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Hatching Failure and Success of Hawksbill Sea Turtles (*Eretmochelys imbricata*) in Semi-Natural Nests Karimunjawa

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Abstract. Hatching success and failure is influenced by the interaction between biotic and abiotic factors during the incubation period. During the eggs incubation period in non-optimal nest are susceptible to bacterial contamination affecting hatchling morphology. The purpose of the study is to understand the relationship between environmental conditions of semi-natural nest with bacteria contaminants, hatchlings allometry and abnormality. The method used was quantitative descriptive which are nest temperature and humidity measurement, allometry measurement of hatchlings, observation of Gram negative bacteria using EMB (Eosin Methylene Blue) agar, TPC (Total Plate Count), bacteria characteristic morphology and observation of hatchling abnormality. The result showed that semi natural nest have high temperature (>31˚C) and high humidity (>70%) which caused smaller hatchlings and negative allometry, hatchling abnormality and bacterial contaminants in the eggs. TPC showed that the number of negative bacteria in unhatched eggs (1.42×10^7) are higher than in hatched eggs (1.03×10^7). The bacteria colonies were suspected to be from the *Enterobacteriaceae* family according to microscopic and macroscopic morphological characteristics. Macroscopically, the of the bacterial colony is dominated by a round shape, concentric surface, flat edges, flat elevation and pink color, while the microscopic form is bacilli. There is significant correlation between environmental conditions of semi natural nest with hatchlings growth, hatchlings abnormality and eggshells bacteria. This research contributes to the ecological conservation of endangered species, especially in discovering the factors that cause hatchling failure of hawksbill turtles (*Eretmochelys imbricata*) from microbiological and morphological aspects.

Keywords: Abnormality; Allometric; Bacterial Morphology; *Enterobacteriaceae*

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INTRODUCTION

The decline in sea turtle populations is due to various human activities, unsustainable utilization, predation, disease, pollution and natural factors. These impacts can affect the success and failure of egg hatching and reduce the number of hatchlings released into the wild. According to Mazaris et al. (2017) sea turtles are vulnerable to extinction due to overexploitation factors (e.g., fishing and hunting), habitat loss, disease and climate change. According to Miguel et al. (2022) climate change causes an increase in temperature in semi-natural nests, which lead to failure of embryo development, and a longer incubation period. It is difficult to increase the percentage of sea turtle population as a migratory biota, so conservation is needed starting from the incubation period until hatching.

Hatching success and failure are influenced by the interaction between biotic and abiotic factors during the incubation period. Biotic factors are bacterial contamination of the eggs or nest. Abiotic factors are temperature and humidity of the nest sand (D’Alba, 2021). Semi-natural nest environmental conditions that are not optimal are susceptible to bacterial contamination of the eggs during the incubation period, causing hatching failure and leading to damaged and rotten eggs. According to Nursanty et al. (2019) the influence of the semi-natural nest environment can affect microbial growth in turtle eggs. Bacteria on eggs are toxic to the embryo and interferes with the development during the incubation period.

The environmental conditions of the nest during incubation affect the size and morphology of hatchlings after hatched. Hatchlings with smaller weights are more vulnerable to predators than those with more body weights. According to Banerjee et al. (2020) a heavier body size or weight has a greater chance of survival to avoid predators. Extreme environmental conditions are indicated by the presence of malformations. This makes hatchlings born with abnormalities or malformations of their body and unable to survive in the wild. According to Compo et al. (2018) embryonic development in sea turtles depends on environmental conditions in which affects either normal or abnormal development. Other than embryos, hatchlings can also experience abnormalities in their body parts.

Conservation of sea turtle bio-resources is one of the strategies to prevent sea turtle extinction caused by anthropogenic and natural factors. Karimunjawa National Park is a conservation area and habitat for green turtles (*Chelonia mydas*) and hawksbill sea turtles (*Eretmochelys imbricata*). Karimunjawa National Park's conservation activities to preserve sea turtles from extinction is semi-natural hatching. According to Wyneken and Salmon (2022), conservation is implemented to emphasize factors that affect all sea turtle species, as well as factors that occur in their environment. Karimunjawa National Park semi-natural hatchery relocates eggs from natural nests to semi-natural nests. Semi-natural hatcheries are established to reduce threats to the eggs (Sumaryati and Kuswadi, 2017).

There are potential threats to eggs and hatchlings that require special attention during the incubation period. According to Gleason et al. (2020) hawksbill sea turtle eggs and hatchlings are facing several threats in their lives, such as predators, microbes on eggs that cause rotting and nest condition factors that can affect the failure and success of hatching in semi-natural turtle nests. It is thus important to perform further research on the failure and success of hatching eggs and hatchlings during the incubation period associated with semi-natural nest condition factors.

This study aims to determine the relationship between semi-natural nest conditions with hatchling failure caused by the potential contamination of total abundance of Gram-negative bacteria on the shells of turtle eggs that failed to hatch, hatchling abnormality and relationship with hatching success, such as allometric growth of hatchlings after hatching. This research contribute in ecological conservation of endangered species, especially in discovering the factors that cause hatchling failure of hawksbill turtles (*E. imbricata*) from microbiological and morphological aspects.

METHODS

This study used quantitative descriptive methods through observation, measurement, correlation and descriptive. Samples were collected from three semi natural nest after hawksbill sea turtle eggs after hatched and emerged. Data were obtained from temperature and humidity measurements of semi-natural nests, microbial observations of hatched and unhatched eggshell samples, allometric measurements of hatchling length and weight and observations of hatchling abnormalities after hatching. Data obtained were correlated with data on nest environmental conditions.

Temperature and Humidity Semi – natural Nest

Nest temperature and humidity measurements were taken twice a day between 09:00 - 10:00 am and 12:00 - 13:00 pm using a hygrometer. Nest temperature an humidity was measured by placing hygrometer in a semi-natural nest with the same nest depth in the center of the egg pile. Measurements were taken during the incubation period of hawksbill sea turtle eggs until the eggs hatched. According to Clabough et al. (2022) temperature monitoring can provide information on temperature fluctuations in semi-natural nests that have an impact on egg development and the hatching period of sea turtle eggs. According to Matthew et al. (2021) moisture is measured by digging a semi-natural nest and inserting a hygrometer moisture tool into the sand. Semi-natural nest moisture and temperature measurements below the surface are expected to represent the conditions in the nest.

Allometric of Hatchlings Hawksbill Sea Turtle

Length measurements of hatchlings were measured using a caliper with an accuracy of 0.01 mm and weight measurements W (Weight) using digital scales with an accuracy of 0.01 g. Morphometric measurements of hatchlings were taken linearly on length measurements SCL (standard carapace length), CW (Carapace Width), PW (Plastron Width), SPL (Standard

Plastron Length), FLL (Forelimb Length), HLL (Hindlimb Length) and BD (Body Depth) can be seen in Figure 1.

