupung sub-district. These two river areas are one of the water resources that have important benefits to human life.

The Reuleueng and Bale rivers are located close to residential areas. Increasing economic development activities, land-use change, and increasing population growth cause complex problems. The activities of residents can affect the quality of the river. In general, residents who live around rivers throw household waste into rivers, causing river water pollution, besides that garbage that settles in rivers, it will have an impact on river water silting [2].

Bale river is not only located close to the settlement but also passes through a cement factory. Disposal of factory waste into the river causes a decrease in river water quality and is polluted by heavy metals. The results of research by [3] showed the presence of high levels of Pb and Zn metals both in aquatic sediments and in the biota. Heavy metal contamination is a risk factor for toxicity in humans and animals [4], [5].

Heavy metal pollution, especially Pb and Zn, can hurt the aquatic environment, especially the

organisms that live in it and humans who consume polluted organisms [6]. Bioaccumulation of heavy metals in the body interferes with biochemical processes and inhibits the absorption of essential nutrients [7]. Heavy metals are not biodegradable, are easily absorbed by aquatic biota, and accumulate in tissues. This causes damage to the aquatic environment, is toxic to aquatic biota, and is bad for humans who depend on aquatic products as a source of food.

F. ater is one of the macrozoobenthos that is often consumed by the community, especially in the Leupung and Lhoknga areas, Aceh Besar District. The proximate analysis results showed that Faunns ater meat contained 76.92% water content, 4.88% water, 10.46% protein, and 0.82% fat [8].

F. ater belongs to the Pachychilidae family that lives in brackish waters [9]. The waters of the Balee and Reuleung rivers are known to be abundant. The results of [10] show that about 17% of the macrozoobenthos components in the Reuleung river are *F. ater* and have been tested to contain Pb and Zn in it [11]. The survival of these animals is highly dependent on the quality of the surrounding waters.

The presence of Pb and Zn is suspected to be able to damage the body cells of *F. ater*, especially the ovotestis organ. Ovotestis is an organ in mollusks in general that can produce egg cells and sperm cells simultaneously. However, groups of animals that have ovotestis are not able to auto fertilize [12]. Histopathological data of ovotestis *F. ater* in polluted rivers have not been found. Therefore, this research is very important to do considering for *F. ater* which is one of the aquatic commodities used by the surrounding community in Aceh Besar Regency for consumption. It is also feared that river water pollution will disrupt the balance of the river water ecosystem by decreasing the *F. ater* population in the future.

Material and Methods

F. ater samples were taken from the Balee River, Lhoknga District, and the Reuleung River, Leupung District, Aceh Besar District. Each river is divided into three stations and each station, three samples of *F. ater* were taken. Ovotestical histopathological analysis was carried out at the Histology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University. The preparation of histology ovotestis Faunus ater was carried out using the paraffin method. *F. ater* was terminated and carcass surgery was performed.

The shell of *F. ater* opened slowly so that only get meat from *F. ater*. The meat washed thoroughly with running water. The ovotestis organ was immediately put into Bouin's fixative solution, then dehydrated using 70% alcohol series to absolute alcohol, clearing in xylol, infiltration, and embedding in paraffin block 56–58°C. The embedding preparation was slashed to a thickness of 5 microns. Each sampling made four incisions with an interval of 10 and stained with the Hematoxylin Eosin staining method [13].Microscopic observation of ovotestis faunus ater at 10 x 40 magnification with three fields of view for each incision.

Observation of the level of damage to the ovotestis tissue of *F. ater* was carried out using a cell damage scoring system, namely, the level of damage, the type of damage, and the scoring value. The damage category refers to the modification of the cell damage method [14] (Table 1).

Table 1: Liver damage score

Damage rate	Damage type	Scoring score	Damage category
Level I	Hypertrophy	1	light
Level II	hypertrophy and Lysis	3	medium
Level III	Necrosis, hypertrophy, and Lysis	6	heavy
Level IV	Necrosis, hypertrophy, Lysis, and Cirrhosis	10	awfully

Result and Discussion

The results showed that there was a change in the ovotestis tissue of *F. ater* in the Balee River, Lhoknga District, and the Rheuleng River, Leupung District, Aceh Besar District (Figures 1 and 2). In Figure 1, it can be seen that the shape of the cells is irregular in the tissue and the presence of necrotic spermatocytes. Figure 2 also shows lobules with reduced oogonia, a collection

