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The Relationship Between Egg Weight of Olive Ridley Sea Turtle (*Lepidochelys Olivacea*) on Hatchability using "Maticgator" (Automatic Turtle Egg Incubator)

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Abstract: Sea turtles are reptile animals that live primarily in oceans but migrate to sandy beaches to produce and lay eggs in large quantities. However, the hundreds of eggs produced, only a dozen hatch and return to the sea. Also, factors of climate change and global warming have an impact on decreasing the wild sea turtle population, where female turtles are generated from the hatched eggs. These factors have resulted in an unbalanced population, making it difficult to locate mates during breeding. The importance of protecting this population and efforts to prevent these creatures from extinction should be emphasized. This study aimed to determine the relationship between the weight of the olive ridley sea turtles' eggs (*Lepidochelys olivacea)* and hatchability using Maticgator technology (Automatic Turtle Egg Incubator). An experimental method was used by conducting a test on ten different groups of egg weights containing 40 eggs. Subsequently, samples that have been insulated or divided into four parts were inputted into the incubator nest media and then each part was coded KI, KII, KIII, KIV into the Maticgator box. The weights were marked as box code I or (KI), II or (KII), III or (KIII) and IV or (KIV) for 32.5 g, 27 g, 30.5 g and 26 g egg weights and then placed into the Maticgator until the turtle eggs hatched. Consequently, the correlation results showed a significant relationship between the egg and hatchling weight of 0.491, hence the association was sufficient. Furthermore, there was a moderate correlation of 0.709 between the egg weight and carapace length, but no critical relationship was observed with carapace width. Also, 33 (82.5%) out of the 40 eggs were successfully hatched. The highest number of eggs hatched was recorded in box III, box I, box IV and box II, with the hatching percentages of 100, 90, 80 and 60%, respectively.

Keywords: Olive Ridley Sea Turtle, Eggs Weight, Maticgator, Hatching Percentage

Introduction

Sea turtles primarily live in the sea and occasionally migrate to shores to spawn (Robinson *et al*., 2013). There are several environmental factors chosen by these creatures when they are ready to lay eggs, such as the tides of the sea, coastal vegetation closure, width of the coast and sand type (Zavaleta-Lizárraga and Morales-Mávil, 2013). These animals lay eggs in large numbers and of the hundreds produced by a single sea turtle, only a dozen hatches and make their way to the sea. Indonesia is a country rich in marine life with six of the seven sea turtle species (Ranan, 2015). Generally, different scientists stated that there were 8 types of sea turtles, but only 7 have been approved. These species include Lepidochelys kempii, olive ridley, green, hawksbill, leatherback, loggerhead and flat turtle, where Chelonia agassizii (Galapagoz Green Turtle) has been categorized as part of Chelonia mydas (Khashman *et al*., 2016). Currently, the population of sea turtles is decreasing and on the verge of extinction (Ario *et al*., 2016). Specifically, the gray turtle (*Lepidochelys olivacea*), which are commonly found on many shorelines in relatively small amounts compared to the green and hawksbill variant (Tapilatu *et al*., 2016).

Among the vast variety of marine life, sea turtles are a protected species because of their low population. Globally, these creatures are on the red list of the International Union for the Conservation Nature (IUCN) and classified as Appendix I Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Therefore, their existence is threatened and all forms of its usage and distribution must receive serious attention to prevent extinction (Ríosa *et al*., 2015).

Several factors, such as climate change and global warming result in the degradation of the sea turtle population and impact the sea ecosystem and toxic residue which usually impacting the aquatic organism (Maftuch *et al.*, 2017). Consequently, climate change affects the sea turtles' nutrient source and their ability to spawn, while global warming influences the eggs on the spawning ground (Butt *et al*., 2016). Temperature, humidity and sand moisture majorly influence the hatching results of these creatures (Tilley *et al*., 2019). Also, these eggs produce female turtles due to climate change and global warming, when the earth's warm temperature exceeds 29°C. These factors contribute to an unstable wild sea turtle's population and the difficulty in attracting a partner during the mating season (Lamont *et al*., 2020).