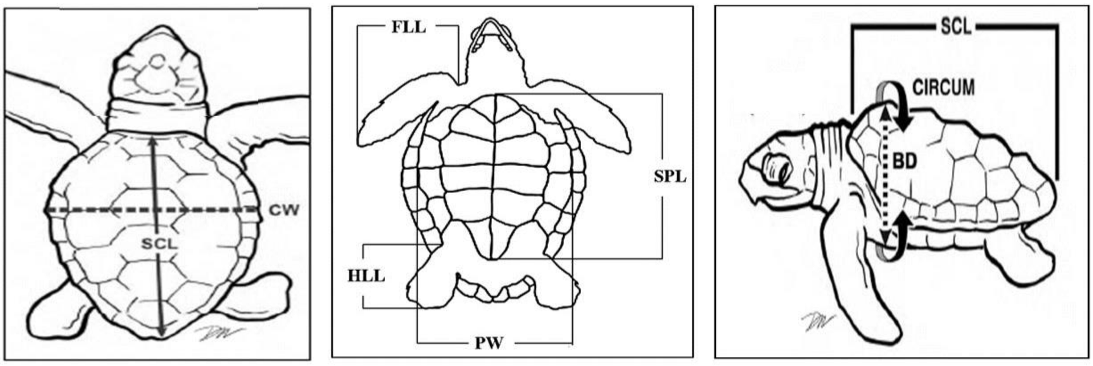

Figure 1. Morphometric measurements of hatchlings hawksbill sea turtle (*E. imbricata*). (Wyneken, 2001)

Data analysis of allometric growth in hatchling body parts can be estimated through non-linear regression equations, using the formula (Karyati et al., 2019):

$$Y = a(X)^b$$

where Y is the dependent variable, X is the independent variable and a and b are the slope and intercept of the regression graph obtained from the logarithmic equation:

$$\text{Log } Y = \text{Log } a + b \text{ Log } X$$

b describes the isometric growth of the two variables being compared and is expressed as the theoretical number β (Syuhaida et al., 2019). If b > β means Y grows relatively faster than X and the growth pattern is categorized as positive allometry. If b < β which means Y grows relatively slower than X, then the growth pattern is negative allometry. If b = β or isometric, it means that variables Y and X grow with relatively the same growth rate. The value of β = 3 if weight (Y) is compared to length (X), but if length is compared to weight then β = 1/3, β = 1 if length (Y) is compared to length (X). Comparisons using t-test ($p < 0.05$) calculated with Microsoft Office Excel software and statistical tests to determine deviations from isometric growth patterns of the variables being compared:

$$\text{tobs(n-1)df} = \frac{(b-\beta)}{Serror\ b} \text{ (Afiati, 2005)}$$

Bacterial Isolation

The observation of bacterial samples were hawksbill sea turtle (*E. imbricata*) eggs from failed and successfully hatched eggshells in semi-natural nests. Capri *et al*. (2023) explained that eggs are opened using a scalpel to separate the contents and eggshells. According to Guzman et al. (2020) egg samples that failed to hatch were cleaned of sand using aquabides. Egg samples were pounded using a sterile mortar and pestle until one gram of wet eggshell sample was obtained.

Dilution was made by taking a sample of 1 ml of solution that had been homogenized with one gram of wet eggshell, sample was put into a test tube containing 9 ml of 0.9% NaCl diluent solution and dilution was made until 10^{-5}. Bacterial isolation using spread method on EMBA (Eosin Methylen Blue Agar) media and *bacterial isolate* was made in *duplo*. According to Chuen et al. (2019) in their research, using selective media EMBA (Eosin Methylene Blue Agar) for growing Gram negative bacteria, especially from the Enterobacteriaceae that often contaminate seaturtle eggs. Samples were streaked using a spreader rod and incubated for 24 hours at 37°C. When several different types of colonies were obtained, isolate purification followed, as according to Nursanty et al. (2019)

TPC (Total Plate Count) is a technique used to count the number of bacterial colonies in the culture results after incubation is finished. The number of microbial colonies from the sample is calculated using the following formula:

$$\text{CFU/ml} = \sum \text{colonies per Petri } \times \frac{1}{\text{dilution factor}} \text{ (Speck, 1976)}$$

Macroscopic observations were examined directly on the morphology of bacteria that grew on petri dishes. Macroscopic characteristics can be seen from the shape of the colonial margin (entire, undulate, filamentous, lobate, circular, serrated), colonial surface (concentric, radiated, contoured, smooth, wrinkled), colonial edge (circular, irregular, pintpoint, filamentous, rizhoid), colonial elevation (convex, raised, flat, umbonate, pulvinate) and colony color (white, beige, yellow, black, gray, red, green, and clear). According to Silaban et al. (2020) morphological observations of each isolate include color, shape and edge of the colony observed from above petri dish, while the surface of the colony is observed from the side petri dish.

Microscopic observation of bacteria was using a microscope after Gram staining. Kusdarwati et al. (2021) the bacterial isolate was tested for Gram staining which aims to determine the shape of the bacteria during observation in a microscope with a magnification of 1000x using emersion oil. According to Dinata et al. (2021) Gram positive bacteria are bacteria that retain a crystal violet color when stained. Gram negative bacteria are bacteria that do not retain violet color and will turn red when stained. There are three basic forms of bacteria microscopically, such as cocci, bacilli and spiral. According to Gufe et al. (2019) bacteria are observed for morphological characteristics, Gram staining and bacterial morphology.

Hatchlings Abnormality

Abnormalities were directly observed on the morphology of the hatchlings after emerged from the surface of the semi-natural nest. Body parts of the hatchlings were observed with criteria such as carapace shape, head shape, number of flipper, number of eyes, number of nails, beak shape, and presence of pigmentation abnormalities. According to Köhnk et al. (2021) the most common abnormality is scutes on the carapace. All hatchlings in the nest with abnormalities were documented. According to Compo et al. (2018) analyzed abnormality data by describing some of the abnormalities encountered in sea turtle hatchlings, possible causes of abnormalities associated with nest environmental condition data and some actions to reduce abnormal development in hatchlings.

RESULTS AND DISCUSSION

Temperature and Humidity Semi – Natural Nest

The success and failure of Legon Janten Karimunjawa semi-natural nests is influenced by the physical conditions of the nest, such as temperature and humidity. The samples of semi-natural nests of hatchlings hawksbill sea turtle (E. *imbricata*) obtained were three nests, Nest I (n = 70 eggs), Nest II (n = 79 eggs) and Nest III (n = 80 eggs). The average temperature and humidity of each nest has a different range in the semi-natural turtle hatchery, the result can be seen in Table 1.

Table 1. Average temperature and humidity of semi-natural nests

Nest	Temperature (°C)		Humidity (%)	
	09.00 – 10.00	12.00 -13.00	09.00 – 10.00	12.00 -13.00
I	32.8	33.9	86	79
II	32.6	33.8	86	80
III	32.4	33.2	87	83

The temperature in Nest I is higher than Nest II and Nest III, this is because according to Topping and Nicole (2021) the temperature difference in each nest is due to the difference in sunlight intensity received by the surface of the nest, meaning that Nest I gets more sunlight than Nest II and Nest III. The location of the semi-natural nest also affects the amount of light intensity that enters, this is because Nest I is in an exposed areas close to the window while Nest II and Nest are in shaded areas receiving less sunlight. This is confirmed by Camargo et al. (2020) the placement of the nest location affects the hatchings success by the environmental conditions of the nest (humidity and temperature). The results of this study are relevant to Sullivan et al. (2022) research that high average temperatures are associated with open nest locations or high radiation intensity at the nest site, while lower temperatures are associated with shaded nest locations, or locations exposed to less sunlight.

Sand temperature affects sand moisture, such as if the sand temperature is high, then if the sand temperature is high then it is followed by low sand moisture. This is supported by Muliani et al. (2022) an increase in temperature will induce evaporation and have an impact on decreasing humidity. However, in the semi-natural nests in Legon Janten, the temperature and humidity of the nests are high (79 - 87%), this is because the humidity is influenced by heavy rainfall. The incubation period of hawksbill sea turtle (*E.imbricata*) eggs occurs in April, which is the rainy season with heavy rainfall (BMKG, 2023). According to Martins et al. (2020) natural factors such as heavy rainfall can also influenced in increase humidity due to higher absorption of water content in the nest sand.

According to Aguirre et al. (2020) the optimal incubation temperature for hawksbill sea turtles is 29.5˚C - 31˚C. Temperatures in semi-natural nests are quite high in all nests. The incubation temperature of semi-natural nests has a temperature ≥ 31˚C and possibility to produce female hatchlings rather than male hatchlings. According to Sukandar et al. (2020) the optimal humidity for sea turtle egg nests is ranging from 70 - 80% with success rate of embryo development reaches 90%. Humidity in Nest I, Nest II and Nest III is the condition of the nest with optimal humidity (79 - 87%). Temperature conditions of semi-natural hawksbill turtle (*E.imbricata*) nests in Nest I, Nest II and Nest III in Legon Janten Karimunjawa are optimal for the growth of egg embryos with a high hatching success rate reaching 86 - 88%. The percentage of hatching success of semi-natural nests is shown in Table 2.

