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HATCHING SUCCESS OF OLIVE RIDLEY SEA TURTLES (*Lepidochelys olivacea*) IN ARTIFICIAL HATCHERY IN TURTLE CONSERVATION AND EDUCATION CENTER (TCEC) SERANGAN

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Abstract: **Sea turtles are marine aquatic animals whose lives range from shallow marine waters to deep-sea waters. All types of turtles are considered rare and protected. Conservation is an activity that is expected to prevent the extinction of turtles. One of the efforts to save and preserve turtles is through Semi Natural Hatching. Hatching of turtle eggs at long distances and long travel times has never been done at the hatching of olive ridley turtle eggs. The purpose of this study was to determine the success rate of egg hatching, the factors, and conditions that caused the eggs of olive ridley turtles do not hatch, as well as the sex ratio of the hatchlings. This research was conducted by measuring the depth of the nest, and calculating the number of hatchlings, as well as sampling and making zoological specimens for reproductive organs. The benefit of data collection on hatchery numbers is to provide information, knowledge and scientific evidence regarding the hatching success of olive ridley turtle eggs (Lepidochelys olivacea) incubated at the Turtle Conservation and Education Center (TCEC), Serangan.**

Keyword: **Olive Ridley Turtle, Eggs, Hatching Success, Sex Ratio, TCEC.**

I. INTRODUCTION

Sea turtles are marine aquatic animals whose lives range from deep-sea waters to shallow marine waters. Sometimes sea turtles are also in the coastal area to lay eggs in the sands of nesting beaches. In Indonesia, there are 6 types of turtles, namely Green Turtle (Chelonia mydas), Leatherback Turtle (Dermochelys coriaceae), Loggerhead Sea Turtle (Caretta caretta), Hawksbill Sea Turtle (Eretmochelys imbricata), Flatback Turtle (Natator depressus), and Olive Ridley Turtle (Lepidochelys olivacea).

[1]All types of sea turtles are considered rare and protected. In the IUCN Red Data Book (International Union for Conservation Nature and Natural Resources) they have been recorded in the Endangered category, actively threatened with extinction, which means that these animals are on the verge of danger because they are endangered.

[2]Olive Ridley turtles (Olive Ridley turtle, Lepidochelys olivacea) are included in kingdom Animalia, phylum Chordata, reptile class, order: Testudines, family: Cheloniidae, and species: Lepidochelys olivacea. [3],[4]The carapace is covered with large keratin scales totaling six or more costal scute pairs. The presence of more than 5 costal scales and inframarginal pores is a way of identifying this species.

Serangan Island, along with the village of Tanjung Benoa, has been known for decades as the biggest black market for the meat and other turtle products. Serangan was a port for hundreds of turtle fishing vessels that sailed to Derawan, East Kalimantan and the Papua bird's head region. This massive trade and hunting not only destroyed the sea turtle population around the Bali region but also caused ecological impacts on many regions in Indonesia.

In its heyday until 2000, this business captured around 30,000 sea turtles per year and was taken to the island. The combination of adaptive strategies, in line with advocacy, and a grounded community strengthening program carried out by WWF and local authorities for several years, not only can reduce the number of turtles traded but also can mobilize support from the local community, which can slowly get rid of the big turtle traders.

Conservation is one of the activities that are expected to prevent the extinction of turtle habitat, prevent the use of turtles for commercial interests such as the sale of eggs, meat and shells and can be a means of sharing knowledge or educating the public at large about the importance of turtle conservation in order to preserve turtle habitat in Indonesia so as not to become extinct.

Rescue and preservation of turtles, among others, can be done through Semi-Natural Hatching, Translocation Habitat Protection (in situ conservation), Law Enforcement, and Empowerment of Surrounding Communities. Hatching of turtle eggs at long distances and long travel times has been successfully carried out in Green turtle eggs (Chelonia mydas) hatching [5], but has never been done on Olive Ridley turtles. Therefore, the researcher was interested in doing the same thing but with Olive Ridley turtles (Lepidochelys olivacea).

II. MATERIALS AND METHODS

Object of Research

The objects used in this study were 13 incubation nests of olive ridley eggs at the Turtle Conservation and Education Center (TCEC), Serangan Village, South Denpasar District, Denpasar City, Bali Province.

Research Materials

The materials used in this study consisted of: NBF 10%, alcohol 70%, alcohol 80%, alcohol 90%, alcohol 96%, toluene 1 and 2, formic acid 75%, absolute alcohol, liquid paraffin 56°C, paraffin block, Permount adhesive, xylol 1 and 2, lithium carbonate, and dye hematoxylin-eosin (HE).

Research Equipment

The equipment used in this study consisted of a measuring tape (150 cm in length), books, stationery, scalpels, scissors, tweezers, small pots, paper tissue, object glasses, cover glasses, embedding sets, microscopes, and cameras for documentation.

