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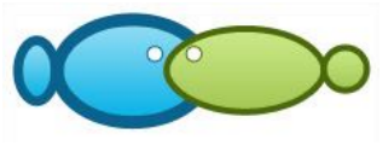

The histopathology of antique ark's mantle (*Anadara antiquata*) post-depuration with the shells' filtration

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Abstract. Cockles are marine organisms which have the character of filter feeders so that heavy metals can be neutralized naturally through their shells. However, not all heavy metals can be neutralized, so depuration needs to be done. After depuration, histopathological analysis is needed to determine the condition of the soft tissue of the shells so that the disease can be diagnosed through structural changes that occur in the organs that are the main target of pollutants. This study aims to determine the histopathology of antique ark's mantle (*Anadara antiquata*) after post-depuration with the filtration of the cockles' shells. This research method applies an experimental method with scoring histological damage to antique ark's mantle that ranges from 0 to 3, depending on the level and extent of the changes that occur. After that, the distribution of normal and non-homogeneous data was obtained, and then the Kruskal-Wallis non-parametric test was conducted. The main parameter is the histopathology of the antique ark's mantle. Supporting parameters include water quality, namely temperature, dissolved oxygen (DO), nitrate, nitrite, ammonia, salinity, levels of heavy metals Pb and Cd, total suspended solid (TSS) and total dissolved solid (TDS). The results of the Kruskal-Wallis statistical analysis shows no significant difference between treatments P0 (Control), P1 (Filter 25%), P2 (Filter 50%), P3 (Filter 75%), and P4 (Filter 100%). The histopathological features of the antique ark's mantle organ tissue found were edema, hyperplasia, necrosis, and atrophy.
Key Words: histopathology, mantle, antique ark (*Anadara antiquata*), filter feeder.

Introduction. Cockles are one of the marine organisms that are susceptible to environmental changes, because of their passive or sedentary movements. Each species of cockle has a tolerance level to different environmental changes. One of them is the clam from the Arcidae family. Arcidae members are filter feeder inhabiting intertidal waters with sandy mud substrates in a water depth of between 2-20 m. Utilization of antique ark (*Anadara antiquata*) has not been intensive until now. The price is still relatively cheap and production is low. Productivity in East Java, Indonesia in 2010 reached 9,737.6 tons or it is equivalent to 2.87% of total fisheries production in East Java, Indonesia for cockle products and 3,746.7 tons or equivalent to 1.11% for *Anadara* sp. commodities caught by a total of 247,802 fishermen in 2010 (KKP 2014).

Generally, Indonesian people are less aware that consuming cockles needs to be vigilant, because of the ability of the cockles itself to absorb heavy metals that endanger public health. Toxic and decomposed properties of heavy metals are influenced by environmental factors, and the characteristics of these metals. Toxic properties of metals are due to their effectiveness with sulfhydryl (SH) group in cellular enzymes, then forming metalloenzymes and metalloproteins so as to stop the function of the enzyme. Shells naturally detoxify metals in the body through metabolic processes, but also through induction (Gabr & Gab-Alla 2008).

The higher the concentration of heavy metals in the waters, the bioaccumulation of heavy metals in the body of shellfish also increases. That is because some types of heavy metals cannot be metabolized by the body and other types of metals have a high affinity for the formation of tissues that are rich in non-lipid compounds. The Pb content can be consumed < 1.5 mg kg^{-1} while Cd is 1.0 mg kg^{-1} (DKP 2004). Prevention and

efforts to reduce and also to determine the levels of heavy metals in antique ark clams are very necessary.

One effort that can be done is through the depuration process. Depuration is a technique used to eliminate pollutants in marine products. This would be very useful to help Bivalvia expel and separate heavy metal contaminants (El-Gamal 2009). The shells are formed by biomineralization and most consist of calcium carbonate with a small amount of organic matrix (Yao et al 2014). The high content of calcium carbonate makes the shells capable of cleaning water, and can even reduce the levels of iron, manganese and other metals (Simaremare et al 2013). Meanwhile, the mantle is the body part that covers the shell on the inside and this part is often exposed to direct contact with the aquatic environment. Histopathology can be applied as a biomarker to determine the condition of cockles' soft tissue in diagnosing diseases through structural changes. It usually occurs in organs that are the main target of pollutants. Therefore, it is necessary to do research on the histopathology of the antique ark's mantle post-depuration with the shells' filtration. It is expected that the depuration process can provide a result of changes in tissue structure. Based on the statement of the problem, the purpose of this study is to determine the histopathology of antique ark's mantle (*Anadara antiquata*) post depuration with shells' filtration.