Immediate action must be taken to protect the sea turtle population from extinction (Lovemore *et al*., 2020). Consequently, sea turtle eggs can be saved from their habitat and kept safe through the use of incubators (Oh and Kim, 2017). This device is used to hatch the eggs and control the hatching process in the event of global warming and climate change (Pike, 2014).

The Maticgator machine is a Campus Intellectual Product Business Development Program, made by a team from the Agricultural Training Center in the Faculty of Fisheries and Marine Science, Universitas Brawijaya (Brawijaya University). Also, the early phase of developing this device was completed after 3 years, while the official launch and research were conducted in August 2015 by the Community Supervisor Group of Wonocoyo Village. This was achieved to conserve the sea turtle eggs at the Kili-Kili Park Beach (Pantai Taman Kili-Kili) and other regions located in East Java, such as Blitar, Pacitan and Malang District (Pratama *et al*., 2020).

The Maticgator is an automatic turtle egg incubator employed to automatically hatch sea turtle eggs. This technology is expected to help in the hatching process of reptile eggs, such as sea turtles and maintain its population from extinction. Therefore, further study to determine the Maticgator stability by monitoring the effect of sea turtle egg weight on hatchability is required, while automatically controlling the nest temperature and humidity (Hendra *et al*., 2020). In this study, hatchability is the percentage of sea turtle eggs that successfully hatched in the Maticgator and became hatchlings. This factor is influenced by temperature, humidity and the hatching medium in the nest (Pazira *et al*., 2016).

Materials and Methods

Time and Place of Research

This study was conducted between 15th July and 24th September 2020. The egg samples were obtained from the sea turtle conservation at Kawasan Konservasi Penyu Pantai Taman Kili-Kili, Wonocoyo Village, Trenggalek District, East Java Province of Indonesia.

Data Collection and Data Processing

Data collection and process was conducted after these creatures had finished laying eggs and returned to the sea at 01:00 AM (GMT + 7). The entire egg transfer was conducted without changing their original position in the nest during the collection, hence the process was semi-natural (Rudiana *et al*., 2012).

A total of 40 eggs were obtained from their nest and weighed immediately. These samples contained 10 each and were codenamed KI, KII, KIII and KIV, as well as weighed 32.5 g, 27 g, 30.5 g and 26 g, respectively. This was aimed to group the eggs into four containers and mark their tops to prevent them from being inverted. The incubation scheme based on the egg weight inside the Maticgator is displayed in Fig. 1. While automatic sea turtle egg incubator device in Fig. 2 and part of it in Table 1.

Table 1: Parts of maticgator

Name	Function
LCD	Displays the temperature, humidity and the gender of the turtle cubs that are going to hatch.
4 Menu buttons	Run commands from the microcontroller, such as on/off (red button), male turtle cubs (blue button), female turtle cubs (yellow button), ok/start incubating (white button)
2 Fans	As a cooler to control the room temperature when it exceeds the threshold
Aluminum foil	A medium covering the container conducting heat to control the temperature and humidity.
2 Light bulbs	Heat source of the container, automatically controlled and run by a programming language.
Water suply control	Water suply control is an automated water sprayer to stabilize the humidity in the container.
Egg containers	Places to lay down the eggs inside the device, where they are covered with sand to simulate the natural environment.
Temperature censor	Measures and determines the temperature that is displayed on the LCD.
Humidity censor	Measures and determines the humidity that is displayed on the LCD.
Microcontroller	A Sequence of electrical system inside the device to control the entire system making sure that it is running perfectly and automatically.

