UDC 594

THE EXPRESSION OF THE HORMONE ESTROGEN IN THE GONADS OF GREEN ASIAN GREEN MUSSEL (PERNA VIRIDIS) AS BIOMARKER OF ESTROGENIC COMPOUND ON THE BANYU URIP S AND KENJERAN WATERS, EAST JAVA

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ABSTRACT
Asian Green Mussel is one the ideal bivalves to investigate the estrogenic contamination because of its life that is sedentary, attached, and sensitive to environmental changes. Estrogenic compound is very harmful to organisms because it can resemble the estrogen hormone (xenoestrogen) and it has high accumulation power in the body. Xenoestrogen can bind to ER expressed in the E2 form. The research method used in this research was ELISA kit. Based on the research result, the ER in the body of Asian Green Mussel in *Banyu Urip* is 0.125 ng/l in male and 8.2 ng/l in the female. In *Kenjeran* waters, *Perna Viridis* is 5.8 ng/l in male and 2.9 ng/l in the female, the estradiol concentrations in water and body of male and female of Asian Green Mussel in *Banyu Urip* are 4 ng/l; 0.25 ng/l and 30.6 ng/l, respectively. Meanwhile, in *Kenjeran* are 80 ng/l; 39.9 ng/l and 5.9 ng/l, respectively. E2 Gonads of male Asian Green Mussel in *Banyu Urip* tend to be lower than in *Kenjeran*. This is due to the presence of estrogenic compounds that will disrupt the hormone system within the Asian Green Mussel's body. Thus the Asian Green Mussel synthesizes the estrogen hormone more than the usual level. If this condition continues, it can change the Asian Green Mussel that is supposed to be male changes in female due to excessive synthesis of estrogen hormone. This change may cause an unbalanced condition that will affect the population within an aquatic ecosystem.

KEY WORDS
Estrogenic compund, perna viridis, biomarker, hormones.

Kenjeran waters are the estuaries of Brantas River and Surabaya River, thus the pollutants will empty into the waters and cause environmental contamination. Environmental contamination due to waste discharged into the waters causes the presence of estrogenic compounds. According to Syamsuri (2006), Brantas River has experienced estrogenic pollution that causes the feminization in fish. According to Shobikhuliatul (2013), the Java Barb fish (Barbus goionotus Bleeker) in Surabaya suffer from the abnormality in its estrogen hormone synthesis, due to xenoestrogen contamination in the river.

Estrogen hormone-related environmental contamination (estrogenic activity) may affect humans and wild animals' reproduction (Richardson et al., 1999). Household waste such as detergent, pesticide, urine, plastic, feces, and medicines contain chemicals with estrogenic activity. Such contaminants can enter the body and disrupt the endocrine system. The material is an endocrine disruptor or endocrine function disorder which is known as the Endocrine Disrupting Chemicals (EDC) (Sharpe dan Skakkebaek, 1993; Man, 2002; Bayond, 2010). Pollution problem will cause negative effects on biological, chemical and even physical condition. The biological condition occurs in waters include death, structure and function defects of organ, and even abnormalities (Pratiwi, 2010).

The organism especially filter feeder will filter all foods enter its body. Bivalve is an ideal organism to investigate environmental contamination in aquatic invertebrates (Chakraborty, et al., 2013), fluctuations change in salinity, temperature and oxygen (Chandurvelan, et al., 2015), and bioindicators of heavy metal and pesticide contamination in waters (Miller, et al. 2005). Filter feeder bivalve is more sensitive and excellent as bioindicators, it lives in a sedentary and abundant way, and it has annual availability which makes it excellent as bioindicator (Cima and Ballarin, 2015; Chandurvelan, et al., 2015) and can show the relationship between heavy metal and organism in waters (Zairinayati, 2016). Bivalve is a sedentary organism and filter feeder which become the main target in the chemical estrogenic contamination, therefore, they potentially accumulate many contaminants and have low biotransformation to contaminants (Caseni, et al 2007). Asian Green Mussel is one of the bivalvia classes live in *Kenjeran* and *Banyu Urip* waters.

Estrogen receptor is a member of the receptor core of superfamily that has common characteristics and proteins, ER can be divided into several different domains. In fish of vertebrate ovipar type, it is clear that the expression of vitellogenin is regulated by 17β-estradiol (E2) via ER (Matsumoto, et al., 2007). Some contaminations such as xenoestrogen appears to disrupt Estrogen Receptor and become an alternative to endocrine systems of marine organisms including mollusks (Raingerad, et al., 2013). In invertebrates, estrogen receptors have been studied previously in molluscs, Aplysia California (Thornton, et al., 2003), Thais Calvigera (Kajiwara, et al., 2006) and immunoreactive with antihuman estrogen receptors (ER) antibodies can be detected in the reproductive system of female Octopus vulgaris (Keay, et al., 2006), Mytilus galloprovincialis (Raigeard, et al., 2013) and Neomysis japonica (Yang, et al., 2012).