Table 2. Percentage of Hatching Success of Hawksbill Turtle Eggs

Nest	Nest origin	Egg Count			Percentage of Success
		Unhatched	Hatched	Total	
I	Krakal Island	10	60	70	85.7
II	Cilik Island	11	68	79	86.1
III	Cilik Island	10	70	80	87.5

Based on the result in Table 2, the percentage of hatching success of semi-natural nests of hawksbill turtles (*E.imbricata*) has a relatively high hatching success rate. The highest percentage of hatching success is in Nest III from Cilik Island with a success rate of 87.5%. The lowest hatching success was in Nest I from Krakal Island with a success rate of 85.7%. This explains that very high temperatures in the nest I result in a decreasing percentage of hatching success. According to Aguirre et al. (2023) nests with low humidity causes dryness in the nest during the incubation period and results in insufficient uptake of hatchling eggs for successful embryonic development. According to Pena and Julia (2021) temperature sea turtle nest at 33˚C as the maximum thermal limit for hatching to occur, a percentage of hatching success of 71% at an average temperature of 33˚C.

Allometric of Hatchlings Hawksbill Sea Turtle

The total sample length-weight allometry of hawksbill sea turtle (*E. imbricata*) hatchlings were collected 198 individuals in three different nests at Legon Janten Karimunjawa semi-natural hatchery. The results of the length-weight allometry analysis of hawksbill sea turtle hatchlings (*E. imbricata*) can be seen in Table 3.

Tabel 3. Length-weight allometry of hawksbill sea turtle (*E. imbricata*)

Nest	n	Average SCL length (mm)	Average Weight (W) (mm)	Intercept (a)	Slope (b)	r	Formula
I	60	38.00 ± 2.01	12.85 ± 0.96	1.358	0.200	0.271	$1.358W^{0.200}$
II	68	38.18 ± 0.98	13.30 ± 1.10	1.326	0.226	0.132	$1.326W^{0.226}$
III	70	38.40 ± 0.89	13.37 ± 0.62	1.518	0.059	0.121	$1.518W^{0.059}$

The length and weight of each nest are different, with the result that the standard deviation is smaller than the mean, indicating that the variable data are distributed equally. The correlation coefficient (r) showed the relationship between length and had a weak correlation. This is supported with the research of Andrastea et al. (2018) the value of the correlation coefficient (r) is categorized into four, which are: r = 0.00-0.25 indicates no relationship or weak relationship, r = 0.26-0.50 indicates a moderate relationship, r = 0.51-0.75 indicates a strong relationship, and r = 0.76-1.00 indicates a very strong or perfect relationship.

Based on the results of length-weight allometry analysis, Hawksbill turtle (*E. imbricata*) hatchlings in Nest I, Nest II and Nest III in Legon Janten Karimunjawa National Park showed that the allometry of carapace length (SCL) and weight (W) of hatchlings had negative allometry, i.e. length growth (SCL) was slower than weight growth (W). According to Mawardi et al. (2022) the negative allometric growth means that length growth is faster than weight, so these animals tend to be smaller. According to Rodrıguez and William (2019) that the positive allometry of weight (W) growth is faster than length indicating that the body size of hatchlings is larger.

The allometric of two variables from the body parts of hawksbill sea turtle (*E. imbricata*) hatchlings in Nest I, Nest II and Nest III had a negative allometric growth type. The results of hatchling allometry measurements are presented in Table 4, Table 5 and Table 6.

Table 4. Allometric of Hatchlings in Nest I, n = 60

Variable		a	b	β	Seb	r	tobs	Allometric
Dependent	Independent							
SCL	CW	0.811	0.534	1	0.118	0.509	-3.937*	-
	FLL	1.128	0.318	1	0.097	0.394	-7.025*	-
	HLL	1.240	0.281	1	0.064	0.499	-11.202*	-
	SPL	0.833	0.513	1	0.101	0.553	-4.795*	-
	PW	0.999	0.415	1	0.113	0.435	-5.192*	-
	BD	1.262	0.264	1	0.099	0.332	-7.458*	-
	W	1.358	0.200	0.333	0.093	0.271	-1.426ns	-
CW	FLL	0.850	0.414	1	0.085	0.539	-6.899*	-
	HLL	1.175	0.218	1	0.065	0.406	-12.108*	-
	SPL	0.723	0.492	1	0.097	0.556	-5.255*	-
	PW	0.501	0.670	1	0.081	0.737	-4.081*	-
	BD	1.005	0.361	1	0.088	0.475	-7.277*	-
	W	1.266	0.156	0.333	0.090	0.221	-1.967ns	-
FLL	HLL	1.077	0.286	1	0.084	0.408	-8.507*	-
	SPL	0.832	0.406	1	0.142	0.352	-4.196*	-
	PW	0.905	0.370	1	0.148	0.312	-4.265*	-
	BD	1.020	0.335	1	0.122	0.338	-5.443*	-
	W	1.369	0.048	0.333	0.120	0.052	-2.750*	-
HLL	SPL	0.100	0.761	1	0.192	0.462	-1.249ns	-
	PW	0.277	0.664	1	0.204	0.393	-1.645ns	-
	BD	0.632	0.477	1	0.175	0.338	-2.994*	-
	W	1.213	-0.007	0.333	0.172	0.005	-1.981ns	-
SPL	PW	0.533	0.658	1	0.104	0.640	-3.297*	-
	BD	1.037	0.346	1	0.103	0.404	-6.338*	-
	W	1.321	0.119	0.333	0.104	0.150	-2.057*	-
PW	BD	1.005	0.327	1	0.101	0.392	-6.669*	-
	W	1.428	-0.027	0.333	0.101	0.035	-3.546*	-
W	BD	0.823	0.237	3	0.138	0.220	-19.969*	-

Notes:
* = t-statistic significantly different at p<0.05 (n = 60, t0.05 = 2.002)
ns = t-statistic not significantly different at p<0.05
\- = negative allometry
\+ = positive allometry

Table 5. Allometric of Hatchlings in Nest II, n = 68

Variable		a	b	β	Seb	r	tobs	Allometric
Dependent	Independent							
SCL	CW	1.370	0.148	1	0.077	0.229	-11.018*	-
	FLL	1.414	0.116	1	0.161	0.051	-10.054*	-
	HLL	1.520	0.050	1	0.058	0.106	-16.284*	-
	SPL	1.117	0.319	1	0.067	0.509	-10.226*	-
	PW	1.395	0.133	1	0.082	0.195	-10.533*	-
	BD	1.917	-0.290	1	0.552	0.065	-2.337*	-
	W	1.326	0.226	0.333	0.085	0.312	-1.256ns	-
CW	FLL	1.584	-0.103	1	0.137	0.092	-8.029*	-
	HLL	1.438	-0.001	1	0.091	0.002	-11.027*	-
	SPL	1.042	0.271	1	0.115	0.278	-6.341*	-
	PW	1.018	0.296	1	0.125	0.281	-5.638*	-
	BD	1.465	-0.024	1	0.079	0.038	-12.935*	-
	W	1.164	0.240	0.333	0.135	0.214	-0.686ns	-
FLL	HLL	1.303	0.113	1	0.080	0.172	-11.103*	-
	SPL	1.383	0.040	1	0.107	0.046	-8.996*	-
	PW	1.412	0.020	1	0.116	0.022	-8.444*	-
	BD	1.419	0.018	1	0.071	0.031	-13.897*	-
	W	1.516	-0.067	0.333	0.123	0.067	-3.249*	-
HLL	SPL	0.929	0.198	1	0.160	0.150	-5.001*	-
	PW	0.689	0.374	1	0.170	0.262	-3.679*	-
	BD	1.271	-0.045	1	0.107	0.052	-9.747*	-
	W	1.529	-0.277	0.333	0.184	0.182	-3.309*	-
SPL	PW	0.904	0.391	1	0.125	0.360	-4.8858*	-
	BD	1.491	-0.030	1	0.081	0.046	-12.662*	-
	W	1.236	0.193	0.333	0.140	0.167	-0.997ns	-
PW	BD	1.633	-0.187	1	0.071	0.306	-16.617*	-
	W	1.296	0.099	0.333	0.130	0.093	-1.798ns	-
W	BD	1.014	0.097	3	0.070	0.169	-41.754*	-

