ISBN 978-979-792-665-6
International Proceeding

Histopathological Changes in Gonads of Blue Spotted Ray (*Dasyatis kuhlii*) Due to Heavy Metal Mercury (Hg)

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ABSTRACT

The aim of study was to analyze the effect of mercury on gonads of blue spotted ray *Dasyatis kuhlii* using a histological observation. The rays were placed in five tanks filled by seawater with three fishes for each tank. Blue spotted rays, *Dasyatis kuhlii* were treated for 12 days by using different concentrations of mercury (Hg), those are, 0 ppm (treatment A), 0.0025 (treatment B), 0.0050 ppm (treatment C), 0.010 ppm (treatment D) and 0,020 ppm (treatment E). Results of the study show that Hg exposure altered the gonads microscopic structure. Histopathological change in ovary is detachment of vitellin layer. Histopathological changes in testicular are hyperplasia of germinal epithelium, cloudy swelling, blood clots and distorted of lobules.

Keywords: *Histopathological changes, heavy metal mercury (Hg), fish gonads, ovary, testicular, Blue Spotted Ray Dasyatis kuhlii.*

INTRODUCTION

Faster population growth, on the one hand has resulted in the residential and industrial sectors development. This has put heavy pressure on natural resources and the environment and on the other hand lead to increased food needs. Fishery resources is one resource that gets heavy pressure. In addition to land due to the narrower, also due to the tendency water pollution continues to increase from year to year. Blue spotted ray (*Dasyatis kuhlii*) is the one commodity that is economical, and is one of the foods that need to be maintained, preserved and developed to support the growing community needs. This type of fish live in shallow coastal areas near the tropical region, which includes demersal fish. Where in the area allows for contamination by pollution from industrial waste and household waste that much experience precipitation in the bottom waters. Contamination can lead to a termination of a chain of environmental order or destruction of a type of organism that the level will eventually destroy the ecosystem.

Mercury (Hg) is a pollutant, including group metals that are harmful to living beings, and where utility is widely used in industry for the development of optimal management of natural resources. Metal compounds such as $HgCl_2$ can get in very easily and quickly in implanting in the body and can accumulate in the body of aquatic organisms. It can also be toxic to humans if consumed fish like these. This group metal toxicity to aquatic organisms is no doubt that the damage to the tissues of aquatic organisms occur in organs such as gills and intestines, then into the inner tissues such as the liver and kidneys where the metal accumulates (Darmono, 1995). Beginning of the mercury-contaminated marine life is the inclusion of industrial waste containing mercury into the oceans. Furthermore, the presence of biomagnification process that works in the oceans, the concentration of mercury that enters will be improved in addition to the continuous addition of mill effluent. Mercury that enters the system is then associated with the food chain, so it goes into the body of aquatic biota (Palar, 1994). The process of metal accumulation in tissues occurs after absorption of metals from

water or through contaminated feed, the metal absorbed by blood, binds to blood proteins that then distributed to all body tissues (Palar, 2001). Metal toxicity may affect reproductive processes, among others, can lower sperm fertilization, lowering the oviduct contraction to eject eggs and caused the destruction of germinal epithelium (Darmono, 1995).

By looking at the dangers that can be posed, it is necessary to study to get a clear image of the damage to gonads, as a result of exposure to heavy metal mercury. The objective of study was to analyze the effect of various Hg concentrations on gonads of blue spotted ray, *D. kuhlii* using a histological observation.

MATERIALS AND METHODS

The total length and weight of blue spotted ray used were from 39 to 65 cm and from 230 to 700 g, respectively. Blue spotted rays (*D. kuhlii),* were treated for 12 days by using different concentrations of Mercury (Hg), those are, 0 ppm as control (treatment A), 0.0025 ppm (treatment B), 0.0050 ppm (treatment C), 0.01 ppm (treatment D) and 0,02 ppm (treatment E). Gonad specimens for histological preparate were conserved and fixed by using Bouin Solution, haematoxylin and eosin according to Carson (1990). The study was a descriptive analysis by using a histological observation to determine the gonad alterations. The alterations were determined by using histological features of Takashima and Hibiya (1995).

RESULTS AND DISCUSSION

There are some histopathological changes occur in gonads of Blue spotted rays (*D. Kuhlii)* after the treatment (Fig.1).