One of the estrogen hormones that affect reproduction is 17β-estradiol. Some compounds have estrogen-like structures that can bind to estrogen receptors, which can trigger biological impacts similar to how they are affected by the hormone estrogen (Syamsuri, 2005). 17β-Estradiol can cause feminization, slow down the spawning and other reproductive disorders that affect fish (Scott and Sloman, 2004). In invertebrates, estrogen plays a role in the process of gametogenesis (Casein et al., 2006) and reproduction (Matsumoto, et al., 2007). 17β-estradiol in shellfish is detected in gonads, pedal ganglion and hemolyne (Keuy, 2006), Oyster and scallop on ovaries (Matsumoto, et al., 2007).

The use of biomarkers of invertebrate species including Asian Green Mussel is interesting. This is because the habitat of the Asian Green Mussel is permanent. Additionally, they can filter all the waters in large quantities, which render them as the main target of estrogenic contamination in the marine environment (Rifferser and Hock, 2002). Research on estrogen receptor and 17β-estradiol on the Asian Green Mussel has not been performed by many. Therefore, it is necessary to research on the estrogen receptors and 17β-estradiol in Asian Green Mussel in the waters suspected to be contaminated by estrogenic activity (*Kenjeran*) and that has not been contaminated (*Banyu Urip*).

MATERIAL AND METHODS OF RESEARCH

Asian Green Mussels of 4-5 cm in size were obtained from the *Banyu Urip* and *Kenjeran* waters in February 2018. The same side of shells between females and males were collected. 4 male and female shells each were taken at each. Asian Green Mussels were acclimatized on the aquarium for 2 days, dissected to take the gonads.

The sexes of Asian Green Mussels can be distinguished by looking at the color of the gonads. The differences between male and female shells cannot be seen visually or externally from their shells.

Gonads of Asian Green Mussels were weighed by 0.1 grams and PBS washing was performed. They were then centrifuged and stored in the freezer in -8°C (5 days), 20°C (1 month) and 80°C (3 months) until preparations were used.

Samples of gonads that had been weighed and washed with PBS were inserted one by one on mortar pestle to be crushed. Next, 50 µl lysis buffer was added and inserted into the tube. They were incubated for 30 minutes, centrifuged for 20 minutes at 10000 rpm. The supernatant was separated into tubes and stored in a freezer.

For ER analysis, ER fish kit with Cat.No.E00105 was used, while estradiol used Estradiol fish kit with Cat.No. E0050FI.

The working procedure with ELISA was based on Bioassay Technology Laboratory 2018 method. The diluent standards in this research were 320 ng/l, 160 ng/l, 80 ng/l, 40 ng/l, 20 ng/l, 10 ng/l, 5 ng/l, and 2.5 ng/l. Then, the isolated standard and sample were inserted into the well. Follow the procedures or intrusions written on the ELISA kit.

The prepared well was included in the ELISA reader with a wavelength of 450 nm. Readings with ELISA reader were not more than 30 minutes. Analysis of ELISA reader results was processed using M.excel.

The analysis was using the descriptive method, which is by drawing and explaining the observation data located in the research location.

RESULTS OF STUDY

Banyu Urip is an Asian Green Mussels cultivation site far from community activity. Asian Green Mussels cultivation in *Banyu Urip* is using a step chart system. The *Kenjeran* waters have been heavily affected by human activity. The dense industries and households cause the *Kenjeran* waters to be polluted. The results of estradiol analysis in *Banyu Urip* waters are about 4 ng/l while in *Kenjeran* of 80 ng/l.

In *Banyu Urip* waters, Gresik Regency, the average results of ER analysis on gonads of Asian Green Mussels are 0.175 ng/l in males and 8.2 ng/l in females. In *Kenjeran* waters, the average ER levels are 5.8 ng in males, and 2.9 ng/l in females.

The female Asian Green Mussels have higher ER levels than males in *Banyu Urip* waters. Different results showed in *Kenjeran* waters, ER level of *Perna Viridis* in males is higher compared to females.

The average level of estradiol in male Asian Green Mussel species (*Perna Viridis*) in clean water is 0.25 ng/l; while estradiol levels in contaminated water is 30.6 ng/l. The average level of estradiol in female Asian Green Mussel species in clean water is 39.2 ng/l; while estradiol level of female Asian Green Mussels in contaminated water is 5.9 ng/l.

Estradiol content in male Gonads of Asian Green Mussel (*Perna Viridis*) in clean waters tends to be lower than those in contaminated waters .While female Gonads of Asian Green Mussel tend to be higher in Banyu than those found in *Kenjeran* waters.