Notes:
* = t-statistic significantly different at p<0.05 (n = 68, t0.05 = 1.997)
ns = t-statistic not significantly different at p<0.05
- = negative allometry
+ = positive allometry

Tabel 6. Allometric of Hatchlings in Nest III, n = 70

Variable		a	b	β	Seb	r	tobs	Alometric
Dependent	Independent							
SCL	CW	1.240	0.238	1	0.063	0.418	-12.117*	-
	FLL	1.345	0.167	1	0.058	0.328	-14.236*	-
	HLL	1.615	-0.025	1	0.082	0.037	-12.501*	-
	SPL	1.175	0.280	1	0.082	0.382	-8.790*	-
	PW	1.381	0.143	1	0.074	0.229	-11.620*	-
	BD	1.623	-0.032	1	0.061	0.065	-17.020*	-
	W	1.518	0.059	0.333	0.058	0.121	-4.699*	-
CW	FLL	0.997	0.311	1	0.102	0.348	-6.770*	-
	HLL	1.338	0.086	1	0.143	0.073	-6.368*	-
	SPL	1.121	0.220	1	0.153	0.172	-5.093*	-
	PW	1.088	0.251	1	0.129	0.229	-5.798*	-
	BD	1.411	0.027	1	0.107	0.031	-9.137*	-
	W	1.373	0.062	0.333	0.104	0.072	-2.605*	-
FLL	HLL	0.979	0.374	1	0.154	0.282	-4.061*	-
	SPL	1.003	0.294	1	0.170	0.205	-4.153*	-
	PW	1.348	0.060	1	0.148	0.049	-6.345*	-
	BD	1.239	0.162	1	0.117	0.165	-7.134*	-
	W	1.250	0.162	0.333	0.115	0.169	-1.489*	-
HLL	SPL	1.250	-0.024	1	0.131	0.022	-7.813*	-
	PW	1.158	0.040	1	0.112	0.044	-8.582*	-
	BD	1.215	0.000	1	0.090	0.000	-11.136*	-
	W	1.164	0.045	0.333	0.088	0.063	-3.277*	-
SPL	PW	1.096	0.259	1	0.099	0.303	-7.509*	-
	BD	1.437	0.022	1	0.083	0.031	-11.778*	-
	W	1.107	0.316	0.333	0.072	0.472	-0.232ns	-
PW	BD	1.360	0.049	1	0.097	0.061	-9.792*	-
	W	1.124	0.261	0.333	0.090	0.333	-0.799ns	-
W	BD	0.908	0.182	3	0.122	0.178	-23.108*	-

Notes:
* = t-statistic significantly different at p<0.05 (n = 68, t0.05 = 1.995)
ns = t-statistic not significantly different at p<0.05
- = negative allometry
+ = positive allometry

Growth of standard carapace length (SCL) and growth of carapace width (CW), were negative allometry to other body parts. This indicates that carapace length growth is slower than other body parts. Slower growth in length causes hatchlings to appear thinner and smaller. According to Gouvello et al. (2020) smaller hatchlings are more vulnerable to predators and hatchlings with larger sizes are fitter than hatchlings with small sizes. Standard plastron length (SPL) and carapace width (CW) had negative allometric growth relative to other body parts. This showed that the growth of the plastron was slower than other body parts. Hatchling plastron becomes smaller due to slower growth. According to Zhang et al. (2019) a larger plastron may offer a larger radius of protection from predator attack for the ventral side of the turtle when upside down. Forelimb length (FLL) and hindlimb length (HLL) growth of hatchlings were negative allometry. This indicates that the growth of flipper length is slower than the other body parts resulted in shorter and smaller flipper length. According to Tanabe et al. (2021) hatchlings with smaller flipper take longer to swim and crawl to the sea.

Hatchling weight is negatively allometric (slower growth) to other body parts. This can be concluded that hawksbill sea turtle (*E. imbricata*) hatchlings in Karimunjawa are small and light. According to Josimovich et al. (2021) hatchlings with light body weight are vulnerable to predator attacks but according to Sönmez (2019) lighter weight hatchlings are faster to swim. According to Salmon et al. (2018) hawksbill turtle hatchlings have other strategies to avoid predators, such as more effective morphological forms. The strategy is the development of carapace ridges that overlap between the five central scutes to increase protection against predators. According to Lovich and Whit (2021), a larger body volume increases self-protection because the carapace ridge also becomes higher.

According to Diaz et al. (2022) length and weight as well as size, morphology, growth of hatchlings after hatching are influenced by different temperatures and humidity during the incubation period. According to Wei et al. (2020) hatchlings incubated at 32℃ have smaller body size and mass than hatchlings incubated with a temperature range of 23 - 29℃. Nest I with the highest temperature and low humidity produced the smallest hatchling size and negative allometry growth. Nest II and Nest III with lower temperature and high humidity produced larger hatchling size and negative allometry growth type. According to Gatto and Richard (2022) higher humidity produce larger and heavier hatchlings during incubation, meanwhile low humidity levels produce hatchlings of smaller size.

Bacterial isolation of Hawksbill Sea Turtle Eggshell (E. imbricata)

Total Plate Count (TPC) of bacterial colonies in hawksbill sea turtle hatched and unhatched eggshells that have grown on EMBA (Eosin Methylene Blue Agar) selective media was calculated manually on petri dishes with duplo dilutions. The calculation results of Total Plate Count (TPC) bacterial colonies are presented in Table 7.

Table 7. Total Plate Count (TPC) bacteria colony eggshell

Sample	Dilutions	Colony Number		Average	(CFU/ml)
		Petri 1	Petri 2		
HE (Hatched Eggshell)	10^{-5}	43	60	51.5 x 10^{-5}	1.03 x 10^{7}
UE (Unhatched Eggshell)	10^{-5}	64	78	71 x 10^{-5}	1.42 x 10^{7}

Total Plate Count (TPC) results showed that total number of bacterial colonies in Petri dishes of hatched eggshell (HE) was 1.03 x 10^{7} CFU/ml and unhatched eggshell (UE) was 1.42 x 10^{7} CFU/ml. The calculation results show that the number of hatched eggshell colonies (HE) was less than the unhatched eggshells (UE). This result matches with McMaken et al. (2023) research that there are differences in the number of bacteria found in sea turtle eggshells, that the total number of bacterial colonies in unhatched eggshells is higher than hatched eggshells. This shows that the number of bacterial colonies affects the failure of turtle egg hatching. According to Praja et al. (2021) one of the causes the death of turtle embryos due to the high microbial load from contaminated eggs can be pathogenic.

Identification of turtle eggshell bacterial characteristics was carried out by conventional methods, such as macroscopic and microscopic observations of bacterial colonies. The morphological macroscopic observations of bacterial colonies in hatched and unhatched eggshells that have grown on the EMBA (Eosin Methylene Blue Agar) plates after incubation presented in Table 8.

Table 8. Macroscopic morphology of bacterial colonies

Sample	Code	Edge	Surface	Margin	Elevation	Colour
HE (Hatched Eggshell)	HE01	circular	concentric	entire	flat	pink
	HE02	circular	concentric	undulate	umbonate	pink
	HE03	irregular	smooth	entire	flat	transparent
	HE04	circular	concentric	serrated	umbonate	pink
	HE05	circular	concentric	entire	convex	pink
	HE06	circular	concentric	entire	convex	pink
	HE07	circular	concentric	entire	flat	pink
	HE08	irregular	concentric	undulate	flat	pink
UE (Unhatched Eggshell)	UE01	circular	concentric	entire	flat	pink
	UE02	circular	concentric	entire	umbonate	pink
	UE03	irregular	smooth	undulate	convex	transparent
	UE04	irregular	concentric	undulate	umbonate	pink
	UE05	irregular	contoured	lobate	flat	pink
	UE06	irregular	contoured	curled	flat	transparent

The morphological microscopic observation of bacteria colonies in hatched and unhatched eggshells that have grown on the EMBA (Eosin Methylene Blue Agar) plates after incubation, the colonies form of bacterial isolates were Gram-negative. The results of microscopic observation of hawksbill sea turtle eggshell bacteria (*E. imbricata*) in semi-natural nest hatching can be seen in Figure 2.

