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Survey of Hawksbill Turtle (*Eretmochelys imbricate*) Health Condition in Terms of Parasites and Microbes in Alas Purwo National Park, Indonesia

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ABSTRACT

Indonesian waters have six types of turtles that can live, spawn and breed. Sea turtle conservation becomes an important and urgent program to be done in order to protect and save sea turtle population in Indonesia. One of the factors that most affect the turtle population is the cause of degradation of pathogenic factors. Alas Purwo National Park, East Java, there is some communities that have activities turtle conservation. Conservation is done by securing and protecting turtle eggs. Turtle eggs that have been hatched are released into the sea once it is ready. This study aims to determine the type of bacteria and fungi infecting hatchlings and environmental factors that influence. This research is descriptive method to Hawksbill turtle (*Eretmochelys imbricate*) is by way of random sampling. Sampling of microbes in sea turtle was conducted using cotton swab method and then microbes were cultured and indentified in laboratory. The results showed the kind of parasites and microbes which were indentified in hatching and adult Hawksbill sea turtles were fungus with genus *Aspergillus* sp., *Geotrichum* sp., *Fusarium* sp., and *Gliocladium* sp.; bacterias are *Pseudomonas aeruginosa* and *Enterobacter cloaceae*; and parasite is *Chelonibia testudinaria* barnacles. The parameter average value of water in pond indicated 28.1-29.2°C for temperature, 32-34‰ for salinity, 7.78–8.2 for pH, and 3.86–4.21 mg/L for DO.

Keywords: Parasites, microbes, turtle, environment.

INTRODUCTION

Indonesia is an archipelago with 70% consists of marine and consists of 15,508 islands. It has the biological resources that are invaluable. Indonesian water is a unique region in the world, where coastal and ocean Indonesia has a strategic geographical location (Solihin et al*.,* 2016). This is evident from the presence of sea turtles in the world, recorded in the waters of Indonesia, there are 6 of the 7 species of sea turtles in the world. There are six types of turtles: 4 species of them: green turtle (*Chelonia mydas*), hawksbill (*E. imbricata*), olive ridley turtles (*Lepidochelys olivaceae*) and leather backs (*Dermocelys coriaceae*). Has been known to breed in Indonesia, while other species, logger head (*Caretta caretta*) all agedly also breed here. The sixth type, flat turtle (Natatorepresus) known to breed only in Australia, but it has been observed foraging in the waters of Indonesia (WWF, 2015).

At this time, turtle conservation efforts become important and urgent program to be done in order to protect and save the sea turtle population, especially in Indonesia. It is a habitat and nesting place for six of the seven species of sea turtles that still exist in the wild today. Given the presence of turtles

in the sea has long been end angered, then the national government to give status to the turtle as an animal protected by the State as stated in Law No. 5 of 1990 on Conservation of Natural Resources and Ecosystems and Government Regulation No. 7 of 1999 on Preserving types of Plants and Animals (Department of Marine and Fisheries, 2009).

Fungi and bacteria become one pathogen that can cause health problems for sea turtles. Increased disease in turtles related to the environment and a place of life. Factors that affect the area where live coral and toxins from algae blooming. Besides the influence of pesticides, contamination from industry and climate change also affect patterns of life and health of sea turtles. How to control the health of the turtle depends on the method and procedures used (Herbest and Jacobson, 2003).

The bacteria can cause several diseases including ulcerative Traumatic disease, bronchopneumonia and Ulcerative stomatis, diseases are caused by the bacterium *Vibrio alginiolyticus, Aeromonas hydrophia, Pseudomonas spp. and Flavobacterium*. Symptoms of the disease can be seen in hatchlings aged 5 to 9 weeks (Glazebrok and Campbell, 1990).

From the above explanation, it is important to do research on the identification of the bacteria on the hawksbill in the National Park Alas Purwo.

MATERIALS AND METHODS

Material Research.

The tools used in this study are a conical tube, pH meter, saline meter, DO meter, digital thermometer, cameras, GPS, cool box, metre ruler, Petri dishes, test tubes and measuring cups. Materials used in this study is the hawksbill, cotton swabs, paper labels, ice cubes, TSA, Xylene.

Research Methods.

This study used a sample analysis by in-situ both water quality and making the bacteria carried in the study site is in the National Park Alas Purwo, Banyuwangi, while analysis of samples of ex-situ i.e bacteria samples analyzed in the laboratory Diseases and Fish Health Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang. Water quality measurement and test bacteria include in-situ measurement methods. Bacterial identification was done in a laboratory.

Ex-situ conservation deals with protection of biological diversity components outside their natural habitats. It is the process of protecting an engangered species of plant or animal by removing part of the population from a threatened habitat and placing it in a new location, which may be a wild area or within the care of humans (Borokini, 2013).

Research Procedure.

Sampling.

The first thing to do when going to take a sample is to determine what samples to be taken. The samples to be taken must be able to represent and cover all aspects related to the research topic. In this study, samples taken are in the form of maintenance of pool water, seawater and hawksbill were taken by random sampling. Random sampling is a simple method in sampling a population that is done randomly without regard to strata that exist in the population (Creswell, 2014).

