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BACTERIAL IN VARIOUS DEPTHS OF SEMI-NATURAL NESTS TOWARDS HATCHING RATE OF GREEN TURTLES (*CHELONIA MYDAS*) AT PANGUMBAHAN BEACH, SUKABUMI

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Green Turtle, Hatching Rate, Bacteria, Sea Turtles Nest, Various Depth, Pangumbahan

ABSTRACT

Green turtle (*Chelonia mydas* (Linnaeus 1758)) has been categorized endangered in Red Data Book IUCN and needs to be protected from eggs to adult turtles. The purpose of this research is to get the morphology of bacteria found in sand as hatching media in various depths of green turtle nests and analyze the effect of bacteria on the success of hatching of green turtle eggs at Pangumbahan Beach, Sukabumi. The method used in this research is experimental method and the result data is analyzed descriptively quantitative. The method of identifying bacterial morphology using gram staining and analyzed under a microscope, while for quantitative data using Total Plate Count method. Early pre-incubation bacterial morphology was identified as having a form of cocci, diplococcus, and bacillus in 30cm depth, diplococcus in 40cm depth, and diplococcus, bacillus in 50 depth. Whereas the bacterial morphology after incubation has the form of cocci, diplococcus, coccobacillus in-depth 30cm, coccobacillus and diplococcus in 40cm depth, and then bacillus, diplococcus, and streptobacillus in 50cm depth. All bacteria are known to be gram-negative and aerobic. The success of the hatching rate is influenced by early pre-incubation bacteria (97%) and after incubation (98%) well in the calculated linear regression model.

Introduction

Six types of sea turtles live in Indonesia, which are included in the IUCN Red Data Book, included the green turtle (*Chelonia mydas*) (Nuitja, 1992). Breeding is one way to keep turtles safe from predators. However, unsupportive environmental conditions can also prevent eggs from hatching optimally. According to research conducted by Wyneken et al. (1988), the nests with the lowest hatching rates have the highest number of bacterial microorganisms. This shows that the presence of microbes from the media, which is sand is to be fathomed to be one of the causes of failure of hatching turtle eggs.

Microorganisms can enter and contaminate eggs through egg pores which act as a place for gas exchange and water absorption (Al-Bahry et al. 2011). That way, the bacteria in the egg will develop quickly and cause the eggs to rot quickly (Santoro et al. 2006). Elfidasari (2017) states that the prediction of bacteria plays a role in decreasing the hatching results. This is related to the depth of the semi-natural nest in captivity, because of the increasingly different oxygen content.

Bacteria that live in the soil have different amounts, spreads, and types depending on certain factors. Fierer et al. (2003) stated that along with the increase in soil depth, generally the number and biomass of aerobic bacteria will decrease. The distribution of bacteria in the soil is influenced by soil depth and profile.

Efforts to preserve sea turtles in Pangumbahan Beach must continue to be done to reduce the decline in turtle populations. The purpose of this research is to determine the morphology of aerobic bacteria found in the sand as a hatching medium at various depths of the nest and to find out how much influence the number of aerobic bacteria on the sand on the success of hatching of green turtle eggs.

Materials and Methods

A. Research Place and Time

The research was held from March until May 2019, including data collecting, analysis at a laboratory, and final writing.