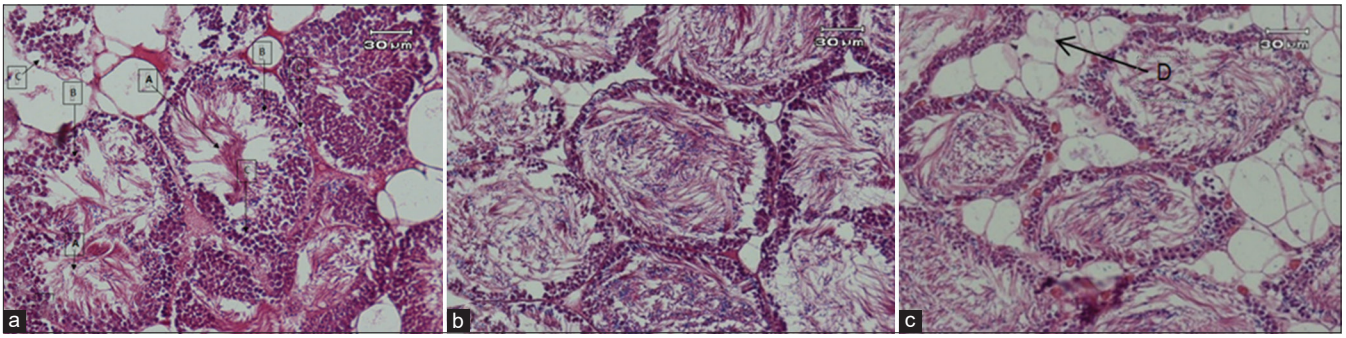

Figure 1: Ovotestis histology description in the form of male gonads F. ater (a): Spermatozoa in the lumen, (b): Spermatids, (c): Spermatogenic undergoes necrosis), and (d): Lipid droplets

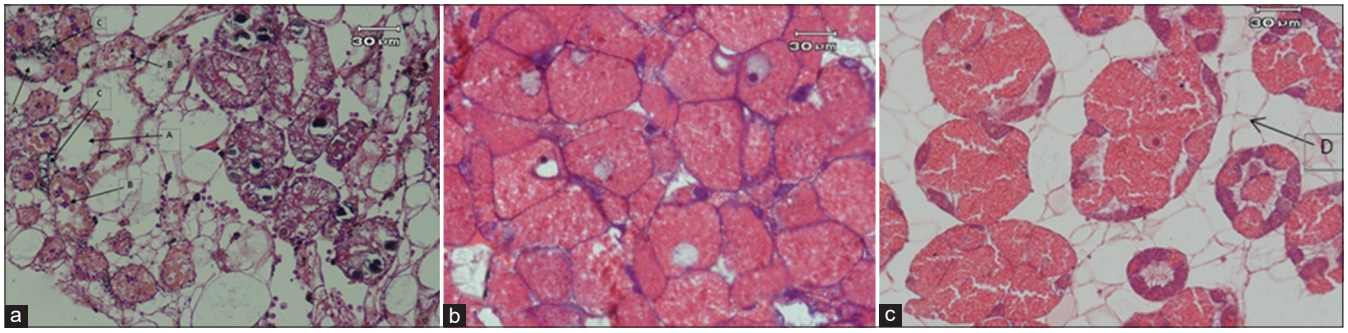

Figure 2: Ovotestis histology description in the form of female gonads F. ater (a): Lobules with reduced oogonia, almost empty lobules appear, (b): Oogonia cell count is rare, (c): Collections of inflammatory cells around the lobules, (d): Lipid droplets

of inflammatory cells in response to inflammation. Lipid droplets were also found around the lobules so that the lobules were seen infrequently.

The results of data analysis also showed that there had been broken to the gonadal cells (spermatocytes and oocytes) of the *F. ater* at that location. Table 2 shows that the percentage of damage to the gonads of *F. ater* from the Bale river was higher than that of the Reuleung river, namely, 19.027% for the male gonads and 42.687% for the female gonads in the Bale river.While the highest percentage of damage to ovotestis organ occurred in Krueng Reuleung 15,489% for male gonads and 40,695% for female gonads. This damage caused by the chemical content contained in it. It has been proven that there is a high content of Pb and Zn in river water, sediments, and the body of Faunus ater which has a habitat in it [11].

Bale river contains higher Pb and higher Zn in *F. ater* than Reuleung river, namely, 0–54,190 mg-Pb/kg and 0–84.53 mg-Zn/kg for Bale river [15] and 0–9.651 mg-Pb/kg and 16,428–147.90 mg-Zn/kg for the Reuleung river [3]. Data analysis of the level of damage to gonadal cells in ovotestis *F. ater* in the Bale river and Reuleung river was at Level III with a score of 6 (Table 3) in the severe category.

The high content of Pb and Zn in river water causes osmotic disturbances in cells that trigger various kinds of damage. Accumulation of heavy metals in organisms can occur through the food chain [16] thus disrupting the metabolic system. Heavy metals that enter the body will trigger the formation of reactive oxygen species ROS as a result of the deactivation of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase which function as antioxidants, thus easily damaging components, biomolecules that built up cells [17]. The damage caused can be in the form of cell death, mutagenesis, carcinogenic, and aging [18].

It is assumed that the Pb and Zn metals contained in river water are the cause of the formation of ROS in the body of *F. ater* in the Bale and Reuleung rivers which damage gonadal cells. Damage to cells in tissues occurs as a result of an imbalance between endogenous antioxidants in counteracting ROS [19].

Damage to gonadal cells in the ovotestis *F. ater* is a cell response to both physiological and pathological stimuli as a result of environmental changes. Damage was observed in the form of hypertrophy, lysis, and necrosis of gonadal cells. Hypertrophy is tissue damage characterized by an increase in organ size as a result of the increase in the size of the cell nucleus [14], [20].