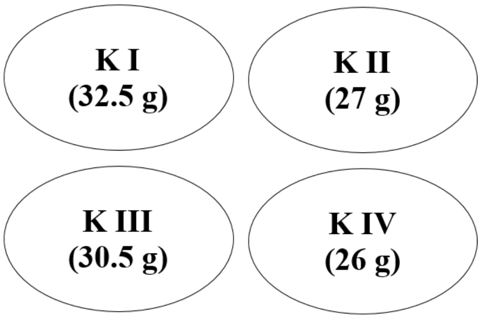


Fig. 1: Sea turtle egg layout scheme

Fig. 2: Automatic sea turtle egg incubator device

Maticgator (Automated Turtle Egg Incubator)

Maticgator is a box-shaped automated turtle egg incubator device that is made of a multiplex, coated with an aluminum foil on the interior. The device has an $80 \times 70 \times 60$ cm dimension with handles on both sides and wheels on the bottom to provide mobility. In addition, the drawer is located on the top as a compartment for electronic components programmed with a micro-controller system, which automatically controls temperature and humidity.

Incubation Period

During the incubation, samples were monitored by taking note of the temperature and humidity displayed on the screen at 06:00 am, 12:00 pm and 06:00 pm during the day until the eggs hatched. This process also included the room and sand temperatures, as well as humidity. In addition, the hatched eggs were removed from the container and the weight, carapace length and width of the turtle cubs were measured. Subsequently, the cubs disconnected from their umbilical cord were moved and released into the sea.

Hatching Results

The hatched eggs that developed into turtle cubs were analyzed. This data analysis was conducted to determine the percentage of successfully hatched olive ridley sea turtle eggs, based on the ratio between the hatched and the number of incubated eggs (Rofiah *et al*., 2012). The following equation was used:

$$HSs = \frac{JS}{JS + TM} \times 100\%$$

Where:
HSs = Hatching results
JS = Number of hatched eggs
TM = Number of unsuccessful hatches

Data Analysis

A bar chart of the average numbers display was used to represent the temperature and humidity analysis of the censor, room and sand. Moreover, testing the correlation between egg weight and hatchling size (weight, width and length of the carapace) was conducted through the SPSS application 16.0. The X variable was the weights of the egg while the Y variable was the weight of the hatchling (Y1), length (Y2) and width of the carapace (Y3).

Results

During the use of the Maticgator technology, the effect of egg weight on the hatching results was influenced by humidity and temperature inside the incubator. Each factor will be elaborated below for further details:

Effect of Egg Weight on Hatching Results

The study used a total of 40 olive ridley sea turtle eggs obtained from the same mother at Kili-Kili Park Beach, Wonocoyo Village, Trenggalek District. These eggs were divided into 4 containers comprising of 10 eggs each. Subsequently, their weights were measured based on the codenames KI, KII, KIII and KIV and 10 eggs from each group weighed 32.5, 27, 30.5 and 26 g, respectively (Fig. 3).

During the incubation until the hatching day, 33 (82.5%) eggs were successfully hatched. The third container (KIII) had the highest hatching result of 100% compared to the other three. Meanwhile, in the first (KI) and fourth container (KIV), 9 and 8 eggs were successfully hatched with a 90 and 80% result, respectively. However, the lowest result was recorded in the second container (KII), with only 60% result and 6 hatched eggs. Therefore, the study on the effect of egg weight on hatching results was successful because the outcome was higher than 50%. These effects on sea turtle eggs are generally influenced by temperature and humidity, not egg weight. Table 2, shows the hatching results of the olive ridley turtle.

Table 2: Hatchability on olive ridley sea turtle eggs

I	II	III	IV	V	VI
10	I (32.5)	9	1	0	90
10	II (27.0)	6	2	2	60
10	III (30.5)	10	0	0	100
10	IV (26.0)	8	1	1	80

Description:
I : Number of eggs
II : Containers/boxes (egg weight in grams)
III : Number of hatched eggs
IV : Number of developed embryo
V : Number of defected embryo
VI : Hatching percentage (%)

Monitoring on Maticgator

During the incubation, the eggs were monitored by taking note of the temperature and humidity displayed on the screen at 06:00 am, 12:00 pm and 06:00 pm until the eggs hatched. This monitoring also included both the room and sand temperatures and humidity.