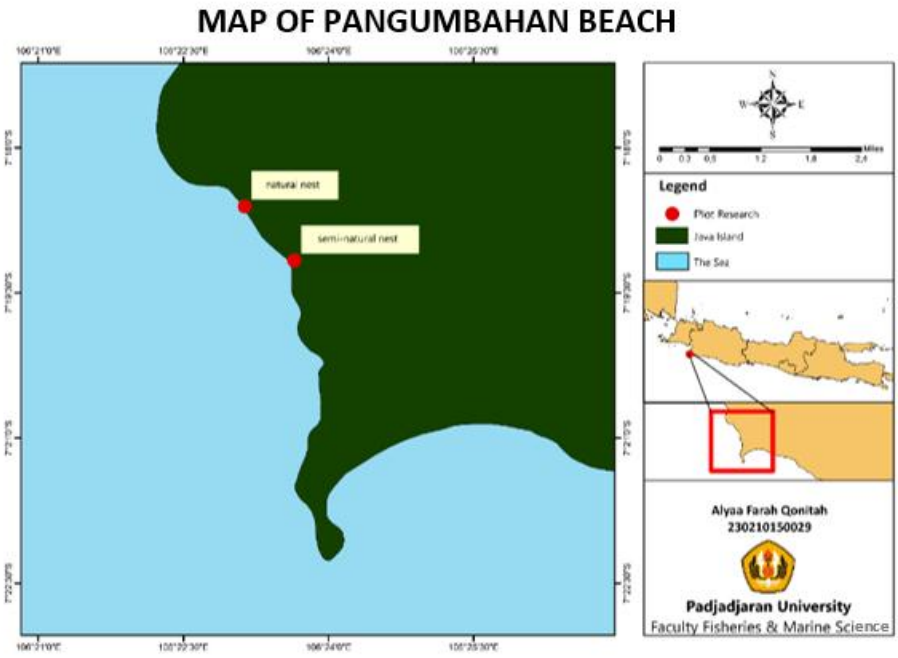


Figure 1. Map of Research Location

B. Research Method

This study uses an experimental method and then analyzed in a quantitative descriptive. The experimental method is carried out by giving experimental treatment in the form of differences depth of semi-natural nest. This difference in nest treatment is done randomly. Then the sand is taken at the beginning (pre-incubation) and end of the egg incubation period. Samples are then taken to the laboratory for bacterial content testing through the process of isolation and morphological identification. Bacterial identification includes gram testing and morphological examination through a microscope. The type of bacterial content in the sand sample will be

identified and its characteristics sought. After that, the data is analyzed using a multiple correlation formula to determine the effect of the number of bacteria and the depth of the semi-natural nest.

C. Media Preparation Procedure

Media preparation is done by making nests with depths of 30cm, 40cm and 50cm with each repetition 3 times. When the mother turtle lands and finishes laying in the natural nest, the eggs are moved to nine semi-natural nests that have been made. Before the semi-natural nest is closed again, each nest is measured by temperature and soil pH. Then the nest is closed and left through the incubation period within 45-60 days.

D. Data Collection Procedure

After going through the incubation period, the eggs hatch and hatchlings will appear to the surface of the nest. Hatch success data is obtained by counting the number of hatchlings that successfully hatched. Then measuring the temperature and pH of the nest again.

E. Sand Sampling Procedure

Sand samples were taken twice at each semi-natural nest, namely at the beginning before the incubation period begins and at the end of the incubation period is complete. The sample is put into a 15ml plastic tube.

F. Isolation and Identification Bacteria Procedure

All samples were taken 1 gram and then made 10 times dilution in a test tube containing 9 ml of sterile seawater. Bacterial isolation was carried out using the spread plate method. Bacterial isolation takes 50 µl at 10 dilutions and is spread on Nutrient Agar media. The cup contains suspense that has been flat incubated for 24 hours.

After 24 hours, the growing bacteria are counted by the TPC (Total Plate Count) method. Then the shape, color, and size of each bacterium were examined and then purification was done by the scratch method. The results of purification were taken 1 colony with an ose needle flattened on a glass object assisted by physiological NaCl and allowed to stand and pass on fire. One drop of gentian violet drops left for 20 seconds and washed with distilled water. 1 drop of iodine solution, let stand for 1 minute and washed with alcohol then distilled water. 1 drop of safranin or fuchsin, leave for 20 seconds and washed with distilled water. Dried and then observed on a microscope with a magnification of 100x objective lens.

G. Data Analysis

Data in the form of the number of bacterial colonies and the success of hatching will be calculated using the correlation and regression formula to determine the relationship and the closeness of the two using Microsoft Excel. The results of morphological identification were analyzed descriptively.

Result and Discussion

Overview of Research Locations

After the mother turtle lays eggs, nine semi-natural nests are made with three different depths of 30, 40 and 50cm. The temperature and pH of the nest are measured and have a range that is not too far away at each depth (Table 1)

Table 1. Nest Parameters Measurement Average.