Hypertrophy and lysis are early symptoms of necrosis. Necrosis is cell death characterized by pyknosis (nuclear shrinkage), karyohexis (nuclear destruction and chromatin fragments scattered in the cell), and karyolysis (loss of nucleus) [21]. The presence of necrosis will cause an inflammatory response in living tissue.

Table 2: Percentage of damage to *F. ater* gonad cells in Bale river and Reuleung river, Aceh Besar district, Aceh province, Indonesia

No	Location	Sample	Male gonads				Female gonads			
			Spermatocyte count		Damage%	Average % damage	Oocyte count		Damage%	Average % damage
			The damaged one	Total			The damaged one	Total		
1	Bale River	1	31	214	14.486	19.027	4	5	80.000	42.687
		2	32	199	16.080		1	3	33.333	
		3	29	219	13.242		4	6	67	
		4	29	209	13.876		3	4	75	
		5	23	165	13.939		7	13	53.846	
		6	45	280	16.071		0	0	1	
		7	48	156	30.769		0	0	1	
		8	46	196	23.469		4	10	40	
		9	34	116	29.310		5	15	33.333	
2	Reuleung River	1	48	180	26.667	15.489	1	6	16.667	40.695
		2	48	177	27.119		4	14	28.571	
		3	39	277	14.079		4	14	28.571	
		4	33	324	10.185		2	5	40	
		5	37	299	12.375		4	8	50.000	
		6	42	256	16.406		3	13	23.077	
		7	24	253	9.486		8	9	89	
		8	37	287	12.892		4	12	33.333	
		9	16	157	10.191		4	7	57.143	

Table 3: Histopathological damage level of female gonads in Bale river and Reuleung river, Aceh Besar district, Aceh province, Indonesia

No	Location	Sample	Histopathological feature	Female gonads		Male gonads	
				Scoring score	Damage rate	Scoring score	Damage Rate
1	Bale rivers	1	Necrosis, Hypertrophy, and Lysis	5,5	III	6	III
		2	Necrosis, Hypertrophy, and Lysis	6	III	5,75	III
		3	Necrosis, Hypertrophy, and Lysis	4,3	III	5,5	III
		4	Necrosis, Hypertrophy, and Lysis	6	III	6	III
		5	Necrosis, Hypertrophy, and Lysis	5,75	III	5,5	III
		6	Hypertrophy and Lysis	3,83	II	6	III
		7	Necrosis, Hypertrophy, and Lysis	6	III	6	III
		8	Necrosis, Hypertrophy, and Lysis	5	III	6	III
		9	Necrosis, Hypertrophy, and Lysis	6	III	6	III
2	Reuleung River	1	Necrosis, Hypertrophy, and Lysis	6	III	6	III
		2	Necrosis, Hypertrophy, and Lysis	4,75	III	4,75	III
		3	Necrosis, Hypertrophy, and Lysis	4,3	III	6	III
		4	Necrosis, Hypertrophy, and Lysis	6	III	6	III
		5	Necrosis, Hypertrophy, and Lysis	5,75	III	5,75	III
		6	Hypertrophy and Lysis	3,03	II	6	III
		7	Necrosis, Hypertrophy, and Lysis	6	III	6	III
		8	Necrosis, Hypertrophy, and Lysis	5	III	5,25	III
		9	Necrosis, Hypertrophy, and Lysis	5,5	III	5	III

Heavy metals can damage follicular cells so that their number continues to decrease [22] (Figure 2). Inhibition of ovum development is a result of cell damage. Cell damage in the ovotestis of F. ater in the Balee and Rheuleung rivers occurs through a complex system. The content of heavy metals in river water can affect the hormonal system in *F. ater* thereby reducing the number of gamete cell production, especially ovum cells, and decreasing the hatchability of eggs [23]. The mechanism of hormonal disturbance in Faunus ater begins with the inhibition of the release of gonadotropin-releasing hormone so that the secretion of other gonadal hormones such as progesterone, FSH, estrogen, and testosterone decreases [24].

Another source explained that heavy metals that enter the body, after turning into free radicals can directly damage follicle cells in the ovaries so that their numbers continue to decrease [22]. It is also seen that the size of the lobules becomes smaller, thereby reducing the production of gonadal cells so that their weight is low [25].

Actually, heavy metals enter the waters through the stages of crystallization in the air with the help of rainwater. Heavy metals enter into *F. ater* metals form complex compounds that cannot be excreted. The presence of heavy metals above the threshold has a negative impact on aquatic ecosystems, including *F. ater* because it causes a decrease in biodiversity until extinction occurs.

Conclusion

There was damage to the gonads of *F. ater* in Krueng Bale and Krueng Reuleung based on the number of fully-formed oocytes/sperm, the number of incompletely formed oocytes/sperm, and the number of damaged oocytes/sperm. The level of tissue damage to the ovotestis organ of *F. ater* was at Level III with a score of 6. The highest percentage of damage occurred in Krueng Bale, namely, 19.027% for male gonads and 42.687% for female gonads. While the highest percentage of damage to ovotestis organ occurred in Krueng Reuleung 15,489% for male gonads and 40,695% for female gonads.

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