Material and Method. This research was conducted from September to November 2016 at the Faculty of Fisheries and Marine in Universitas Airlangga, Surabaya, Indonesia. The tools used during the study were 5 tubs measuring 95 cm x 70 cm x 75 cm, 20 shelves with sizes 42 cm x 33 cm x 14 cm, pipes, 5 pieces of filter tub with a height of 45 cm and the diameter is 26 cm, 5 pieces of aerator, 5 flow meters, 5 UV meters, calipers, analytical scale Ohauss Pioner Px-224, water quality measuring devices (Hanna Instrument HI98107 pH Pen and YSI 550A DO test kit made in USA, ammonia and nitrite-nitrate test kit by SERA), 10 pieces of pot specimen containers, microtomes and microscopes. The materials used in the study were antique ark originating from water areas in the Sedati region of Sidoarjo, Indonesia. The antique ark used is 15 grams with 1200 shells in each tank. In each tub, there are 4 shelves. The media needed in the maintenance of antique ark is seawater with a salinity of 30 ppt. The materials used for making histological preparations for antique ark are 10% formalin, liquid paraffin, alcohol 70%, 80%, 90%, 95%, absolute alcohol, xylene, acidic alcohol, and Hematoxylin-Eosin dyes.

There are two types of variables in this study. The independent variable here is the antique ark (*Anadara antiquata*), while the dependent variable in this study is the histopathological structure of the antique ark. The research method used is an experimental method to determine the effect of giving antique ark's shells A, B, C, D, and E to the structure of the tissue on the antique ark with different percentages for 3 days of maintenance. The study design was used to observe the results of damage to the mantle of antique ark organ tissue. Specifically, it was applied with the semi-quantitative scoring method and continued with the Kruskal-Wallis analysis test. The Kruskal-Wallis statistical test is used to determine whether k samples are independent of different populations (Siegel 2011). Furthermore, this study used five treatments with four replications including:

1. P0: depuration without giving a shell's filtration on the antique ark;
2. P1: depuration by giving 25% of shell's filtration on the antique ark;
3. P2: depuration by giving 50% of shell's filtration on the antique ark;
4. P3: depuration by giving 75% of shell's filtration on the antique ark;
5. P4: depuration by giving 100% shell's filtration on the antique ark.

The shell of the antique ark that will be used as a filter is washed first and then crushed into a size of 0.5-2 cm. After that, the shells are inserted into each tube as a filter. The shells used are 15.3 kg for filters 100%, 11.5 kg for filters 75%, 7.7 kg for filters 50% and 3.8 kg for filters 25%. This study uses five tubs, each tub consists of four shelves and each rack contains 2.5 kg of shells, the total shell as the test material used is 50 kg. The mantle of antique ark samples is taken before and after a 48-hour depuration

process. Then, the mantle of antique ark sample is made into histological preparations carried out at the Pathology Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia.

Microscopic observations were carried out on the preparations of a histological slice of antique ark gills using a digital microscope. The observation of preparations was conducted in March 2017 at the Microbiology Laboratory of the Faculty of Fisheries and Marine Airlangga University, Surabaya, Indonesia. The parameters in the study include the main parameters and supporting parameters. The main parameter is the histopathology of the mantle in the antique ark. The supporting parameters include factors of the water quality itself, namely temperature, dissolved oxygen (DO), nitrate, nitrite, ammonia, salinity, levels of heavy metals Pb and Cd, total suspended solid (TSS) and total dissolved solid (TDS). The analysis of the main parameter used is the result of the mantle structure on the antique ark using preparations and observations of microscopic preparations. The analysis of supporting parameters utilizes a thermometer, refractometer, pH pen, DO test kit, ammonia test kit, nitrite nitrate test kit, and spectrophotometer (Human X-Ma 1200).

The data obtained were analyzed descriptively after previously scoring with quantitative methods, namely the analysis carried out by calculating the percentage of the damage to the mantle of antique ark studied. Scoring used is the Pantung method which aims to determine the level of damage to gill histology that ranges from 0 to 3, depending on the level and extent of the changes that occur. Histopathological symptoms observed included edema, hyperplasia, necrosis and atrophy. The following are the percentage scoring values (Pantung et al 2008) presented in Table 1. After obtaining the distribution of normal and non-homogeneous data, a Kruskal-Wallis non-parametric test was conducted. If $p < 0.05$ then there are significant differences.