Temperature

Based on the results, the automatic temperature yielded an average value higher than the temperature of sand, which was the lowest. The temperature difference between each data collection during the morning, afternoon and evening was not significant. The average automated temperature at 06:00 am was 30.5°C, with 29.53°C on room and 28.58°C on sand. Also, at 12:00 pm the value was 30.5°C, with room temperature 29.97°C and sand 29.47°C. Lastly, at 06:00 pm the automated temperature was 30.55°C, with 30°C and 29.46°C on room and sand, respectively. Figure 4 displays the graphic of the average temperature.

The Effect of Room and Sand Temperature

Based on the analysis results, a correlation (R) value of 0.648 was obtained, where the whole output had a determinant coefficient (R square) of 0.420. Therefore, the effect of the independent variable (room temperature) on the dependent (temperature in the sand) was 42% and the calculated F value was 154.9 with a significance of 0.000 at the 0.05 level (Fig. 5). This result shows that there was a significant relationship between these variables on the sea turtle egg hatching because the significance value was <0.05. Furthermore, the association between these temperatures was beneficial, as seen from the correlation value. The constant value (a) was 1.430 while the room temperature value (b or regression coefficient) was 0.930. Hence, the regression equation can be written as follows:

$$Y = a + bX$$
$$Y = 1.430 + 0.930X$$

Humidity

The chart in Fig. 6 shows that the automatic humidity yields a higher number than the room humidity and sand moisture. Meanwhile, the lowest average value was generated on the sand temperature. The average level of humidity measurements was 59% automatic, 51% room and 44% sand moisture at 06:00 am while at 12:00 pm, the value for sand moisture was 43%. Meanwhile, at 06:00 pm, the humidity changed to 61 and 52% for automatic and room humidity, respectively, but remained at 43% for sand moisture.

The Effect of Room Humidity on Sand Moisture

The data of the automated humidity and room, as well as sand humidity inside the device, is displayed in Fig. 5. While the regression equation and graph can be seen in Fig. 7.

In the Maticgator device, the relationship between room humidity and sand moisture toward the olive ridley sea turtle egg hatchability was denoted with the linear regression equation of $Y = 1.7\ X\text{-}44.4$. Therefore, assuming the independent variable (X) value is 29-40%, then the dependent variable (Y) will rise by approximately 4.9%.

Room and Sand Temperature

The relationship between room and sand temperature in the Maticgator toward hatchability was denoted with the linear regression equation of $Y = 1.43+0.93X$. Therefore, assuming the independent variable (X) or the room temperature reaches 28-30°C, then the dependent value (Y) or the sand temperature will drop to approximately 0.6°C.

Based on the analysis result, the coefficient value (R) of 0.95 was obtained from the output with a determinant coefficient (R Square) of 0.906. Therefore, the effect of the independent variable (room temperature) on the dependent (sand temperature) was 90% and the calculated F value was 2.05 with a significance value of 0.000 at 5% or 0.05 level. These results show there were significant correlations between these variables on sea turtle egg hatching because the significance value was <0.05.

The comparison of the temperature in maticgator with the temperature in nature shows a significant difference in data, namely the value of sig (2-tailed) 0.000 less than 0.05 (0.000<0.05). This finding shows the temperature results on the maticgator (X) has a significant difference to the nature temperature (Y) (Table 3).

The Effect of Egg Weight on Hatchling Weight and Carapace Length and Width

The correlation test with SPSS 16.0 application was used to analyze the data in this study. Based on the correlation results shown in Table 4, different values of significance were obtained.