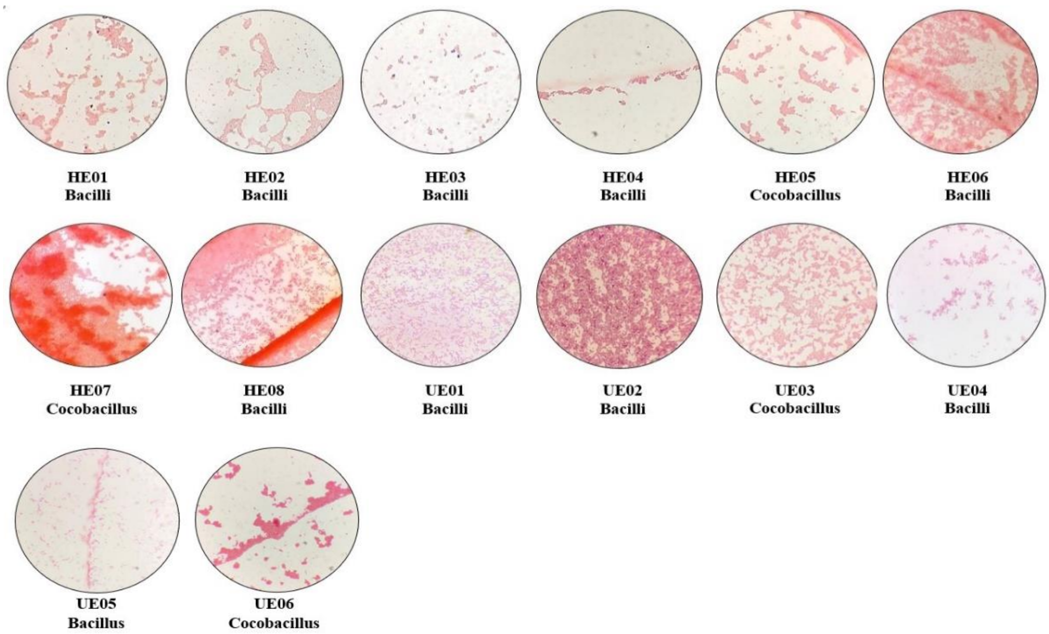

Figure 2. Microscopic observation of bacteria with 1000x magnification.

There are 14 bacterial isolates that have been observed mascroscopically and microscopically. The result of macroscopic observations on bacteria eggshells matches with Nursanty et al. (2019) research that the morphology of bacterial colonies on hatched and unhatched seaturtle eggshells were round colony shapes, undulate colony margin, convex elevations and pink in color. According to Badieyan et al. (2018) that the differences in the morphological characteristics of bacterial colonies can indicate that the bacterial colonies come from different species. The microscopic observations of bacterial colonies on hatched eggshells and unhatched eggshells were Gram negative colonies with colony shapes bacilli and coco bacillus (short rod bacilli). Gifari et al. (2018) showed that microscopically the gram staining results were Gram negative *Salmonella* spp., with a bacillus colony shape. According to Choi et al. (2020) there are Gram negative bacteria found in turtle eggshells, including *Enterobacter* spp. with the form of coco bacillus bacteria.

Based on the measurement of temperature (32.4 - 33.9°C) and humidity (79 - 87%) in semi-natural nests of hawksbill sea turtles (*E. imbricata*) are optimal nest conditions for bacterial growth. The colonies were suspected to be from *Enterobacteriaceae* family according to microscopic and macroscopic morphological characteristics and environmental semi-natural nests condition. According to research by Qiu et al. (2022) bacteria that grow at temperatures ranging from 26 - 34°C with humidity 70 - 90% are *Escherichia coli*, *Shigella* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Citrobacter* spp., *Aeromonas* spp., and *Pseudomonas* spp. According to Talepour et al. (2020) bacteria that grow above a temperature of 24°C with a humidity 70 - 90% were *Escherichia coli*, *Salmonella* spp., *Serratia* spp., and *Pseudomonas* spp. According to Katni et al. (2022) explained that pathogenic bacterial contamination can be obtained from the polluted environment of the mother's habitat and transferred to the turtle eggs. This is supported by the research of Ratu et al. (2023) that polluted rivers carried domestic waste into the sea and carried several types of bacteria such as *Aeromonas* spp., and *Pseudomonas* spp. Beside that the environmental factors also give impact on the abundance of pathogenic bacteria (Sulardiono et al. 2019)

Hatchlings Abnormality of Hawksbill Sea Turtle (*E. imbricata*)

Based on the abnormality observation, in Nest I there were 17 hatchlings that experienced abnormalities out of 60 hatchlings that successfully hatched. Nest II had 15 abnormal hatchlings out of 68 hatchlings that successfully hatched and Nest III had 6 abnormal hatchlings out of 70 hatchlings that successfully hatched. Results of abnormalities observation on hawksbill turtle (*E. imbricata*) hatchlings can be seen in Figure 3, Figure 4 and Figure 5.

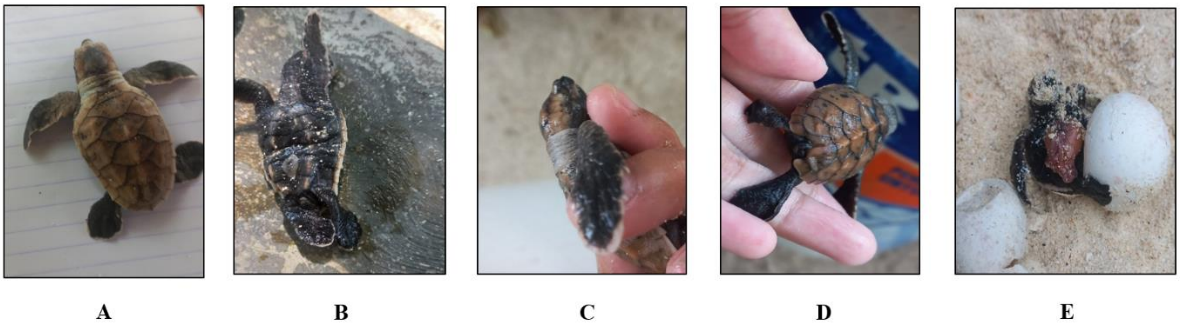

Figure 3. *Abnormalities of hatchlings hawksbill sea turtle found in Nest I. A. Abnormal carapace. B. Abnormal plastron. C.* Cyclopia. D. Anury. E. Hatchlings with large yolk sacs

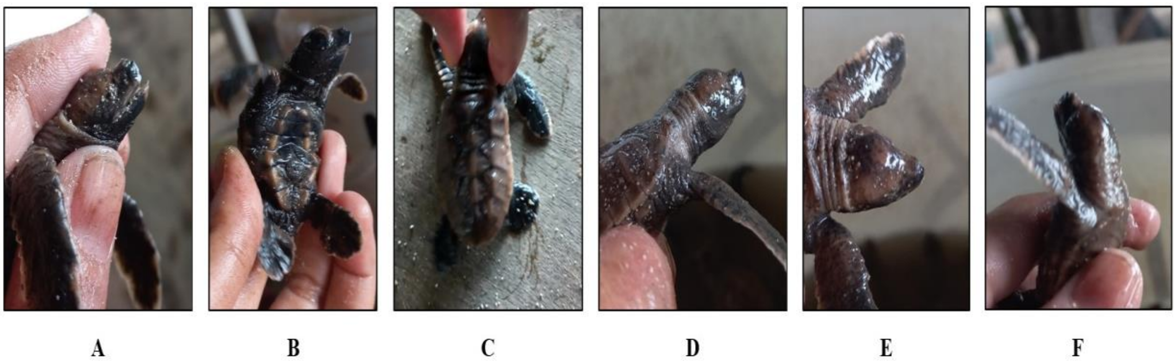

Figure 4. *Abnormalities of hatchlings hawksbill sea turtle found in Nest II. A. Abnormal beak and* mikrophthalmia. *B. Abnormal plastron. C. Abnormal carapace.* D. Microphthalmia. E. Anophthalmia. F. Cyclopia