Table 1

Scoring for histological changes in antique ark (*Anadara antiquata*)

Parameter	*Score 0 (normal)*	*Score 1 (mild)*	*Score 2 (medium)*	*Score 3 (heavy)*
Edema (E)	None	Less than 30% of the area of view.	30-70% of the area of view.	More than 70% of the area of view.
Hyperplasia (L)	None	Less than 30% of the area of view.	30-70% of the area of view.	More than 70% of the area of view.
Necrosis (N)	None	Less than 30% of the area of view.	30-70% of the area of view.	More than 70% of the area of view.
Atrophy (A)	None	Less than 30% of the area of view.	30-70% of the area of view.	More than 70% of the area of view.

Results

Antique ark (Anadara antiquata) description. The antique ark used in this study has the characteristics of red and soft flesh, has 2 valves of shells which have fine fur as the main feature of the antique ark clam. The part of the mantle attaches to the inside of the shell of the clam (Figure 1).

Figure 1. Morphology of antique ark clam (*A. antiquata*), A = clam's shell, B = mantle.

To determine the morphometrics of feather shells, the measurements of length, width, height, and weight were carried out. Average Morphometric Measurement Data for antique ark clam consists of 40 samples. The antique ark clams used in this study had an average length of 3.38 cm; average width of 2.51 cm; the average height of 1.78 cm; and the average weight is 9.545 g.

Histopathology of antique ark clam (Anadara antiquata). The Figure 1 is a histopathological observation of post-depuration of antique ark clam. Based on the picture, it can be seen that the mantle of antique ark clam has the same cell structure in general, consisting of ciliated surface epithelium, goblet cells, fat cells, eosinophilic granular cells, and muscle fibers.

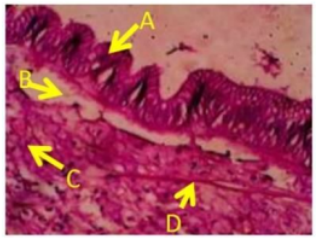

Figure 2. Histology of antique ark clam (*Anadara antiquata*). Goblet cells (A), fat cells (B), eosinophilic-granular cells (C), and muscle fibers (D). Coloring HE. 400x magnification.

The histopathological observations of antique ark's mantle on its post-depuration show there are damage namely edema, hyperplasia, necrosis, and atrophy. The histopathological observations of antique ark's mantle before depuration also suffered damage, namely edema, hyperplasia, necrosis, and atrophy which can be seen in Figure 3. It aims to see the changes that occur after depuration.

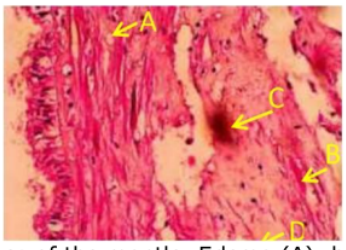

Figure 3. Histological changes of the mantle. Edema (A), hyperplasia (B), necrosis (C) and atrophy (D). Coloring HE. 400x magnification.

The damages to the mantle of antique ark clam are determined by calculating the amount of damage in one area of view which called the scoring method. Each treatment given shows significant results which can be seen in Figure 4A for pretreatment before depuration, Figure 4B for treatment 0 or without filter, Figure 4C for treatment 1 with filter 25%, Figure 4D for treatment 2 with filter 50%, Figure 4E for treatment 3 with a 75% filter, and Figure 4F for treatment 4 with a 25% filter.