No	Nests depth	Temperature		pH	
		Initial	Final	Initial	Final
1	30cm	31,3 °C	31,2 °C	8,69	8,64
2	40cm	30,8 °C	30,8 °C	8,71	8,70
3	50cm	30,5 °C	30,8 °C	8,66	8,64

On average, all nest conditions have temperatures between 30-32°C. nest conditions have a pH that tends to be alkaline because it is in the pH range of 8.60-8.75. Marine bacteria live in the pH range of around 6.5-8.5 and grow optimally at pH 7.2-8.2 (Salle, 1961). Kushartono (2014) stated that a good temperature for success during the incubation period is around 25 - 33°C.

The Number of Aerobic Bacteria for Successful Hatching

The result of counting the number of bacterial colonies is used as one of the quantitative data to determine its relationship to the success of hatching. From the calculation results, the results of the correlation coefficient for the initial TPC worth -0.98 and the final TPC worth 0, -99. Because the correlation coefficient obtained is negative (-), meaning that the two have opposite directions. The correlation calculation results show that both the initial and final TPC count results have a close relationship with the success of the hatching rate (HR) because it is in the range 0.8 - 1 (Sugiyono, 2015) (Table 2). The higher the percentage of HR, the lower the amount of TPC.

Table 2. Calculation bacteria of Sample

No	Initial TPC	Correlation	Final TPC	Correlation	*HR*	Depth
1	$87,07x10^{12}$	-0,98	$96,27x10^{12}$	-0,99	71,7%	30 cm
2	$86,67x10^{12}$		$92,13x10^{12}$		75,7%	40 cm
3	$70,6\ x10^{12}$		$81,67x10^{12}$		93,9%	50 cm

The results obtained show that there are differences in calculations from the samples in each depth, the final TPC is more than the initial TPC (Figure 2).

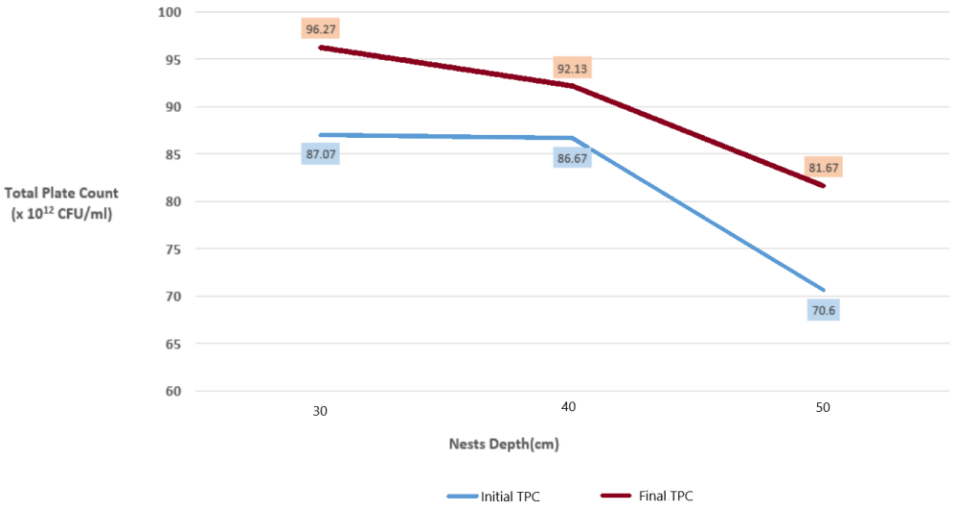


Figure 2. Number of Initial and Final TPC

Referring to Elfidasari (2017), an increase in the number of bacterial colonies in semi-natural nest sand when the incubation period has been completed due to the excavation process that makes microbes dispersed throughout the nest.

In addition, the number of bacteria is getting smaller and deeper. Singh (2015) states that the deeper the soil depth, the number and distribution of aerobic bacteria will decrease, while anaerobic bacteria will dominate. Bhattarai *et al.* (2015), the deeper the soil, the more solid the soil condition is, the less pore space, the reduced oxygen content of the soil and the level of water infiltration and percolation.