Water quality is an important factor to support life and reproduction of an organism. Water quality determines the health condition of the Asian Green Mussel because it is the place of direct contact. The following table shows the results of KA measurement from two research sites, *Kenjeran* and *Banyu Urip.*

Table 1 – Water quality measurement results

Station	Water Quality Measurement			
	pH	Temperature	DO	Salinity
Banyu Urip	7.9	30	6.18	32
Kenjeran	8.0	31	5.32	25
Quality Standard	28-32	7-8.5	≥ 5	33-40

The temperature measurement results were 30°C in *Banyu Urip* and 32°C in *Kenjeran*. Temperature can increase the toxicity of pollutants (household and industrial waste) for the aquatic organisms. Industrial and households wastewater containing pollutants will have higher toxicity as water temperatures rise.

The pH measurement result of *Kenjeran* water is higher than *Banyu Urip.* Changes in pH of water greatly affect the physical, chemical, and biological processes of the organisms living in it. The acidity level is thought to greatly affect the toxicity of pollutants and the solubility of some gases, and determine the form of substances in the water. Water pH values are used to express the acidity (hydrogen ion concentrations) and alkalinity conditions of waters. Low pH values can increase toxicity.

Temperatures of *Banyu Urip* waters is 8.1 mg/l, while in *Kenjeran* in 6.2 mg/l. High dissolved oxygen can improve the reshuffling process of pollutants which makes it reduced.

The salinity in two research stations is 33 ppt in *Banyu Urip* and 25 ppt in *Kenjeran* waters. Salinity is higher in *Banyu Urip* due to the location of Asian Green Mussel's cultivation is rather far toward the sea.

DISCUSSION OF RESULTS

Estrogen Receptor. The salinity in two research stations is 33 ppt in *Banyu Urip* and 25 ppt in *Kenjeran* waters. Salinity is higher in *Banyu Urip* due to the location of Asian Green Mussel's cultivation is rather far toward the sea. Research on two research sites showed different levels if Estrogen Receptor. In *Banyu Urip* waters, the male shells have lower Estrogen Receptor levels compared to those in *Kenjeran* waters. This is due to the fact that *Kenjeran* waters have been contaminated with estrogenic compounds derived from household, industry and agriculture and livestock activities. Human activity can contribute to the waste, especially estrogenic compounds that can disrupt the estrogen receptor bond. The estrogen-like compound will be bound by the receptor of the Asian Green Mussel (*Perna Viridis*).

According to Alisopp et al. (1997), the receptor estrogen mechanism binds to xenoestrogen via two stages, they are:

- Binds to an estrogen-like hormone. Estrogen receptors can only bind to estrogen or artificial estrogen. After binding, it is then sent to the hypothalamus to force the pituitary gland to receive the additional estrogen hormone.
- Blocking so that the estrogen that supposed to bind its receptors is hindered. If it is blocked or hindered, it cannot be expressed to estradiol or estrogen hormone cannot function properly.

Estrogen receptors are the core receptors associated with the reproductive system in organisms. Every organism has an Estrogen Receptor. Increased ER in an organism is affected by the presence of injection or additional estrogen hormone. All types of estrogen hormones will be bound by estrogen receptors. When there is an estrogen hormone or an imitation of estrogen, then ER will also active and will convey to the hypothalamus to be passed on to the reproductive organs of Gonad.

Xenoestrogens (artificial estrogens) will enter the liver that can resemble the real estrogen hormone, and then damage the hormone receptors in the body and signal disturbance occurs in the formation of estrogen in the liver. Estrogen in the liver carries the messages for vitellogenin to be immediately synthesized. Vitellogenin is a raw material for the production of egg yolk which is later secreted to the liver cells, then taken to the gonads through the blood. The estrogen hormone receptor is activated when the artificial Estrogen (xenoestrogen) enters the target organ (gonad). When it is active, the hypothalamus will order to produce the hormone.

Estradiol-17β. The concentration of estradiol in water is very different. It is higher in *Kenjeran* waters than *Banyu Urip* waters. The high concentration in *Kenjeran* waters is because the location of the research which experiencing estrogenic contamination and the location of Asian Green Mussel's habitat is adjacent to the estuary. Rivers in Surabaya with estrogenic contamination will flow and empties into Kenjeren Waters so that estrogenic compounds from the river will accumulate in it. According to Shobikhuliatul (2013), that Java Barb fish in Surabaya River has suffered from the abnormality in estrogen hormone synthesis, due to xenoestrogen contamination in the river. According to Syamsuri (2006), Brantas River has experienced estrogenic pollution that affects the feminization in fish.