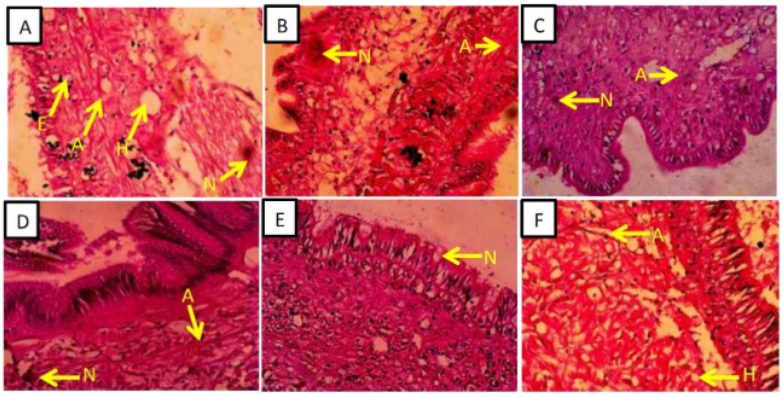

Figure 4. Histopathological changes in post-depuration of antique ark clam. A. The mantle of antique ark clam that has not been depurated, edema score (< 30%), hyperplasia (30-70%), necrosis (30-70%), atrophy (< 30%); B. The mantle of antique ark clam that has depurated P0, necrosis (30-70%), atrophy (< 30%); C. P1 necrosis (30-70%), atrophy (< 30%); D. P2 necrosis (30-70%), atrophy (< 30%); E. P3 necrosis (30-70%), atrophy (< 30%); E. necrosis (30-70%); F. hyperplasia (30-70%), atrophy (30-70%). Coloring HE. 400x magnification.

The average score of the damage to edema, hyperplasia, necrosis, and atrophy in each treatment is presented in Table 2. This average score is the result of observing the antique ark clam organ tissues on a microscope that was repeated twice per treatment.

Table 2
The average values of scoring to the damage edema, hyperplasia, necrosis, and atrophy in each treatment

No.	*Treatment*	*Average values of scoring*			
		Edema	*Hyperplasia*	*Necrosis*	*Atrophy*
1	P	1.375	1.25	1.875	0.5
2	P0	1.25	0.875	2	0.75
3	P1	1.5	1	2.25	0.875
4	P2	1.5	1.625	2.125	1.25
5	P3	1.75	1.625	2.5	1.5
6	P4	2	2.375	2.375	1.375

Scoring results are analyzed statistically which show a difference in each treatment given. Statistical data analysis using the Kruskal-Wallis test is presented in Table 3.

Table 3
The results of Kruskal-Wallis statistical analysis between treatments in histopathological observations of antique ark clam (*Anadara antiquata*)

No.	*Types of damage*	*Kruskal-Wallis statistical analysis between treatments*
1	Edema	0.430
2	Hyperplasia	0.112
3	Necrosis	0.635
4	Atrophy	0.092

In the Kruskal-Wallis statistical test, the lowest damage is at atrophy, which is 0.092 and the highest necrosis was 0.635 so that it has a value of $p > 0.05$. It means that there is no significant difference between treatments.

Temperature, pH, DO, salinity, ammonia, nitrite, and nitrate. The measurements of water quality based on temperature, pH, DO, salinity, ammonia, nitrite, and nitrate are carried out every 6 hours, namely at 6 AM, 12 AM, 6 PM and 12 PM. Data on the range of water quality parameters for 48 hours depuration of antique ark clam can be seen in Table 4.

Table 4

Range of water quality parameter based on temperature, pH, DO, salinity, ammonia, nitrite, and nitrate during the depuration process of antique ark clam (*Anadara antiquata*)

Parameter	*Treatment for 24 hours*					*Treatment for 48 hours*				
	P0	*P1*	*P2*	*P3*	*P4*	*P0*	*P1*	*P2*	*P3*	*P4*
Temperature (°C)	30.5	30.3	29.9	29.6	30.4	30.7	30.4	29.9	29.7	30.2
pH	7.5	7.4	7.4	7.4	7.4	7.7	7.6	7.5	7.3	7.2
DO (mg L^{-1})	4	4.4	4	4	4	4	4	4	4	4
Salinity (ppt)	29	29.8	28.6	28.3	29.4	29.4	29.2	29.2	29	29
Ammonia (ppm)	0.06	0.05	0.05	0.03	0.03	0.09	0.08	0.05	0.03	0.03
Nitrate (ppm)	0	0	0	0	0	0.2	0	0	0	0
Nitrite (ppm)	0	0	0	0	0	0.1	0	0	0	0

Temperature measurements on antique ark clam media maintenance ranges from 29.6 to 30.7°C. The results of DO measurements do not have a difference that is in the range of 4 mg L^{-1}. Salinity in the depuration process ranges from 28.3 to 29.8 ppt. The highest ammonia value in P0 (control) is 0.09. The results of nitrate and nitrite are almost the same as 0 ppm except in treatment P0 (control) the value of nitrite is 0.1 ppm and P2 while P0 is nitrate value of 0.02 ppm.