Whereas to find out the magnitude of HR affected by initial TPC is obtained by regression calculation so that it counts 0.97 (Figure 3).

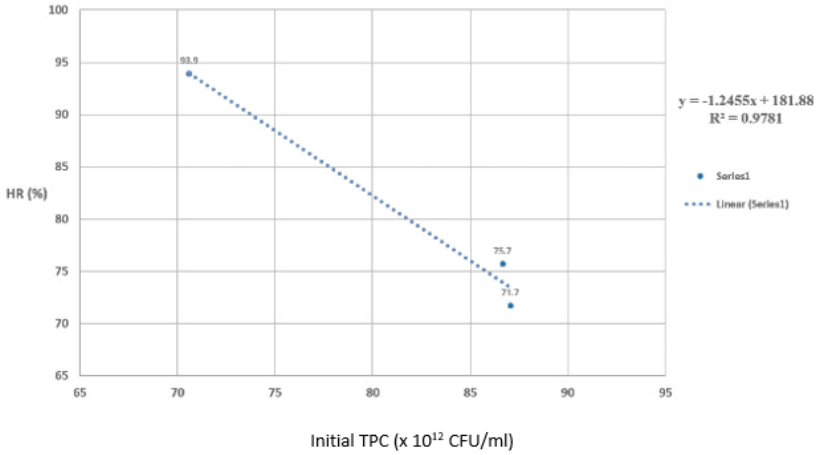


Figure 3. Early TPC Regression on Successful Hatching

In contrast to the initial TPC, HR is influenced by the higher final TPC, which is 0.98 (Figure 4).

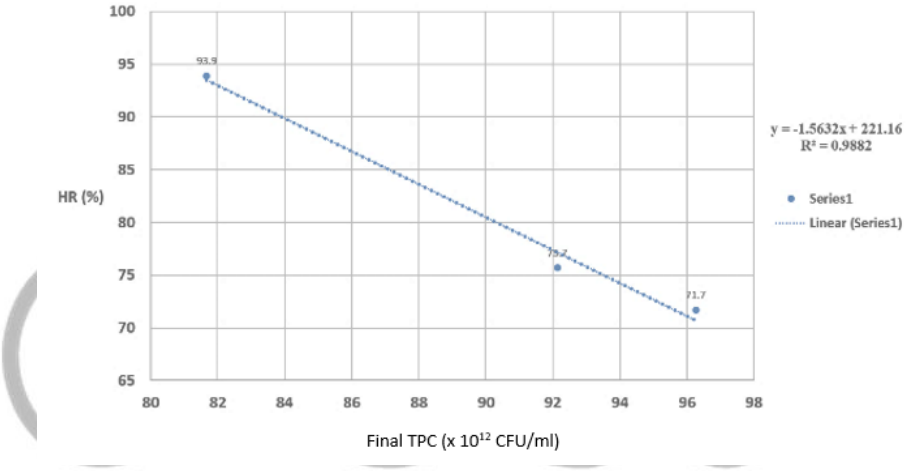


Figure 4. Final TPC Regression for Successful Hatching

Both the initial TPC and the final TPC, have a linear regression model with a high enough value because it is close to 100%, which means the perfect linear model.

Early bacterial identification

From each depth od nests, 10 isolates were identified that had different colony morphologies. Overall, the shape of the circular colony, flat edges, and convex elevation angles. But colors and sizes vary from white, cloudy white, yellowish-white, and murky yellow (Table .3)

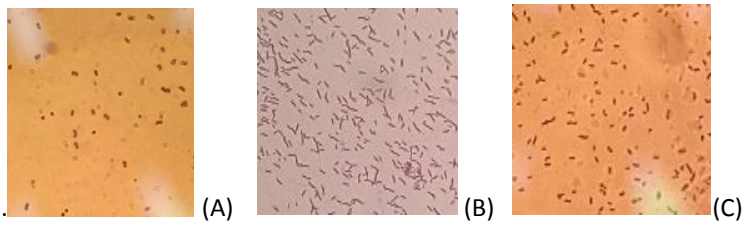
Table 3. Morphological Characteristics of Early Bacteria Samples