A B

Figure 5. *A. Hatchling hawksbill sea turtle with normal plastron. B. Abnormal plastron (irregular depressions) of hawksbill seaturtle hatchling in Nest III.*

Abnormalities found in hawksbill sea turtle (*E. imbricata*) hatchlings in Legon Janten Karimunjawa semi-natural nest were cyclopia (only one eye present), abnormal carapace (irregular depressions), abnormal plastron (irregular depressions) and anury (absence of tail), microphthalmia (reduced eye size), anophthalmia (absence of eyes) and abnormalities in the beak. Some hatchlings still had yolk attached to the plastron. According to Campo et al. (2018) the most common eye malformation is anophthalmia and hatchlings find it difficult to live in the ocean if they do not have two eyes. According to Bhadesiya et al. (2020) abnormal beaks have difficulty in food consumption and inability to defend against predator attacks.

Some hatchlings in Nest I, Nest II and Nest III were found with abnormal plastron conditions with abdominal depressions. According to Ikhwan et al. (2018) hatchlings with a sunken plastron shape indicate that the turtle has caechexia (a health disorder that causes extreme weight loss accompanied by muscle shrinkage) and malnutrition. Hatchlings in Nest I, Nest II and Nest III had asymmetrical carapaces. According to Guerrero and Adán (2021) the asymmetrical shape of the plastron and carapace is due to the relatively slow growth condition of the embryo. According to Zimm et al. (2017) abnormalities can be influenced by genetic differences from the parents or the influence of unsuitable environmental factors during the incubation period of egg nest conditions.

The cause of abnormalities in hatchlings is because of disrupted embryonic growth due to high temperature (32.4 – 33.9˚C) and humidity (79 - 87%) in semi-natural nests. According to Pena and Julia (2021) abiotic factors affect the morphology of hatchlings during embryonic development, such as changes in the shape of the carapace, plastron and tail of hatchlings, causing abnormalities. This showed that in the Legon Janten Karimunjwa semi-natural nest, high temperature and humidity in the nest resulted in morphological changes and abnormalities in hatchlings after hatching. According to Patricio et al. (2021) hatchlings with semi-natural nest temperatures reaching 31˚C - 32˚C and nest humidity of 75 - 80% experience abnormalities in the carapace and plastron. High temperatures can be caused by global warming and high humidity is caused by heavy rainfall.

The novelty of this research is to provide a better understanding of semi-natural hatching conservation activities by studying the failure and success factors of hatching during the incubation period in terms of microbiological and morphological aspects, so it can be used as a reference for further research. The results of this study revealed a variety of factors that can influence the semi-natural hatching of sea turtle eggs during the incubation period and the survival of hatchlings after hatching. Each factor contributes significantly to sea turtle conservation management, showing the influence of nest temperature and humidity on bacterial contamination of eggs, resulting in a decrease in the number of hatchlings released, as well as on the morphological characteristics and abnormalities of hatchlings, showing the weakness of hatchlings defense mechanisms against predators, resulting in a very low percentage of survival at sea. Overall, these results provide the Karimunjawa National Park with a reference on the factors of hatching failure and success during the incubation period, which can help provide input for conservation management strategies for protected biota.

CONCLUSION

The conclusion obtained based on the results of research that has been conducted on semi-natural hatching in Legon Janten Karimunjawa showed that there was a significant correlation between the physical environment of the nest (temperature ranging from 32.4 - 33.9℃ and humidity 79 - 87%) with the success of hatching with a success percentage of 86 - 88%, this indicates that semi-natural nests are quite optimal. However, the effect of allometric growth of hatchlings was negative allometric and smaller hatchling size and hatchlings experienced abnormalities. The environmental conditions of the nest were a suitable for bacterial growth of hawksbill sea turtle eggshells (*E. imbricata*) with total bacteria colony of Gram negative bacteria in unhatched eggshells is higher than hatched eggshells in semi-natural nests. Further research is needed to identify the bacterial contamination of eggs from mothers, sand, or humans, as well as to determine whether there are morphological and abnormality similarities from parents that are passed on to hatchlings.

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REFERENCES

Aguirre, F. C. D., Díaz-Hernández, V., Salgado Ugarte, I. H., Sosa Caballero, L. E., & Méndez de la Cruz, F. R. (2020). Feminization tendency of hawksbill turtles (*Eretmochelys imbricata*) in the western Yucatán Peninsula, Mexico. J. *Amphib Reptile Conserv*, *14(1)*, 190-202. amphibian-reptile-conservation.org

Aguirre, F. C. D., Hernández, D. V., Moreno, D. A. & de la Cruz, F. R. M. (2023). Effect of Moisture, Temperature, and Maternal Influence on The Hatching, Phenotype, and performance of Hawksbill Turtles *Eretmochelys imbricata*. *J. Endangered Species Research*, 50. 217-234. https://doi.org/10.3354/esr01229

Badieyan, S., Dilmaghani-Marand, A., Hajipour, M. J., Ameri, A., Razzaghi, M. R., Rafii-Tabar, H., Mahmoudi, M., & Sasanpour, P. (2018). Detection and Discrimination of Bacterial Colonies with Mueller Matrix Imaging. *J. Scientific Reports*, *8*(1), 1- 10. https://doi.org/10.1038/s41598-018-29059-5

Banerjee, S. M., Frey, A., Kurle, C. M., Perrault, J. R. & Stewart, K. R. (2020). Morphological Variation in Leatherback (*Dermochelys coriacea*) Hatchlings at Sandy Point National Wildlife Refuge, US Virgin Islands. *J. Endangered Species Research*, 41, 361-372. DOI: https://doi.org/10.3354/esr01030

Bhadesiya, C. M., Patel, V. A., Gajjar, P. J. &and Anikar, M. J. (2021). Case Studies on Overgrown Beak in Budgerigars (*Melopsittacus undulatus*). *J. Entomology and Zoology Studies*, 9(1), 1778-1780. https://www.entomoljournal.com/archives/2021/vol9issue1/PartY/9-1-311-602.pdf

Camargo, C. A. J., Álvarez, G. Y., Jaramillo, V. J. & Sánchez, T. F. 2020. Nesting Failure of Sea Turtles in Ecuador Causes of The Loss of Sea Turtle Nests: The Role of The Tide. *J. Coastal Conservation*, 24. 1-10. https://doi.org/10.1007/s11852-020-00775-3

Capri, F. C., Prazzi, E., Casamento, G., Gambino, D., Cassata, G., & Alduina, R. (2023). Correlation Between Microbial Community and Hatching Failure In Loggerhead Sea Turtle *Caretta caretta*. *J. Microbial Ecology*, 86, 1923 -1933. https://doi.org/10.1007/s00248-023-02197-8

Choi, E., Kate, E. C., Kester, L. C., Kimberly, M., Stewart, C. E., Morrall & Michelle M. D. (2020). Research Foundation Leatherback Sea Turtle (*Dermochelys coriacea*) Embryo and Hatchling Pathology in Grenada, with Comparison to St. Kitts. *J. Chelonian Conservation and Biology*, 19(1), 111–123. https://doi.org/10.2744/CCB-1395.1

Chuen-Im, T., Suriyant, D., Sawetsuwannakun, K., & Kitkumthorn, N. (2019). The Occurrence of Vibrionaceae, Staphylococcaceae, and Enterobacteriaceae in Green Turtle *Chelonia mydas* Rearing Seawater. *J. Aquatic Animal Health*, 31(4), 303-310. https://doi.org/10.1002/aah.10082