Analysis result of Pb and Cd heavy metals. The test result for the content of heavy metal Pb and Cd in the water to be used is 1.078 ppm and 0.098 ppm respectively. Testing of Pb and Cd content in the water is carried out to determine the content of heavy metals Pb and Cd in water and in meat. The result of measuring heavy metals in water can be seen in Table 5.

Table 5

Test result for heavy metal content of Pb and Cd in water

No.	*Treatment*	*Pb content (ppm)*		*Cd content (ppm)*	
		Before	*After*	*Before*	*After*
1	P0	1.078	0.250	0.098	0.350
2	P1	1.078	0.374	0.098	0.174
3	P2	1.078	0.186	0.098	0.196
4	P3	1.078	0.167	0.098	0.165
5	P4	1.078	0.294	0.098	0.194

Based on the data in the Table 5, the Pb content in water before depuration is 1.078 ppm and then after the treatment the PB content on the water P0 decrease to 0.025 ppm, P1 is 0.374 ppm, P2 is 0.186 ppm, P3 is 0.167 ppm, P4 is 0.294 ppm. It shows that treatment P1 (filter 25%) has the highest Pb content. Whereas for Cd content in the water itself before depuration is 0.098 ppm lower than P0 which is 0.350 ppm, P1 which is 0.174 ppm, P2 is 0.196 ppm, P3 is 0.165 ppm and P4 is 0.194 ppm. It points out that the highest water content of Cd is P0 as a control.

The following are the results of testing of Pb and Cd heavy metal content in antique ark clam (Table 6).

Table 6

Test result of Pb and Cd heavy metal content in the meat of antique ark clam

No.	Treatment	Pb content (ppm)		Cd content (ppm)	
		Before	After	Before	After
16	P0	0.3598	0.14575	2.144	1.226
2	P1	0.3598	0.1075	2.144	1.365
3	P2	0.3598	0.13225	2.144	1.27575
4	P3	0.3598	0.06125	2.144	1.2495
5	P4	0.3598	0.065	2.144	0.826

The analysis result in the Table 6 shows that Pb content in antique ark clam before depuration is 0.3598 ppm. After depuration, the Pb content in antique ark clam decreased in each treatment. The Pb content with treatment P3 has decreased, from 0.3598 to 0.06125 ppm. Similarly, it happens to P4 the Cd content in the antique ark clam. Before depuration, the Cd content in antique ark clam was 2.144 and it drops to 0.826 ppm after depuration.

Analysis result of TDS and TSS content. In addition to temperature, salinity, acidity or power of hydrogen (pH), DO, ammonia, nitrate and nitrite, the measurements of water quality consist of TDS and TSS. The measurements before and after depuration are performed to determine the amount of concentration of dissolved solids in water. Data from the measurement results of TDS and TSS are presented in Table 7.

Table 7

Measurement result of TDS and TSS

No.	Treatment	Before depuration		After depuration	
		TDS (mg L^{-1})	TSS (mg L^{-1})	TDS (mg L^{-1})	TSS (mg L^{-1})
1	P0	27.040	10	27.260	17
2	P1	26.940	17	27.660	21
3	P2	26.580	40	27.080	39
4	P3	24.700	138	26.040	146.67
5	P4	27.080	68	27.220	56

Based on the Table 7, the highest TDS content before depuration is P4 27080 mg L^{-1}. The highest TSS content before and after depuration is found in P3 which is equal to 146.67 mg L^{-1}.

Analysis result of antique ark clam mortality. The initial number of antique ark clam samples used in the depuration process is 1248 clams/tub. Within 48 hours, each treatment of antique ark clam experiencing death can be seen in Table 8.

Table 8

Measurement result of mortality data

No.	Treatment	Death	Mortality
1	P0	39	3.12%
2	P1	382	30.61%
3	P2	391	31.33%
4	P3	724	58.01%
5	P4	732	58.65%

The lowest analysis of antique ark clam mortality in treatment P0 (unfiltered) was 3.12% while the highest in treatment P4 (100% filter) was 58.65%.

Discussion. In the antique ark clam tissue organ, it occurs inflammation caused by the presence of toxic materials such as heavy metals. Inflammation is not a disease but an infection reaction due to the toxic substances into the bloodstream. There are several causes of inflammation, one of the causes are chemicals such as corrosive, acidic, alkaline, and bacterial toxins. Chemicals that cause corrosives will damage the tissue and then cause an inflammation process. In addition, infectious agents can release specific chemicals that cause irritation and inflammation (Harjono et al 1996).