Based on statistical analysis, the relationship between egg weight and carapace length and width is positive:

a. Hatchling weight

The correlation result (r) between egg and hatchling weight was 0.491 with s significance of 0.004 on the 5% or 0.05 level:

b. Carapace length

The correlation result (r) between egg and carapace length was 0.709 with a significance value of 0.000 on the 5% or 0.05 level. Therefore, the correlation level between these variables was positive:

c. Carapace width

The correlation result (r) between egg and carapace width was 0.042 a with a significance value of 0.818 on the 5% or 0.05 level. Therefore, the correlation level between the variables was not sufficient.

Table 3: Levene's test for equality of variances of temperature in maticgator room and in nature

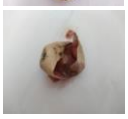
	Levene's test for equality of variances		T-test for equality of means					95% Confidence interval of the difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. Error difference	Lower	Upper
a	24.268	0.000	8.295	430.000	0.000	0.66463	0.08012	0.50715	0.82211
b			8.295	383.999	0.000	0.66463	0.08012	0.50709	0.82217

Description: A. Equal variances assumed; B. Equal variances not assumed

Table 4: Correlation test results

Description	Correlation value (r)	Significant value on 5% level
Hatchling weight	0.491	0.004
Carapace length	0.709	0.000
Carapace width	0.042	0.818

Table 5: Unsuccessful hatches number of olive ridley sea turtles

No.	Image	Container	Numbers	Phase
1		I	1	Stationary on the oval opaca area phase attached to the rounded pellucid area.
2		IV	2	The embryo ended on the post-gastrula phase with the primitive plate extending the lateral area between the opaca and the pellucid region bordering the oval germinative disk. Also, the cephalic process extended ventrally to the anterior neuropore, followed by the appearance of the head (amniotic).
3		II	3	The embryo stops at the stage, where the nerve canal is still open with the amniotic fluid extending the anterior extremity.
4		IV	26	The embryo stopped at the scaly phase, only visible at the edge of the face and fin tip in older specimens, where some pigmentation only occurred at the end of the scales.
5		II	2	The embryo paused in the post-gastrula phase with the primitive plate extending the lateral area between the opaca and pellucid region bordering the oval germinative disk, followed by the appearance of the head (amniotic).
6		II	19	The embryo ceased to be pigmented on the neural and the proximal claw-free plate at the anterior border due to the formation of the rear fin.
7		II	17	The embryo stopped in the two-egg phase with the same score, therefore it ended at the phase of the mandibular process reaching the anterior border of the eye and the encephalon, then the diencephalon started to take shape.

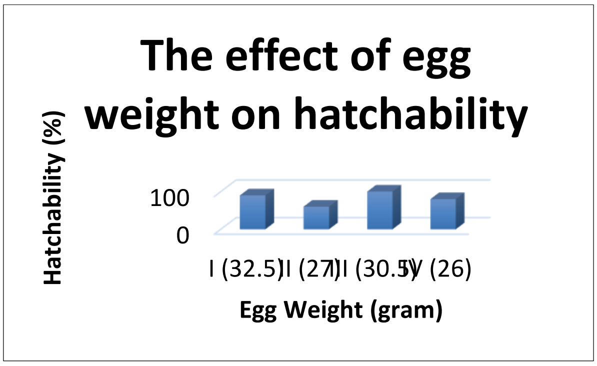


Fig. 3: Diagram of olive ridley sea turtle egg weight (g) effect on hatchability (%)