In the tank A with 0 ppm of Hg (control), the testicular is in normal shape, with the spermatozoa, Sertoli cell and germinal epithelium in the normal position (Figure 1 A). Germinal epithelium is also known as the wall of the seminiferous tubule within the testes. The cells in the epithelium are connected via tight junctions. Sertoli cell located at the cyst periphery. The cyst wall consists of Sertoli cell whose projections are related to the germinal cells forming Sertoli/germinal cell units (Schulz & Miura, 2002 *in* Bizzott and Gudinho, 2007).

At a concentration of 0.0025 ppm shown in Figure 1 B cloudy swelling that occurs in the Sertoli cells are characterized by cell swelling and turbidity on. In this condition, also looks a blood clots that formed from the destruction of blood cells due to infiltration excess substances from the outside. It happens because the Sertoli cells functioned in the fluid entry and exit offset in the cell. After Huang, *et al*., (2002), Sertoli cells with cystoplasmic extension as a cystic structure encompassing spermatocytes in the early reproduction season.

In Figure 1C with a concentration of 0.0050 ppm showed hyperplasia of germinal epithelium and distorted lobule of spermatozoa. After OECD Guidance Document for the Diagnosis of Endocrine-related Histopathology of Fish Gonads (retrieved on 20 June 2014), extensive testicular degeneration may lead to localized or generalized loss of the germinal epithelium.
www.oecd.org/dataoecd/33/29/42140778.pdf,

In Figure 1 D with a concentration of 0.01 ppm shows the development of eggs at the stage of vitellogenesis. This stage is characterized by the proliferation of cytoplasmic volume derived from exogenous vitelogenin forming yolk. The first cortical alveoli oocytes as an indication of the beginning of the vitellogenesis process (Casadevall *et al*., 2009). Mahmoud (2009) suggested that

most of the vacuoles in this stage were connected to each other and formed spaces between the yolk granules in the cytoplasm. The deposition of yolk granules that contain lipoprotein appear at the marginal regions of the maturing oocytes and then spread until the entire central cytoplasm of the oocytes. In Figure 1 D shows a detachment of vitelline layer. Normally, vitelline layer (envelope) serves to protect the eggs, also sperm binding, preventing the polyspermi during fertilization, and prevent cleavage blastomeres. This malfunction caused by the accumulation of organic and inorganic components in vitelogenesis stage, where blood had been contaminated by mercury is absorbed by the cells of the egg through the membrane so that the membrane permeability damage. This has been explained by Sorrensen (1995) that mercury tended to damage the membrane plasma and permeability characteristics of the cells involved. At this stage it is also apparent that the atresi egg mature eggs are spawned but not so removed. This condition may be caused by the release of hormones that are not appropriate.