Edema often happens from excessive exposure to heavy metals. This also occurs in some severe cases due to chemical pollutants and heavy metals in the *Salmo trutta fario* aquaculture namely mucous accumulation, epithelial hyperplasia, followed by epithelial cell death (necrosis), embolism, and inflammatory cell infiltration (Crespo et al 1988).

Edema causes a buildup of fluid in the cavities between the fibers which are distant and stretchy (Takashima & Hibiya 1918). It can be seen in tissue damage to the antique ark clam organ (Figure 3). This shows that there is a buildup of red blood cells that are very dense in blood vessels which will cause blood vessels to block. Indeed, it will allow edema around the blood vessels which can be seen from the expansion of tissue between blood vessels and cells in the mantle of antique ark clams.

Some pathological events found in histopathological observations of antique ark clam, besides edema, are the thickening of granular cells (Figure 3). This thickening is called hyperplasia which makes granular cells look like baseball beats. Hyperplasia usually occurs as a chronic response due to exposure to bacteria, parasites, or chemical agents, one of which is heavy metal. In addition to suppressing blood vessel capillaries in cells, hyperplasia also requires an increase in blood supply to newly formed tissue. Even in chronic conditions, the cell's condition is not normal anymore but sticks together (Takashima & Hibiya 1918). Hyperplasia can occur due to various chemical pollutants and heavy metals, especially Cd, Cu, and Zn. Clams exposed to heavy metals, detergents, ammonia, pesticides, and nitrophenols show cell separation which can lead to the collapse of a single unit of the cell structure (Olurin et al 2006).

If there is a black spot or the cell condition is not intact anymore in the cell structure of the mantle organ itself then it is called necrosis. Necrosis is the death of cells or tissue which also accompanies cell degeneration in every animal life and is the final stage of irreversible degeneration (Plumb 2018). The mantle necrosis that has been depurated is rarely found in tissue repair due to the condition of cells that are no longer able to repair cell damage. Necrosis is the most common pathological form. This is because heavy metals that accumulate the mantle of clam interfere with the cell metabolism and destroy these cells so that the cells are not intact anymore (necrosis).

Atrophy is a process of decreasing the size of a body part or organ due to a reduction in the size or number of cells and the reduction process usually takes place slowly. In the muscle fibers of the antique ark clam which have atrophy, the sarcoplasm becomes thinner and disappears, as well as being released from sarcolemma and endomysium. Atrophy can be caused by hunger or malnutrition (the most common cause), and also lacks adequate blood supply or chronic infection (Plumb 2018). Based on the results of the observation data, the pathological form of atrophy does not decrease. This is presumably because during maintenance there is no food supply to the antique ark clam so that they were starved. After absorbing heavy metals, within one hour there will be muscle contractions and at 3-6 hours there will be an accumulation of hemocytes. The accumulation of hemocytes indicates an inflammatory process. The purpose of inflammation is to clean the area of damage caused by heavy metals (foreign objects) before the healing process takes place (Flynn & Rovee 1982).

The change in damage that is the fastest after the occurrence of muscle contraction is that there will be no cell accumulation or thickening. Edema will begin to diminish with the passage of the body's metabolism of the clam. Furthermore, if there is no cell accumulation, there will be no capillary suppression of blood vessels in the cells which needed to increase the blood supply to the newly formed tissue. Thus, hyperplasia will begin to decrease too. For pathological conditions of necrosis, it is difficult to experience changes with rapid time due to the condition of cells that have died.

Epithelialization of the clams itself begins after three days until the area of damage is cured and regeneration is seen on the twelfth day until the twenty-fourth day (Polakitan et al 2013).

Likewise, with atrophic conditions, it is very difficult to experience improvement fast because the cause of atrophic damage itself comes from hunger or malnutrition (the most common cause), lack of adequate blood supply or chronic infection (Plumb 2018). During the depuration process, the absence of food supply makes the ability of the clams in repairing the damage from atrophy to be very difficult.

Depuration is the state of clams' cultivation in controlled and closed water circulation so that it has the ability to reduce the content of heavy metals in both the meat and the aquaculture water. This is beneficial for the clams. It is because, in depuration conditions, the clams will release heavy metals that accumulate in the body (desorption) so that the body's metabolism of the clams will go well. However, the clams' shell filter used in the depuration process makes the mantle tissue worsen. This is allegedly due to an increase in excess Ca content in the body of the clams.