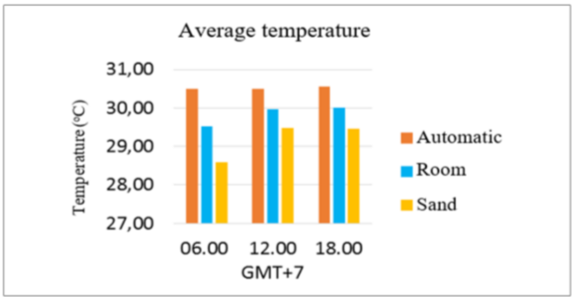


Fig. 4: The average automatic and room temperature, as well as sand moisture

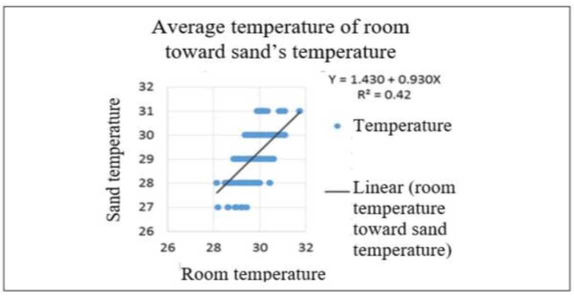


Fig. 5: Linear regression of the average temperature of room temperature toward sand temperature

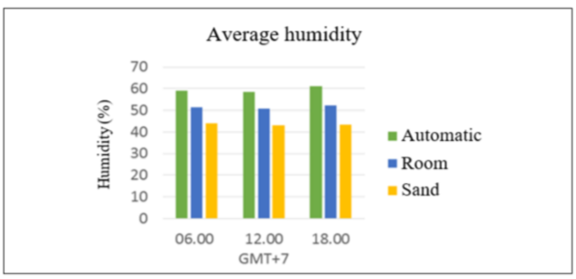


Fig. 6: The average automatic and room humidity, as well as sand moisture

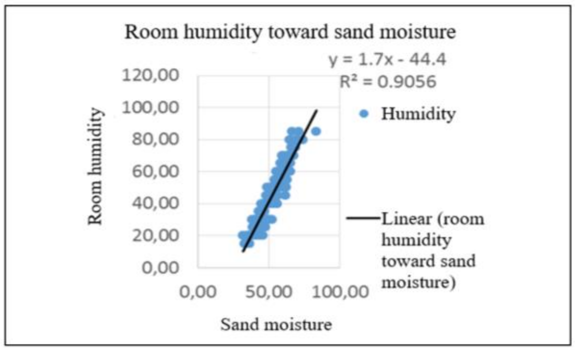


Fig. 7: Linear regression of room humidity toward sand moisture

Unsuccessful Hatches

Based on the Maticgator study, there were 8 unsuccessful hatches. There are 31 phases of the embryo development of sea turtle eggs from the earliest stage to hatching. Therefore, each of the unsuccessful hatches failed to emerge in different phases. Table 5 displays the elaboration of each egg, starting from the lowest score (Cratz, 1982).

Table 5 shows the failure of hatching eggs in each box. The biggest damage is the embryo stopped at the scaly phase, only visible at the edge of the face and fin tip in older specimens, where some pigmentation only occurred at the end of the scales.

Discussion

Artificial Hatching

Saving sea turtle eggs in coastlines by transferring to incubators and hatching them, maintaining and growing the eggs up to a certain size and releasing the cubs to the sea, certainly have an impact on saving their life until adulthood, where they can reproduce (Sulaiman *et al*., 2011). The process of moving eggs must pay attention to the speed of transfer and the position of the eggs. Within 1-12 h of egg transfer, the hatching rate was high. However, this percentage diminished to 0% between 24 and 48 h. According to a discovery by Parmenter (1980), sea turtle eggs are sensitive to rotating motions between 12 to 24 h after oviposition. Maulana *et al*., (2017) stated that the hatching results are quite high if the average number of treatments is above 50%. The results of artificial hatching in this study got more than 50% successful results

Olive Ridley Sea Turtle Egg Weight (g) Effect on Hatchability (%)

The different percentage ratios for each olive ridley sea turtle hatching result, where the highest was on the 30.5 g egg weight with a percentage of 100%. However, the lowest was on the 27 g egg weight with a percentage of 60%. This outcome was due to a defect in the water sprayer; hence the whole surface and bottom of the 4 containers were not covered. Therefore, humidity is the defining factor of the hatching results. Furthermore, other factors that affected these findings, included temperature, the egg's condition and the transfer time of the eggs (relocation from the habitat to the incubator). This finding agreed with study by that sea turtle eggs have a natural trait, which is sensitive to a dry environment, though its carapace is prone to fungal growth in presence of high humidity.