No	Isolate	Color	Size
1	30(a).B1	Yellow	*Small*
2	30(a).B2	Murky white	*Small*
3	30(a).B3	Murky white	*Moderate*
4	40(a).B1	Yellowish white	*Small*
5	40(a).B2	Yellowish white	*Small*
6	40(a).B3	Yellowish white	*Moderate*
7	40(a).B4	Murky yellow	*Small*
8	50(a).B1	Murky white	*Small*
9	50(a).B2	Murky white	*Moderate*
10	50(a).B3	Murky white	*Small*

Furthermore, the identification of gram bacteria and all gram-negative are known. Fardiaz (1989) in Hidayat (2014), the cell

wall of gram-positive bacteria contains peptidoglycan which is soluble due to alcohol and causes purple crystals to survive during coloring. Then Pelczar (1986) states that gram-negative bacteria generally have a cell wall composition in the form of a higher lipid content compared to gram-positive bacteria.

When observed under a microscope, several forms of bacterial cells were obtained, namely coccus (A), bacillus (B), and diplococcus (C) (Figure 5). At a depth of 30cm, all three forms of bacteria were found. At a depth of 40cm diplococcus was found only, while at a depth of 50cm found diplococcus and bacillus.



(A) (B) (C)

Figure 5. Early Bacterial Cell Forms

Fierer *et al.* (2003) revealed the type and dominance of microbial groups are known to differ at each soil depth. Based on Bergey's Manual of Determinative Bacteriology, Eighth Edition, Pelczar (1986) classifies aerobic, gram-negative, bacillus and coccus bacteria.

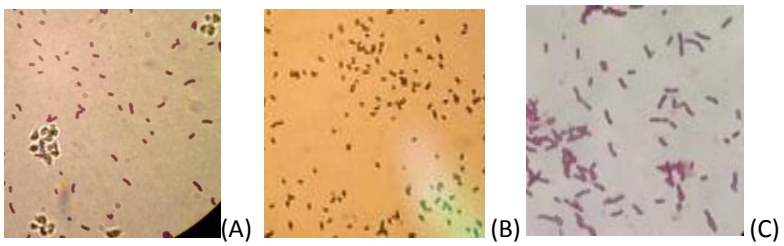
Final Bacteria Identification

In sand samples after the incubation period ends, each depth obtained 11 different colonies. All colonies are circular and have flat edges. In general the convex elevation angle, but there is a flat raised. The size starts from a point, small, and moderate. Whereas white, yellowish-white, clear white, and clear yellow (Table 4).

Table 4. Characteristics of Bacteria Colonies for Final Samples

No	Isolate	Color	Size
1	30(b).B1	White	*Small*
2	30(b).B2	White	*Moderate*
3	30(b).B3	White	*Moderate*
4	40(b).B1	White	*Moderate*
5	40(b).B2	Yellowish white	*Moderate*
6	40(b).B3	Clear white	*Small*
7	40(b).B4	Clear yellow	*Small*
8	40(b). B5	White	*Point*
9	50(b).B1	White	*Small*
10	50(b).B2	White	*Small*
11	50(b).B3	Yellowish white	*Small*

Eleven isolates were gram stained and found to be all gram-negative. Whereas benthic bacterial cells are found in the form of diplococcus (A), coccus (B), coccobacillus (C), streptobacillus (D), and bacillus (E) (Figure 6.



(A) (B) (C)

 (D)  (E)

Figure 6. Shape of the Final Bacterial Cell

At a depth of 30cm diplococcus, coccus, and coccobacillus were found. At a depth of 40cm found coccobacillus, diplococcus, then at a depth of 50cm found streptobacillus, diplococcus, and bacillus. Referring to Bergey's Manual of Determinative Bacteriology Eighth Edition in Pelczar (1986), there are groups of coccobacillus and gram-negative and aerobic coccus.

Elfidasari (2017) found an aerobic bacterial colony similar in morphology to the one tested, which was later identified as *Escherichia coli.* Hidayat (2014) obtained aerobic gram-shaped stem cells and were gram-negative. In his research, bacterial colonies found in sand samples with eggs failing to hatch have a similar morphology. Circular, clear and milky white, and convex which were later identified as *Bacillus cereus, Shigella* sp., and *Salmonella* sp.