The high level of estradiol in male shells in *Kenjeran* is because the waters contain estrogenic so that the Asian Green Mussel suffer from the interruption in synthesizing hormones. This is in accordance with the study by Sumi et al. (2007) that the level of male fish hormone is higher of 653±417 pg/ml in Ishizu river Japan, and also high of 1087±949pg/ml in Teganuma region, Japan. This is similar to Juanda (2013) that E2 in male fish is 580 pg/l in Surabaya River and 720 pg/l in Kalimas River.

The high estrogenic contamination in *Kenjeran* waters derived from urine, in accordance with Johson and Ulahannan (2004) which stated that female menstrual urine is able to produce as much as 129 mg/day of waste and pregnant women is as much as 260 mg/day. According to Syamsuri (2006), buffaloes, cows, and goats contribute estradiol-17, medicines such as birth control pills, paper, and farming industries (DDT and DDE), garbage both baby diapers and sanitary pad can contribute estradiol to the waters which cause male fish to experience abnormalities in synthesizing estrogen hormones. These abnormalities in estradiol synthesis occur due to a disturbance in the endocrine system of the Asian Green Mussel. The estrogenic compound acts as an artificial estrogen hormone or resembles the estrogen hormone so that it enters the body and affects the secretion of hormones present in the Asian Green Mussel's body.

This Xenoestrogen has lipophilic and hydrophobic properties which mean high accumulation if entered into the tissues of aquatic organisms (Arukwe and Goksyr, 1998). Xenoestrogens present in waters will enter along with water when the Asian Green Mussel filters the feeder or breathing. Gills have an epithelium membrane. Through a biochemical process, water entering through the gill will be transferred to the circulatory system. The blood carries an estrogenic compound that is passed directly to the digestive system and then absorbed and carried into the liver, while some estrogenic compounds will flow directly to the whole body including brain and gonads (Pait and Nelson, 2002, Zhei et al., 2009). Through the feeder filter, water that contains estrogenic compounds will enter through the ventral siphon which in the gills, then through the lamella of gills in both coats will select the compound to be inserted to the oesophagus and to the digestion (Suwignyo, 1981).

Relationship between ER and Estradiol-17β. Estradiol is a hormone associated with steroid hormones found in organism body that have specific receptors for the hormone estrogen system. Estradiol formation starts from the presence of environmental stimulation that will be forwarded to the hypothalamus by activating the receptors into the target organ and then there will be the formation of egg yolk or vitellogenin. The receptor in question can bring the message in the form of a core protein, called an estrogen receptor. This estrogen receptor can only bind to estradiol compounds or estradiol-like (xenoestrogen) compounds.

High ER concentrations in female Asian Green Mussels do not match the E2 concentrations in the body. This is due to the interference of compounds that can contaminate hormones in the body so that ER is blocked and can not bring the message to the estrogen hormone. According to Allisop et al. (2007), the mechanism of estrogenic compounds binding to receptors, namely estrogen receptors that must binding are obstructed or closed, which in turn it cannot be expressed.

Water Quality. The water quality found in two study sites is still suitable for the survival of Asian Green Mussel (*Perna Viridis*). Water quality such as temperature, pH, DO, and salinity are still below the standard quality threshold of 2004, this means that it still suitable for biota life. Asian Green Mussel is one of the biotas that are resistant to changes in water quality.

Kenjeran waters temperature is higher than that of *Banyu Urip.* However, the difference is very little because *Kenjeran* and *Banyu Urip* area are still in the same one province of East Java. This temperature is influenced by the sunshine factor that shines in the location.

pH is the level of pH acidity value which greatly affect chemical reactions of compounds or other water quality. The high pH can cause a more toxic compound. pH at the study site tends to be alkaline due to the habitat location of Asian Green Mussel that is close to the sea, especially in *Banyu Urip* waters.

The dissolved oxygen in Banyu Uurip is higher than that of *Kenjeran* because the Asian Green Mussel cultivation location in *Banyu Urip* is closer to the sea and due to the high waves. Meanwhile, in *Kenjeran*, it is close to the estuary as well as due to the high organic matter from household and industrial waste which affects the dissolved oxygen.

Salinity in *Kenjeran* waters differs greatly to *Banyu Urip* do to the location of Asian Green Mussel in *Kenjeran* is close to the estuary while in *Banyu Urip* is far from the estuary. Therefore, the salinity in *Banyu Urip* is higher. The cultivation of Asian Green Mussel in *Banyu Urip* is in the form of a step chart.

CONCLUSION

The conclusions of the study showed that the levels of ER and E2 in Asian Green Mussel gonads found in *Banyu Urip* and *Kenjeran* waters were different both in males and females. In *Kenjeran* waters, the males shells tend to have both ER and E2 levels higher than females and vice versa. If such event occurs continuously, the reversal of sex from males to females will occur. Therefore, there needs to be a processing of estrogenic compounds so as not to pollute the aquatic environment.

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