Currently on turtle sampling required by special methods because turtles including protected animals. For a sampling of animals that protected one of them is with the swab method (Govindarajulu and Schwantje, 2008). The principle of the method is scraping swab the cotton swab on the surface of tortoiseshell and then inserted

into Na physiological solution. Na physiological was used because it contains an electrolyte solution that is capable of maintaining fluid balance inside and outside cells.

Bacteria cultured and Identification.

Sampling of bacteria in sea turtle was conducted using cotton swab method and then microbes was cultured in Trypticase Soy Agar (TSA) with NaCl, Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) agar, Plate Count Agar, Potato Dextrose Agar (PDA) and indentified in laboratory. Samples of bacteria were taken from gross lesions and cultured on a variety of selective and non-selective media. All cultures were incubated at 25°C aerobically.

The identification of a bacterial species is based on many factors, including cell and colony morphology, chemical composition of cell walls, biochemical activities, and nutritional requirements. In order to begin identifying a bacterial species must start with a pure culture (Christopher and Bruno, 2003). Once a pure growth had been obtained, bacteria were identified based on the biochemical profile, vitek®2 machine (BioMérieux), and PCR analysis.

Measurement of Environmental Parameters.

Data retrieval is performed to determine the environmental parameters of water quality and sea water pool maintenance. Environmental parameter measurement data is then used to analyze the relationship between environmental factors with an abundance of bacteria in the water pool maintenance and the hatchlings body. The waters parameters were measured are temperature, salinity, pH and DO (Dissolved Oxygen).

According to research of Bramha et al. (2011), collection water were made during the turtle breeding. Surface water sample were collected using a clean plastic bucket and stored in acid cleaned polythene bottles. The water quality paramater like temperature, pH, salinity, conductivity, turbidity, Dissolved Oxygen (DO) were measured immediately at onboard using batery operated water quacily checker.

The environmental parameters are very important to species of life. According to Purcell (2005), temperature and salinity affect asexual reproduction rates directly through metabolism and indirectly through prey capture.

Data Analysis.

Data analysis was performed with descriptive method, by displaying data in the form of images so as to produce information about the condition of hawksbill (E. *imbricata*) are exposed to the bacteria at the Alas Purwo National Park, Banyuwangi.

Data analyze for descriptive method use qualitative or quantitative data and in an inductive or deductive way. When using content analysis, the aim was to build a model to describe the phenomenon in a conceptual form. Both inductive and deductive analysis processes are represented as three main phases: preparation, organizing and reporting (Elo and Helvy, 2008). The analysis performed in this study is the analysis of PCA analysis (Principal Component Analysis) and Pearson correlation analysis.

RESULTS AND DISCUSSIONS

Description Location Research.

Alas Purwo National Park, Banyuwangi which has an area of 42,420 ha. Geographically this area is located at the eastern end of the island of Java with coordinates 8°26'45''-8°47'00'' LS and 114°20'16''-114°36'00'' BT and administratively located in the district and sub-district Tegaldlimo Purwoharjo District Banyuwangi, East Java Province.

Environmental Parameters.

The results of observations environmental parameters measured in situ can be seen in Table 1.

Tabel 1. The Results of Observations Environmental Parameters.

Station	Salinity ($^0/_{00}$)	pH	DO (mg/l)	Temperature (^{0}C)
Pond	33	8.1	5.2	5.2
Sea	32	8.5	5.6	5.6

Data of Observations Bacteria.

Observation of Bacterial colonies.

Macroscopic observations with attention to form colonies, colonies surface, the color of the colonies and the periphery of the colony. The result shown that form of colonies were dots, round, swim, irregular, similar roots and coils. At hatchling hawksbill (*E. imbricate*) bacteria are spherical, the surface intact, fringe curved, yellow and results, the pool water maintenance hawksbill hatchlings (*E. imbricate*) bacteria are spherical, the surface intact, fringe curved, white and the seawater bacteria are spherical, the surface intact, fringe curved, yellow color.

According to Christopher and Bruno (2003), The Gram stain reaction is dependent on the cell wall structure of the bacteria. The cell wall of Gram-positive bacteria is composed of a thick layer of peptidoglycan that surrounds the plasma or inner membrane. In contrast, a thin layer of peptidoglycan and a second phospholipid bilayer, known as the outer membrane, surround the plasma or inner membrane of Gram-negative bacteria.

The observation that cell morphology and cell shape gram staining was observed using a microscope with a magnification of 10x100. At hatchling hawksbill (*E. imbricate*) bacteria are spherical, the surface intact, fringe curved, yellow and staining results are negative, the pool water maintenance hawksbill hatchlings (*E. imbricate*) bacteria are spherical, the surface intact, curved fringes, white and staining results were negative and the sea water is found bacteria are round, intact surface, curved fringes, yellow and staining results are negative.

Calculation of Total Bacteria.