Clabough, E. B., Kaplan, E., Hermeyer, D., Zimmerman, T., Chamberlin, J., & Wantman, S. (2022). The Secret Life of Baby Turtles: A Novel System to Predict Hatchling Emergence, Detect Infertile Nests, and Remotely Monitor Sea Turtle Nest Events. *J. Plos one*, *17*(10), 1-

21.https://doi.org/10.1371/journal.pone.0275088

Compo, R. M. D., Annelisse, B. I., Itzel, S. R., Raul, L. H. & Alejandra, G. G. (2018). Methylation Status of The Putative PAX6 Promoter in Olive Ridley Sea Turtle Embryos with Eye Defects: An Initial Approach. *J. Elsevier*, Mechanisms of Development, 154, 287-295. DOI: 10.1016/j.mod.2018.08.005

D'Alba, L., Goldenberg, J., Nallapaneni, A., Parkinson, D. Y., Zhu, C., Vanthournout, B., & Shawkey, M. D. (2021). Evolution of Eggshell Structure in Relation to Nesting Ecology in Non-Avian Reptiles. *J. Morphology*, *282*(7), 1066-1079. https://doi.org/10.1002/jmor.21347

Diaz, U. N. M., Phillips-Farfan, B. V., Nava, H., Lopez-Toledo, L., Murata, C., Lajud, N., Vargas, M. A. H., Camacho, C. A. A., Torner, L., Farias, A. L. F & Melendez-Herrera, E. (2022). Negative Effects on Neurogenesis, Ovariogenesis, and Fitness in Sea Turtle Hatchlings Associated to Ex Situ Incubation Management. *J. Frontiers in Ecology and Evolution*, *10*, 850612. https://doi.org/10.3389/fevo.2022.850612

Dinata, G. F., Aini, L. Q., & Kusuma, R. R. (2021). Identification and Characterization of Antagonistic Bacteria from Coffee Plant Litter. *J. Agrotechnology Research, 5(1)*, 32-7. doi:10.20961/agrotechresj.v5i1.49716

Gatto, C. R. & Richard, R. D. (2022). A Review of The Effects of Incubation Conditions on Hatchling Phenotypes in Non-Squamate Reptiles. *J. Comp Physiol B*, Vol. 192, 207–233. https://doi.org/10.1007/s00360-021-01415-4

Gifari, T., Dewi, E. & Irawan, S. (2018). The Effects of Contaminant Microorganism Towards Chelonia Mydas Eggs Hatchery Results in Pangumbahan Green Sea Turtles Conservation, Sukabumi, Indonesia. *J. Biodiversitas*, 19 (4), 1207-1212. https://doi.org/10.13057/biodiv/d190404

Gleason, F. H., Allerstorfer, M., & Lilje, O. (2020). Newly Emerging Diseases of Marine Turtles, Especially Sea Turtle Egg Fusariosis (SEFT), Caused by Species in The *Fusarium solani* Complex (FSSC). *J. Mycology*, *11*(3), 184-194. DOI: 10.1080/21501203.2019.1710303

Gouvello, D. Z. E. M., Ronel, Nel & Anton, E. C. (2020). The Influence of Individual Size on Clutch Size and Hatchling Fitness Traits in Sea Turtles. *J. Experimental Marine Biology and Ecology*, 527(151372).https://doi.org/10.1016/j.jembe.2020.151372

Guerrero, A. & Adán, P. G. (2021). Shell Anomalies in The European Aquatic Stem Turtle *Pleurosternon bullockii* (*Paracryptodira, Pleurosternidae*). *J. Diversity*, 13(11), 1- 10. https://doi.org/10.3390/d13110518

Gufe, C., Tinashe, C. H., Bernard, M., Otlia, M., Jerikias, M., Shuvai, M., Gilbert, J., Pious, V. M. & Jairus, M. (2019). Antimicrobial Profiling of Bacteria Isolated from Fish Sold at Informal Market in Mufakose, Zimbabwe. *J. International Microbiology*, 8759636, 1-7. https://doi.org/10.1155/2019/8759636

Guzman, H. M., Kaiser, S. & Ivan Hinsberg, V. J. (2020). Accumulation of Trace Elements in Leatherback Turtle (*Dermochelys coriacea*) Eggs from the South-Western Caribbean Indicates Potential Health Risks to Consumers. *J. Chemosphere*, 243(125424). https://doi.org/10.1016/j.chemosphere.2019.125424

Ikhwan, F. R., Ida, A. D. K. D., Maulid, D. S. & Dwi, S. (2018). Oral Presentation (WAAC-1) Gastric Obstruction in Stranded Green Turtle (*Chelonia mydas*) in Paloh, Kalimantan Barat at February, 9th 2018. *Proc. of the 20th FAVA CONGRESS and The 15th KIVNAS PDHI*, Bali, 257-258. https://core.ac.uk/download/pdf/230373522

Josimovich, J. M., Bryan, G. F., Alejandro, G. P., Emma B. H., Ian, A. B., Robert, N. R. & Andrea F. C. (2021). Clutch May Predict Growth of Hatchling Burmese Pythons Better Than Food Availability or Sex. *J. Biology Open*, 10(058739) doi:10.1242/bio.058739

Karyati, K., Ipor, I. B., Jusoh, I. & Wasli, M. E. (2019). Allometric Equations to Estimate The Above-Ground Biomass of Trees in The Tropical Secondary Forests of Different Ages. *J. Biological Diversity*, 20(9), 216 – 224. https://doi.org/10.13057/biodiv/d200901

Katni, N. H., Azmi, A. F. M., Abdullah, M. M., Rusli, M. U., Zakaria, Z., Azizan, T.R. P. T., Amat, A. C., Saad, M. Z., Yasin, I. S. M., Nazarudin, M. F & Hassim, H. A. (2022). Nutritional Compositions, Pathogenic Microorganisms and Heavy Metal Concentration in Green Turtle Eggs (*Chelonia mydas*) from Terengganu and Sabah, Malaysia. *J. Front. Mar. Sci*, 9, 1- 16. DOI: 10.3389/fmars.2022.948427

Köhnk, S., Brown, R. & Liddell, A. (2021). Finding of A Two-Headed Green Turtle Embryo During Nest Monitoring In Baa Atoll, Maldives. J.

Veterinary Research, 88(1), 1-8. http://dx.doi.org/10.4102/ojvr.v88i1.1940

Kusdarwati, R., Amin, M., & Wardana, A. B. (2021). DNase and Gelatinase Activities Of β-Hemolysin *Aeromonas hydrophila* Isolated from Catfish (*Clarias batrachus*). *J. Aquaculture and Fish Health*, 10(3), 330 – 340. DOI : 10.20473/jafh.v10i3.25918

Lovich, J. E & Whit, G. (2021). *Turtles of the World: A Guide to Every Family*. Princeton University Press, USA. P. 240

Martins, S., Patino, J., Abella, E., de Santos Loureiro, N., Clarke, L. J., & Marco, A. (2022). Potential Impacts of Sea Level Rise and Beach Flooding on Reproduction of Sea Turtles. *J. Elsevier : Climate Change Ecology*, *3*, 100053. https://doi.org/10.1016/j.ecochg.2022.100053

Matthews, B.L., Christopher, R.G. & Richard, D. R. (2021). Effects of Moisture During Incubation on Green Sea Turtle (*Chelonia mydas*) Development, Morphology and Performance. *J. Endang Species Res*, 46: 253–268. DOI: 10.3354/esr01159

Mawardi, A. L., Teuku, H. W. A., Tri, M. S., Muhammad, K., Muhammad, A. S. & Yusriono. (2022). Growth Patterns of Captive Painted Terrapins *Batagur borneoensis* in The Aceh Province, Indonesia. *J. Biodiversitas*, 23(9), 4872-4878. DOI: 10.13057/biodiv/d230956

Mazaris, A.D., Gail, S., Chrysoula, G., Vasiliki, A., Graeme, C. & Hays. (2017). Global Sea Turtle Conservation Successes. *J. Science Advance*, 3(9), 1-7. DOI: 10.1126/sciadv.1600730

McMaken, C. M., Derek, A. B., Rosanna, J. M. & Jose, V. L. (2023). Potential Impacts of Environmental Bacteria on The Microbiota of Loggerhead (*Caretta caretta*) and Green (Chelonia mydas) Sea Turtle Eggs and Their Hatching Success. *J. Microbiology Open*, 12(3), 1-34. https://doi.org/10.1002/mbo3.1363

Miguel, R. S., Anastácio, R. & Pereira, M. (2022). Sea Turtle Nesting: What Is Known and What Are the Challenges under a Changing Climate Scenario. *Open J. Ecology*, 12, 1-35. DOI: 10.4236/oje.2022.121001.

