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Comparison of Bacterial Contamination in Sea Turtle Natural and Semi-natural Hatching Nest at Boom Beach Banyuwangi

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Abstract

This study aims to investigate the total number of bacterial contamination in sea turtle natural hatching nest and semi-natural hatching nest. Sand samples were taken from sea turtle natural and semi-natural hatching nest located where at Boom beach, Banyuwangi, East Java Province, Indonesia. Total Plate Count (TPC) method was used in this study. The results show a total of 2,9 x 106 colonies were detected from natural hatching nest, while a total of 2,3 x 106 bacterial colonies were detected from semi-natural hatching nest. This research concluded that bacterial colonies were obtained from natural and semi-natural hatching nests. Furthermore, further studies need to be carried out regarding the isolation of specific bacteria using culture media to determine the species of pathogenic bacteria that have the potential to reduce the success of turtle egg hatching.

Keywords: Infectious disease, Marine biodiversity, Sea turtle, Total Plate Count.

Sea turtle are aquatic animals whose existence is protected due to the many threats from nature to human activities (Harahap, *et al*., 2015). All sea turtle species have been designated by IUCN (International Union for Conservation of Nature) in the Red List (IUCN, 2008), while in CITES (Convention on International Tradein Endangered Species of Wild Flora and Fauna) is listed in Appendix I which means that this species is threatened with extinction (CITES, 2015).

Sea turtle are one type of reptile that can live for a very long time and have a fairly wide distribution around the world. There are seven species of Sea turtle living in the world, six of which can be found in Indonesian waters. The sixth type of sea turtles are Flatback sea turtle (Natator depressus),Green (Chelonia mydas), Hawksbill (Eretmochelys imbricate Linnaeus),Leatherback (Dermochelys coriacea), Loggerhead (Caretta caretta), and Olive Ridley (Lepidochelys olivacea) (Wicaksono, *et al*., 2018).

Sea turtle populations tend to decline with each passing season. Failure to hatch sea turtle eggs is also one of the factors in the decline of sea turtle populations. This can happen due to changes in the environment, predators and microorganism attacks. Sea turtle eggs that are in the sand of the nest have a long incubation period and are very susceptible to microbial attack. Bacteria and fungi have been shown to reduce the success of hatching turtle eggs around the world. The number of microorganisms can affect the success of hatching sea turtle eggs (Estika, 2013).

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The presence of microbes in sea turtle nest sand can affect the egg hatching process. As is known sea turtle eggs have a soft structure and have pores that function for gas exchange and water absorption. Although the pores are important for the survival of the embryo, they are also potentially infected by bacteria and fungi because microbes can enter through the pores and contaminate the egg. Generally the bacteria that infect Sea turtle are *Escherichia coli, Aeromonas, Enterobacter sp., Proteus morganii, Salmonella, Citrobacter, Serratia sp., Pseudomonas, Plavobacterium, Horaxella, Micrococcus, and Vibrio alginolyticus*. But the bacteria that often contaminate eggs are mostly from the *Enterobacteriaceae*-like group *Escherichia coli, Shigella, Enterobacter* and *Proteus*. These bacteria are found in soil, water, air, the digestive tract of humans and animals. (Al-Bahry, *et al*., 2011).

The negative effect of microbial abundance on hatching success has long been presumed, no previous research has ever directly quantified microbial abundance and the associated indirect effects on hatching success at a nesting beach. The particularly high microbial load at beaches and its presumed spatial and temporal variability provides a unique opportunity to investigate sea turtle-microbial interactions during embryonic development (Bezy, *et al*., 2015).

According to Wicaksono (2018), the number of microorganisms that contaminate sea turtle eggs greatly affects the success of hatching eggs. The higher the number of bacterial contamination will cause a decrease in the success rate of hatching on sea turtle eggs. According to Cafarchia *et al*. (2020) Several methods can be used to count or measure the number of microorganisms in a suspension or material, one of which is the calculation of the number of cells using the cup count method. The principle of this method is that if living microbial cells are grown on agar medium, the cells will multiply and form colonies that can be seen directly without using a microscope. The method of fertilizing culture in a cup count is the pour-plate method. If the results of the number of colonies have been obtained, then they are adjusted based on the SPC (Standard Plate Count). Banyuwangi Sea Turtle Foundation (BSTF) is a non-profit organization that seeks to preserve the sea turtle population in Banyuwangi, especially at Boom Beach, Banyuwangi. By using semi-natural nests as a medium for hatching sea turtle eggs, but in 2016 and 2017 BSTF experienced a decrease in the success of hatching olive ridley sea turtle eggs (Praja *et al*., 2023). The hatchability produced did not reach the optimum outcome.

Based on the above background, the researchers wanted to compare the amount of bacterial contamination in natural and semi-natural sea turtle nests in the Boom Beach area, Banyuwangi. This research is the basis which is expected to assist in increasing the success of hatchery in sea turtle conservation efforts at Boom Beach, Banyuwangi.

Materials and Methods

Ethical Approval

The sample in this study was sand used by turtles as nests. Therefore, ethical approval was not required for this study.

Sample Collection

Sampling of sand from natural and semi-natural sea turtle hatchery nests belonging to BSTF located in the Boom Beach area, Banyuwangi District, Java. East. Sampling of sand was carried out on the same beach at different location points. The materials used in this study were natural and semi-natural hatching sand with 50 × 50 cm in surface area and 60 cm in depth using roll meter to measure the depth of the nest as much as 25 g within a high density nesting area using a shovel to dig sand. Where there was presumably a high microbial load in the sand (Bezy, 2015). The sample was then inserted into the sampling bag. Then, put in coolbox which has been sterilized and filled with ice gel. This research was conducted at the Laboratory of the Technical Implementation Unit of Quality Testing and Development of Marine and Fishery Products Banyuwangi, Banyuwangi District. The aspect observed was the comparison of the amount of bacterial contamination in the sand nests of natural and semi-natural hatching Sea turtle.