Hatching Success

On March 30, 2019, at 21.05-23.10 a parent was laying eggs. The nesting area is at coordinates -07°32'3" South and 106°38'9" East and brought to the semi-natural nest place which is 1.15km. The eggs are put into nine nests with 11 eggs each. The incubation period ends on May 16, 2019, lasting for 47 days. The incubation period is quite fast because it generally takes around 54-59 days (Ridla, 2007).

From all eggs, 80 hatchlings successfully hatched and appeared on the surface, then calculated the HR of each nest (Table 5).

Table 5. Success of Hatching

Number of Nest	Nests Depth (cm)	*HR*
1	40	72,7%
2	30	100%
3	50	81,8%
4	40	54,5%
5	30	81,8%
6	50	100%
7	40	100%
8	30	33,3%
9	50	100%

In the second nest which has a depth of 30cm, although hatchlings that have succeeded in hatching have a perfect percentage, hatchlings grow to an abnormal size, which is smaller than normal. The eighth nest has the lowest HR compared to the others, this is presumably due to the discovery of red ants in the nest.

Too few eggs may contribute to the success of the turtle hatchery. In the study of Ridla (2007) and Rudiana (2012) included a higher number of eggs, 50 eggs in each nest. In general, the success of hatching increases if the nest of the egg gets deeper. After calculating the Pearson Product Moment Correlation method, a value of 0.93 (Table 6) was obtained.

Table 6. Correlation of Nest Depth and Success of Hatching

No	Nests Depth (cm)	Hatching Rate	Correlation
1	30	71,7%	0,93
2	40	75,7%	
3	50	93,9%	

The correlation coefficient obtained is 0.93, including very strong because it falls into the range 0.8-1 (Sugiyono, 2015). Positive coefficient values indicate the relationship between two variables in the same direction. Whereas the magnitude of influence known through regress is shown by the determinant coefficient value, R^2 of 0.88 (Figure 7). This shows that the y factor (HR) is influenced by x factor (nest depth) of 88%.

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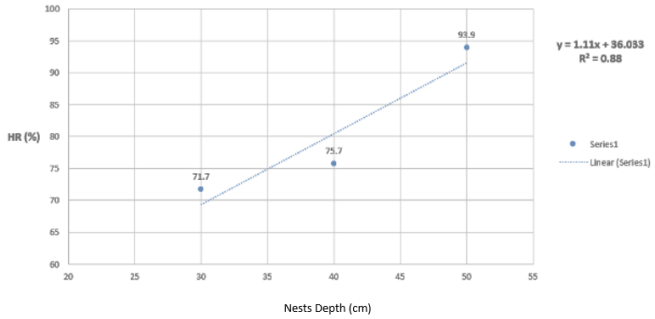

Figure 7. Sarang Depth Regression for Successful Hatching

Kushartono's research (2014), the best HR value is in the nest of 40cm and 80cm. Based on research by Leni (2017) and Kushartono (2014), the depth of the nest influences the length of incubation of turtle eggs but does not have much effect on the success of hatching.

In addition to depth, bacteria are thought to play a role in hatching success. Calculation of the correlation between TPC and hatch success provides a strong estimate of the influence of bacteria present in the nest egg. Hidayat (2014) found pathogenic bacteria in turtle nests that had failed to hatch. Santoro et al. (2003), Elfidasari (2017) found bacteria in green turtle nests. This discovery estimates of the influence of bacteria on the success of hatching turtle eggs.

CONCLUSIONS

Based on research that has been done, it can be concluded that

1. Found bacteria form cells of coccus, diplococcus, and bacillus at a depth of 30cm, diplococcus cell form at a depth of 40cm, and diplococcus and bacillus at a depth of 50cm. In the final sample found bacteria from coccus, diplococcus, and coccobacillus at a depth of 30cm, coccobacillus and diplococcus at a depth of 40cm, and bacillus, diplococcus, and streptobacillus at a depth of 50cm
2. The success of hatching turtle eggs is influenced by 97% of the number of initial aerobic bacteria and 98% of the number of final bacteria

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