Automated, Room and Sand Temperature

Based on the observation, the device managed to stabilize the temperature, which emulates the natural environment with a slightly higher temperature in the evening. This occurrence was related to a findings by, that there was a relatively higher nest temperature in the evening than in the morning due to heat radiation and conduction. The temperature intensified just before the eggs hatched because their metabolism is increased during this period. Temperature also increases at the end of incubation, where the embryo starts to become a turtle cub (Zi-Ye *et al*., 2019). Subsequently, hatchlings require 1-2 days to surface (Aguilera *et al*., 2018). These hatchlings contain egg yolk that coagulates on their abdomen and umbilical cord still connected to the embryo membrane and functions as a food supply until they can reach the surface (Banoet *et al*., 2019).

In the early period of incubation, the high humidity was adjusted to normal. Meanwhile at the end of the process, 3 weeks before hatching, water sprayer systems were no longer used. Therefore, the water content in the eggs is reduced and the hatchability is increased. Furthermore, fungal growth can potentially occur on the eggshell. If the humidity is too high, hence affecting the hatchling's gender (Romeo *et al*., 2018).

The hatching of sea turtle eggs is influenced by the temperature and humidity of the medium or environment, where the hatchability is increased if the average humidity is in the normal range (Wei *et al*., 2021). This is correlated with a statement by Rofiah *et al.* (2012), that hatchability is influenced by sand moisture ranging from 29-40%, hence the chance of success is higher.

According to Parawangsa *et al*. (2018), different sand moisture can be affected by air circulation surrounding the environment on the inside or outside of the nest. Moreover, biological activity from the egg itself can affect sand moisture. The normal development of turtle egg embryos and the biochemical reaction process that occurs within the egg requires matching humidity in the nest. Consequently, the liquid inside the egg can leak due to an excessively dry environment. This phenomenon leads to a lack of liquid during the hatching day, resulting in exhausted hatchlings, which are unable to leave the egg and cannot survive.

Correlation of Egg Weight on Hatchling Weight, Carapace Length and Width

The correlation result shows that egg weight has a significant association with the hatchling weight and carapace length but not with the carapace width. Therefore, the sea turtle egg weight increases with the hatchling size and carapace length and vice versa. According to, a hatchling weight was heavier at low than warm temperatures because the egg was passive at low temperatures. Hendra *et al.* (2020) stated that egg weight influences embryo growth, in relation to the number and size of the cells, where a heavier egg will have bigger limbs.

Also, the unsuccessful hatches were produced by some factors, such as nest humidity. Sea turtle eggs are naturally sensitive to dry environments; however, too much moisture encourages fungal growth on its shell. Furthermore, the transfer process of the eggs from the nest to the incubator and infertile eggs are the others factors that influence this phenomenon. These unsuccessful hatches of olive ridley sea turtles can also be induced by temperature changes and unevenly produced metabolism heat from the egg; hence the embryo was unable to develop (Banoet *et al*., 2019).

Conclusion

The results on the effect of olive ridley sea turtle egg weight on hatchability (29-34°C), imply that there was a significant and sufficient correlation of 0.491 between the egg and hatchling weight. In addition, there was a medium correlation of 0.709 between the egg weight and carapace length. The success percentage was 82.5%, where 33 out of 40 eggs were hatched. Also, the highest number of successful hatches (100%) occurred in container III, with egg weight 30.5 g. However, 90% of the samples were hatched in container I, with 32.5 g egg weight. Meanwhile, 80 and 60% of the eggs were successfully hatched in containers IV and II, with egg weight of 26 g and 27 g, respectively.

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Author's Contributions

Sukandar Sukandar: Conception and design, coordinated the data-analysis and writing of the manuscript.

Sunardi and Zaenal Abidin: Drafting the article, acquisition of data.

Vian Dedi Pratama: Conception and design and acquisition of data.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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