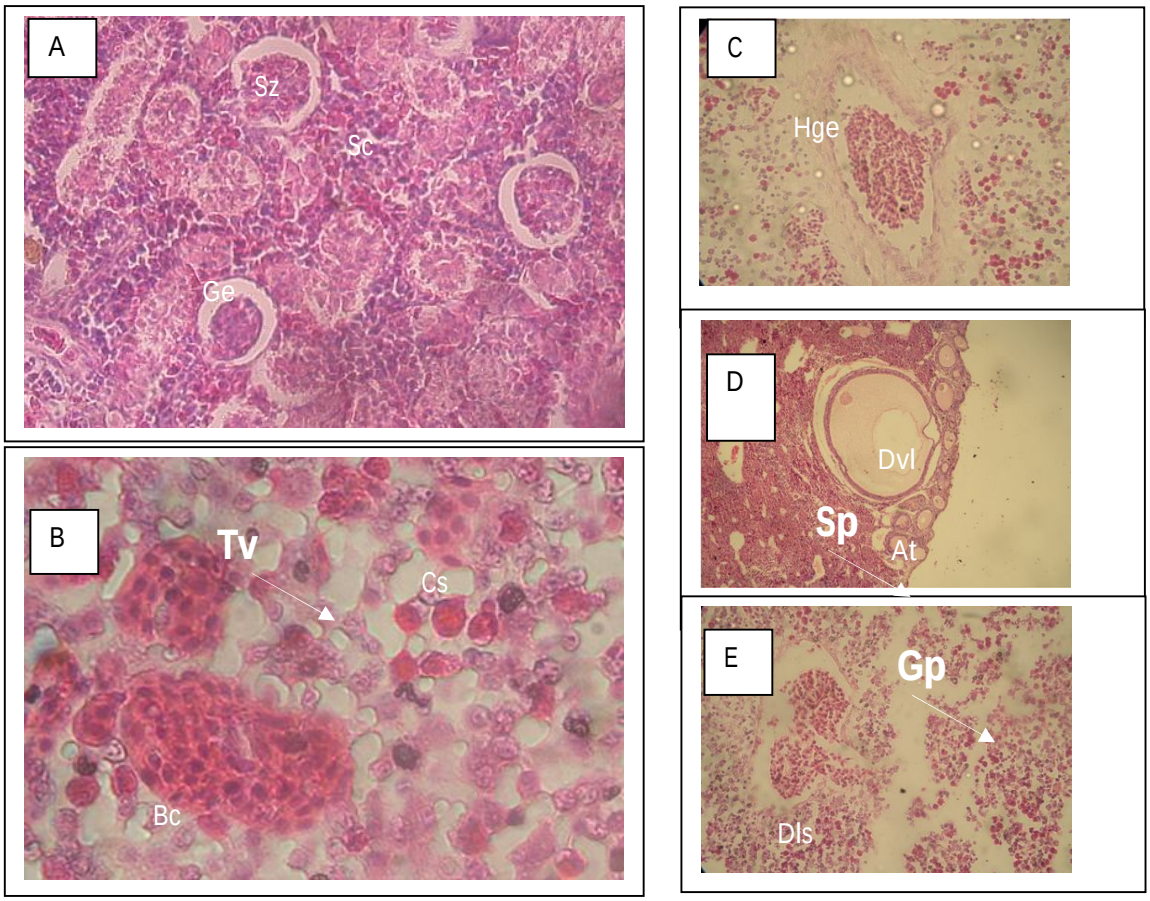

Figure 1. Gonads histological structure of blue spotted ray (*Dasyatis kuhlii)* after exposure at different concentrations of Hg. A, Control; B, 0.0025 ppm; C, 0.0050 ppm; D, 0.010 ppm; E, 0.0020 ppm. Sz : Spermatozoa, Sc : Sertoli cell, Ge : Germinal epithelium, At: Atresia; Hge : Hyperplasia of germinal epithelium, Cs : cloudy swelling; Bc : Blood Clots; Dvl : Detachment of vitellin layer, Dls : Distorted lobule of testicular.

Distorted lobule of testicular occurs in Blue spotted rays (*D. kuhlii*) male in tank E (Figure 1 E). There was a distorted lobule also in testicular of *Colisa fasciatus* that exposure by Potassium chromate. Distorted lobule of testes occurs while the germinal epithelium breaking and dissolution at several places in addition to the intra lobular (Shukla and Shukla, 2013).

Mercury (Hg) is one of the heavy metals that are dangerous to humans and other living organisms. By looking at the forms of damage that occurs based on the results of research, mercury has properties of soluble and bound in the tissues of the body. Where the mercury that enters the body will transported in red blood cells, and in the blood experiencing the oxidation process and will be conducted under the enzyme throughout the body tissues (Palar 1994).

Endocrine-disrupting chemicals such as Hg can have a profound effect on reproduction. And the demersal fish may contained elevated levels of Mercury (Olsvick *et al*., 2013). Darmono argued (1995) that the mercury has properties of soluble and bound in the body tissues and easy absorbed and accumulated in the tissues of organisms water. Mercury is accumulated in the body will damage or stimulate the enzymatic systems which result can lead to a decrease in the ability of adaptation to the animal.

CONCLUSION

From the results of the research can be seen high damage suffered not only depends on high concentrations but also the duration of exposure to the metal. The longer time of heavy metal exposure, the higher damage caused by it. In summary, based on the results obtained, gonads of blue spotted ray (*Dasyatis kuhlii*) changes caused by heavy metal at different concentrations of mercury (Hg). The histopathological changes that occurs in testicular namely: Cloudy swelling, blood clots, hyperplasia of germinal epithelium, and distorted the lobule. And the histopathological changes that occurs in ovary is detachment of vitelline layer.

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