This study uses a descriptive method based on test results with the method Total Plate Count (TPC) and the conventional pour-plate methods for enumerating microorganisms to count the number of bacteria contained in natural and semi-natural hatching sand.

TPC methode are using 25 grams of sand add into 225 ml of Buffered Peptone Water (BPW) (Merck®) diluent. Which is the first dilution or 10-1, then homogenized with a vortex (Thermo Scientific®) and 1 ml from this dilution taken to be put into a second tube containing 9 ml BPW (Merck®), to obtain10-2 dilution. This method was repeated until a dilution of 10-6 was obtained and 0,2 ml was taken and spread pour plate method in a Nutrient Agar (Himedia®), petri dish using hockey stick. Then, incubated media for 24 hours and can be examined later.

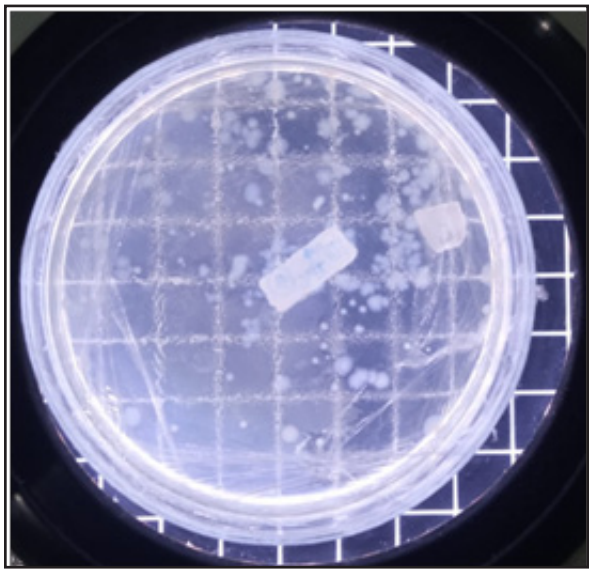

Fig 1. TPC result, the bacterial colonies are white

Results and Discussion

Based on the results of the examination of bacterial contamination in natural and semi-natural sea turtle hatching sand nests in the Boom Beach area, Banyuwangi using the TPC test, a comparison of the number of bacterial colonies from natural and semi-natural sand nests can be seen in Table I.

Based on the results of the TPC test, the comparison of the number of bacterial colonies on sand in natural hatching nests more than semi-natural by the difference 615,000 bacteria. The higher the number of bacterial contamination will cause a decrease in the success rate of hatching on sea turtle eggs. The new experiment told that the bacteria isolated from nest sand and eggs samples were separately counted, the most dominant strains were *Pseudomonas spp.* (41.7%) in egg samples, whereas it was *Bacillus spp.* (46.5%) in nest sand samples (Candan and Candan, 2020).

Bacteria and fungi contamination have been reported to reduce the hatchling success of sea turtle nests worldwide (Bezy *et al.*, 2015; Caron *et al.*,2018; Gifari *et al.*, 2018; Candan and Candan 2020; Hoh *et al.*, 2019). Several bacterial species have even been shown up to 100% nest mortality (Booth and Dustan 2018; Praja, *et al.*, 2021). Several studies have examined the presence of bacteria in association with sea turtle nests. In particular, bacteria and fungi have been cultured and isolated from nest sand and failed eggs (Candan *et al.*, 2021; Gleason *et al.*, 2020; Phillott and Paramenter 2001). According to Samosir *et al.*, (2018), differences in the number of bacterial colonies can be influenced by several factors such as environmental conditions, temperature, humidity and pH. According to Wicaksono (2018), the number of microorganisms that contaminate sea turtle eggs greatly affects the success of hatching eggs.

Soslau *et al.*, (2011) findings that leatherback sea turtle eggs and previous reports with loggerhead and green sea turtle eggs, indicate that eggs laid on beaches by all species of sea Sea turtle are susceptible to lethal microbial infections (natural nest). These microbial infections

Table I. The results of the examination of bacterial contamination in the sea turtle natural and semi-natural hatching sand nest using the TPC method.

Testing Parameter	Test Result	Unit	Test Methods
Microbiology Test TPC Natural	2.975.000	Colony/g	SNI 2332.3:2015
Microbiology Test TPC Semi-natural	2.360.000	Colony/g	SNI 2332.3:2015

are probably major contributors to the natural limitation of viable hatchlings. There are many reports of the use of hatcheries to enhance production of sea turtle hatchlings (semi-natural nest). The hatching failure may be due to the microbial contaminants may be caused by a number of factors. During the hatching process sea turtle, the contaminated cloaca solution makes possible to transfer microorganism into the eggs and natural nesting sand (Gifari, *et al*., 2018). It should also be noted that some environmental factors may influence hatching success of in situ nests, such as humidity, pH, sand type and moisture, predation or the presence of bacteria, insects or fungi (De Andrés, *et al*., 2016).

Conclusion

Based on the research using the TPC method that has been carried out, it can be concluded that there is a difference in the amount of bacterial contamination in natural sand nests with a difference of 615,000 bacteria compared to the amount of bacterial contamination in sea turtle semi-natural sand nests. The semi-natural nesting hatchery thus needs regular sand removals and replacement with new sand or heating the sand to sterilize microorganism. This effort is aimed at increasing the hatchery result and supporting the maximum conservation effort.

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