Nursanty, R., Widya & Sari, S. (2019). Characterization and Identification of Enterobacteriaceae Bacteria in Turtle (*Lepidochelys olivacea*) Eggs Shells from Lhok Pante Tibang, Banda Aceh. *J. Sain Veteriner*, 37(1), 41-48. https://doi.org/10.22146/jsv.48514

Patricio, A. R., Miguel, R. V., Castro, B., Annette C. Br., Maria, B. F. A., Brendan, J. G., Aissa, R., Dominic, T. & Paulo, C. (2018). Nest Site Selection Repeatability of Green Turtles, *Chelonia mydas*, and Consequences for Offspring. *J. Animal Behavior*, 139, 91-102. https://doi.org/10.1016/j.anbehav.2018.03.006.

Pena, R. C.& Julia, A. R. (2021). Incubation Temperature, Hatchings Success and Congenital Abnormalities in Green Turtle Nests from Guanahacabibes *Peninsula*, Cuba. *J. Aquatic Research*, 4(4), 321 – 330. https://doi.org/10.3153/AR21027

Praja, R. N., Aditya, Y., Wiyanto, H. & Vivi, O. (2021). Short Communication: Antimicrobial Properties in Cloacal Fluid of Olive Ridley Sea Turtle (*Lepidochelys olivacea*). *J. Biodiversitas*, 22(9), 3671-3676. https://doi.org/10.13057/biodiv/d220909

Qiu, Y., Yan, Z., Yanfen, C., Xinyue, L., Hui, Z., Xiaorui, L., Ke, Q., Xiaojie, Z. & Ziqiang, L. (2022). The Effects of Ventilation, Humidity, and Temperature on Bacterial Growth and Bacterial Genera Distribution. *Int J Environ Res Public Health*, 19(15345), 1 -13. doi: https:/doi.org/10.3390/ijerph192215345.

Ratu, L. I. N., Seumahu, C. A., & Killay, A. (2023). Biodegradation Test of Polluted River Caused by Domestic Wastewater Using Indigenous Bacteria in the Way Tomu Watershed, Ambon City. *Biosaintifika: J. Biology & Biology Education*, *15*(1), 48-59. DOI: https://doi.org/10.15294/biosaintifika.v15i1.40016

Rodriguez, R. L. & William G. E. (2019). Why the Static Allometry of Sexually-Selected Traits Is So Variable: The Importance of Function. *J. Integrative and Comparative Biology*, 59(5), 1290–1302. doi:10.1093/icb/i

Silaban, S., Marika, D. B. & Simorangkir, M. (2020). March. Isolation and Characterization of Amylase-Producing Amylolytic Bacteria from Rice Soil Samples. *J. Physics: Conference Series*, 1485, 1-7. doi:10.1088/1742-6596/1485/1/012006

Sönmez, B. (2019). Head and Plastron Scalation Patterns of The Green Turtle, *Chelonia mydas*, Hatchlings in Natural and Relocated Nests on Samandağ Beach. *J .Black Sea Mediterranean Environment*, 25(3), 280-293. https://blackmeditjournal.org/wp-content/uploads/3-20193_280-293.pdf

Sukandar., Sunardi., Khalwatu, M., Rahman, M. A., & Abidin, Z. (2020). Design of automatic eggs hatchery as preservation of turtle in coastal of East Java. In *E3S Web of Conferences*, 153, 01002. https://doi.org/10.1051/e3sconf /202015301002

Sulardiono B, Widyorini N, Suprapto D, Ayuningrum D, Rahman A. 2020. Evaluation of antibacterial activity and molecular characterization of bacteria from *Holothuria atra* intestine collected from anthropogenic and non-anthropogenic region in Karimunjawa, Indonesia. Biodiversitas 21: 3149-3155. DOI: 10.13057/biodiv/d210736

Sullivan, S., Heinrich, G. L., Mattheus, N. M., Cassill, D., & Doody, J. S. (2022). Can Reptiles Use Nest Site Choice Behavior to Counter Global Warming Effects on Developing Embryos? Potential Climate Responses in a Turtle. *J. Frontiers in Ecology and Evolution*, *10*, 825110. https://doi.org/10.3389/fevo.2022.825110

Sumaryati, S & Kuswadi. (2017). Community Participation in Sea Turtle Conservation in Karimunjawa National Park, Central Java, Indonesia. *J. Marine Turtle Newsletter*, (152), 11. http://www.seaturtle.org/mtn/archives/mtn152/mtn152-4.shtml

Syuhaida, N. I., Rozihan, M., Akbar, J. B., Akmal, M. S. & Joni, H. D. (2019). Allometry Relationship Of Mangrove Horseshoe Crab, *Carcinoscorpius rotundicauda* from The West Coast of Peninsular Malaysia. *International J. Fisheries and Aquatic Studies*, 7(2), 223-228. https://www.fisheriesjournal.com/archives/2019/vol7issue2/PartC/7-2-10-537

Talepour, N., Mohammad, S. H., Effat, A. M., Seyed, M. L. & Neamat, J. H. F. (2018). Spatio-Temporal Variations of Airborne Bacteria from The Municipal Wastewater Treatment Plant: A Case Study in Ahvaz, Iran. *J. Environ Health Sci Eng*, 18(2), 423–432. https://doi.org/10.1007/s40201-020-00470-3

Tanabe, L. K., Marion, Steenacker., Mohd, U. R. & Michael, L. B. (2021). Implications of Nest Relocation for Morphology and Locomotor Performance of Green Turtle (*Chelonia Mydas)* Hatchlings. *J. Ocean & Coastal Management*, 207(105591). https://doi.org/10.1016/j.ocecoaman.2021.105591

Topping, N. E., & Valenzuela, N. (2021). Turtle Nest-Site Choice, Anthropogenic Challenges, and Evolutionary Potential for Adaptation. *J. Frontiers in Ecology and Evolution*, *9*, 808621, 1-12. https://doi.org/10.3389/fevo.2021.808621

Wei, Y., Gao, Y., Cao, D., Ge, Y., Shi, H. & Gong, S. (2021). Effect of Incubation Temperature and Substrate Moisture on Embryonic Development, Hatchling Phenotypes and Post-Hatching Growth in the Reeves' Turtle, Mauremys reevesii. *J. PeerJ*, 9(2), 1-16. DOI 10.7717/peerj.10553

Wyneken, J. (2001). *The Anatomy of Sea Turtles. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470*, 1-172 pp.

Wyneken, J. & Salmon, M. (2020). Linking Ecology, Morphology, and Behavior to Conservation: Lessons Learned from Studies of Sea Turtles. *J. Integrative and Comparative*, 60(2), 440 – 455. https://doi.org/10.1093/icb/icaa044

Zhang, W., Niu, C., Liu, Y. & Kenneth, B. S. (2019). Positive or Negative? The Shell Alter, The Relationship Among Behavioral Defense Strategy, Energy Metabolic Levels and Antioxidant Capacity in Freshwater Turtles. *J. Front Zool*, 16(3), 1-12. https://doi.org/10.1186/s12983-019-0301-5

Zimm, R., Blair, P.B., Jeanette, W & Jacqueline, E. M. V. (2017). Environmental Causation of Turtle Scute Anomalies in Ovo and in Silico. *J. Integrative and Comparative Biology*, 57(6): 1303–1311. DOI: 10.1093/icb/icx066