Water quality also has an important role in the mortality of antique ark clams. Based on the data obtained, the highest mortality is found in the treatment P4 (100% filter) which is equal to 58.65%. Measurements of the heavy metal content of Pb and Cd in clams are carried out to support the diagnosis. Based on the histopathological results obtained, it has been explained that the mantle of antique ark clam is associated with heavy metal pollution. The results of measurement of Pb and Cd heavy metal content are 2.144 mg kg^{-1} and 0.3598 mg kg^{-1}.

The accumulation of heavy metals in the body of the clams proves the existence of environmental pollution in the area around Sedati waters, Sidoarjo. This is thought to be a trigger for the occurrence of mantle's organ tissue damage, namely edema, hyperplasia, necrosis, and atrophy as shown in Table 2. The values of Pb and Cd heavy metals content are < 1.5 mg kg^{-1} and 1.0 mg kg^{-1} (DKP 2004). Based on the measurement of Pb heavy metal, it has exceeded the safety limit while Cd is still under the security standard.

The content of Pb and Cd that accumulates in the body will affect Ca homeostasis (Javanshir et al 2009). The body will be disrupted due to the presence of heavy metals Pb and Cd which will cause acidosis. Ca ion is thought to undergo mobilization from organs which become Ca deposits so that there will be an increase in Ca concentration in body fluids and then carried through the circulation system to other organs, such as the mantle. When the Ca content from the environment will enter the circulatory system, it will experience reabsorption in the organ. Heavy metals inhibit Ca uptake in organs, one of them is mantle (Sauer & Watanabe 1988). There is competition between heavy metals and Ca at the same binding site, which is on the mantle.

Heavy metals in invertebrates affect the pumping system to Ca in the plasma membrane (Viarengo et al 1988). It will lead to a competition which causes the decrease in Ca pump capacity, either out or into the cell. The high content of Ca in the cell cytoplasm is the response of cell physiology to restore Ca in the cell. In this process, the Ca content in the mantle will increase over time so that it will cause reversible disorders.

Antique ark clams require the same temperature conditions as in their natural habitat. This allows the depuration mechanism to be carried out in conjunction with postharvest activities in a way that conditions are set to resemble their natural habitat.

Water salinity during the depuration process ranges from 28.3 to 29.8 ppt. The decreasing of water salinity increases the toxicity of heavy metals because heavy metals become more soluble (Gupta & Singh 2011). Heavy metals dissolved in water easily enter the body.

The pH of the water in the antique ark clams depuration treatment can be seen in Table 4. The pH values obtained ranged from 7.2 to 7.7. The decrease in pH value can be caused by the presence of the clams' metabolic process which produces ammonia. The pH value of the depurated water to the clams is still within the threshold where they usually live.

The DO values before and after depuration can be seen in Table 4. The DO values before depuration ranged from 4 to 4.4 mg L^{-1}. DO value of water after depuration is 4

mg L^{-1}. The decreasing of DO values can be caused by the process of clams' metabolism which requires oxygen. The DO value of the water itself is still in the range of DO values where the clams can live.

The level of TSS in the depuration process has a higher content of each treatment. It can be seen in Table 7 where the highest treatment is found in P3 which is equal to 146.67 mg L^{-1}. The high content of Ca in the waters is thought to be the cause of high TSS level. An increase in TSS level itself is the result of destabilization of colloidal particles due to excessive doses (Akhtar et al 1997). This restabilization is a process of reversing the load of colloidal particles which in general, almost all colloidal particles in the waters which were initially negatively charged become positive. It becomes an effect of excessive doses which result in a repulsive force between colloidal particles because they are of the same charge. Therefore, it cannot form larger floc and cause an increase in TSS level in the sample.

Comparison of TDS values in each treatment shows that the TDS value is very high even though it had decreased after depuration. The highest TDS post-depuration value is found in treatment P1 (filter 25%) 27660 mg L^{-1}. TDS is usually caused by dissolved organic materials in the form of ions that can be found in the waters. The increase in TDS value has a negative effect on water quality which can result in the death of antique ark clams.

Conclusions. Histopathological features of the antique ark clam's organ tissue (*Anadara antiquata*) found are edema, hyperplasia, necrosis, and atrophy. According to the results of the Kruskal-Wallis statistical analysis, there are no significant differences between treatments. It means that there treatments has no effect on histopathology of the shells.

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