Environmental conditions and the condition of hatchlings influence on the number of bacteria on TSA media. In this study, using a sample of pond water 3 hawksbill hatchlings, hatchlings scales and sea water where the samples tested was the bacteria. Results of the number of bacteria in the pool water hawksbill hatchlings are 167 x 10^8 CFU/ml, hawksbill hatchlings 42 x 10^8 CFU/ml, and seawater 101 x 10^8 CFU/ml. This is in accordance with the opinion of Perez-Ramos and Reynolds (2009), where in the bacterial colony counts the number of colonies of bacteria is best between 30 and 300.

The general ranges in common acceptance for countable numbers of colonies on a plate are 30-300 and 25-250. The originn of those ranges is worth examination. In other words, all plates were counted and each plate's CFU count was used to estimate the original CFU/ml (Sutton, 2011).

Statistical Analysis.

The analysis performed in this study is an analysis of PCA (Principal Component Analysis) and Pearson correlation analysis.

Analysis of Main Kompenen (Principal Component Analysis/PCA).

The results of the statistical analysis of the main components can be seen in Figure 1.

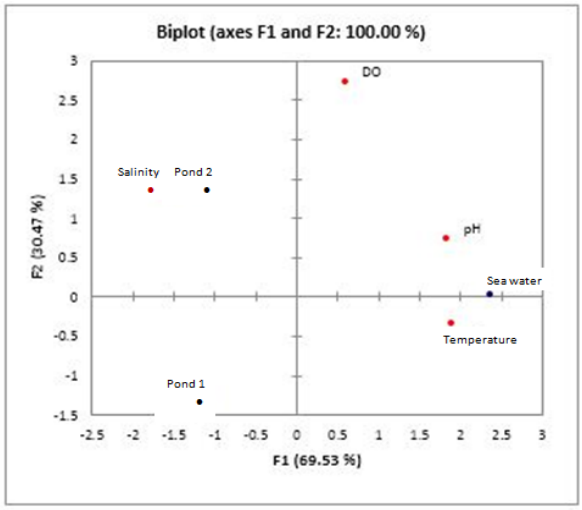

Figure 1. The Results of The Statistical Analysis of The Main Components.

Figure 1 shown that it is known that sea water, DO and pH located in one quadrant is quadrant 1 and 2 and salinity pool located in quadrant 4. While, the temperature is located in quadrant 2, pool 1 is located in quadrant 3. Environmental parameter values and point locations are in one quadrant shows the relationship that is strong enough. Based on the results of the analysis show that the sea water, DO and pH located in one quadrant is quadrant 1, which means the influence of DO and pH of the seawater quite large, as well as with 2 pools and salinity location on one quadrant is the quadrant 4.

While, pool 1 and the temperature can't be an appreciable impact on the value of environmental parameters, because the results of statistical analysis showed the two does not lie in one quadrant.

Correlation Analysis Pearson.

Pearson correlation analysis aims to determine the significance of a variable, which in this study aims to determine the Pearson correlation relationship between environmental parameters and the numbers of bacteria. Values in the table are printed bold indicate that a large influent/stronger of the two variables. The results of the analysis of Pearson in this study can be seen in Table 2.

Table 2. The Results of The Analysis of Pearson.

Variable	Temperature	DO	pH	Salinity
Temperature	**1**	0.196	0.929	-0.926
DO	0.196	**1**	0.545	0.189
pH	0.929	0.545	**1**	-0.721
Salinity	-0.926	0.189	-0.721	**1**

Based on Table 2 note that the values of temperature and pH has a strong influence value, amount to 0.929. The temperature will directly affect the pH value of the water. Temperatures will indirectly affect the pH value of the water. The temperature rises further, the chemical reaction will be faster, while the gas concentration will fall, including oxygen. Value of dissolved carbon dioxide will lead to high pH values down (Casdika, 1998).

Meanwhile, the values of temperature and salinity has a interrelation with each other, amounting to -0.926. The value of high temperature cause of high salinity. According to Reina et al. (2002), salinity affects the biological activity in the osmoregulation process.

CONCLUSIONS

Conclusion.

Based on the results of the research, the bacteria found in hatchlings of hawksbill (E. *imbricata*), the pool water maintenance hawksbill hatchlings (E. *imbricata*) and seawater are gram-negative bacteria. At hawksbill hatchling (E. *imbricata*) bacteria are spherical, the surface intact, fringe curved, yellow and staining results are negative, the pool water maintenance hawksbill hatchlings (E. *imbricata*) bacteria are spherical, the surface intact, curved fringes, white and staining results were negative and the sea water is found bacteria

are round, intact surface, curved fringes, yellow and staining results are negative. Results of water quality of the pool maintenance hawksbill hatchlings (E. *imbricata*) shows the temperature value that is equal to 28.1°C, a salinity of 33 ppt, pH of 8.1 and DO of 5.2 mg/l and the results of sea water quality on show value that is equal to 29°C temperature, salinity of 32 ppt, pH of 8.5 and DO of 5.6.

Suggestions

Suggestions in this study is expected to be capable of doing the handling of diving and turtle eggs because there are four species of turtle with even better treatment in this place.

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