Research Design

The type of research used in this study was observational with a cross-sectional study

How to Collect Data/Sampling

Data collection was carried out by observing and unloading hatching nests in TCEC, Serangan Village, South Denpasar District, Denpasar City, Bali Province. This research was conducted by measuring the depth of the nest, and calculating the number of hatchlings (E, LIN, LPE, DIN, DPE, P, UD, UH, and UHT) and recording the data obtained.

Research Variable

The variables in this study can be divided into independent variables, controlled variables, and dependent variables. In this study, which includes the independent variable is the time of measurement and hatching of eggs. Controlled variables are the type of turtle eggs, planting time, temperature and depth of the nest in TCEC, Serangan, Bali. The dependent variables are the number of hatchlings and the sex of hatchlings.

Research Procedure

Data Collection

The measurement of the length of the incubation and of nest depth was carried out once during the study. The measurement of hatching success can be calculated by dividing the number of eggs that hatch (E + LIN + LPE) with the total number of eggs in the nest then multiplied by one hundred percent. To get the sex ratio data, gonad histology zoological specimen will be made and then compared between male hatchlings and female hatchlings.

Sample Collection

After unloading the nest, samples of reproductive organs are carried out on the DIN (Dead in Nest) hatchlings or 5 hatchlings taken from each nest from hatcheries, then hatchlings are kept for 2 week before gonads are taken. Biopsy of hatchling reproductive organs was carried out using a scalpel knife and surgical scissors, the sample was put into a pot containing 70% formalin solution. Biopsy results are made into histological specimen for microscopic examination.

Making Histological Specimen

Histology specimens are made at the Pathology Laboratory, Veterinary Center, using the Kiernan method (1990). Biopsy samples are dehydrated by immersing the specimen into a multilevel alcohol solution, which starts from a 70%, 80% alcohol solution, 90% absolute alcohol I, absolute II, xylol I, xylol II, paraffin, and finally into paraffin II. Each immersion is carried out for two hours. After that embedding is done, which is planting tissue in liquid paraffin and frozen in the refrigerator to facilitate cutting with the microtome.

The tissue is cut with a thickness of 5-6 µm and the cutting results are placed in warm water to avoid folds due to cutting. The specimen is removed and placed on the object-glass and dried in an incubator at 60˚C for 24 hours. In Hematoxylin-Eosin (HE) staining, specimens on the object-glass were soaked in xylol 1 and 2 for two minutes each for deparaffinization then rehydration with successive soaking in absolute alcohol, 95% alcohol, and alcohol 80% each for two minutes, then wash with running water.

Staining with Hematoxylin was carried out for 8 minutes, then rinsed with running water, then washed with Lithium carbonate for 15-30 seconds, rinsed with running water, and stained with Eosin for 2-3 minutes. The eosin-stained specimen is washed with running water and then dried. The specimen is put into 95% alcohol and absolute alcohol, each specimen dipped 10 times, then into absolute alcohol 2 for 2 minutes. Next to xylol 1 for 1 minute and xylol 2 for 2 minutes. The specimen is then dripped with Permount adhesive and covered with a glass cover and then examined under a microscope.

Sample Check

The sample check was carried out at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University. Histopathological specimens that have undergone the HE staining process were observed under a microscope with a magnification of 40 times and 100 times. Observations were focused on the cortical area of the gonads. Determination of male sex is based on the presence of squamous epithelial layers at the edge of the cortex and in the medullary section there are germ cells (seminiferous cords), while the determinant of female sex is based on the discovery of a complex columnar epithelial at the edge of the cortex and in the medullary part is the medullary cord (Larios, 1999).

Data Analysis

The results of the calculation and tabulation of data on the hatching success of olive ridley turtle eggs were analyzed through qualitative descriptive methods. Data obtained in the form of histological images were analyzed descriptively qualitatively.

Location and Time of Research

The study was conducted at the Turtle Conservation and Education Center (TCEC) Serangan. The research time is ± 8 weeks, namely in April – June 2019.

III. RESULTS AND DISCUSSION

Hatching Success

A total of 13 olive ridleys (L.olivacea) nests that were sampled in this study had spawning ranges in early April and were translocated from Serangan, Klungkung, Jumpai, and Petitenget Beaches. Hatching success ranged from 13-76.7%, with an average of 44.51 ± 20.45%. The lowest hatching success is equal to (13.0%), occurring in nests originating from Petitenget Beach. While the highest (76.7%) was found in nests originating from Pantai Serangan. The hatchery number was generated from an incubation period ranging from 47 - 52 days, with an average of 48.1 ± 1.71 days. All olive ridley turtle eggs are sampled, planted in depths between 40-40cm (36.2 ± 2.98 cm). The full summary is shown in Table I.

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TABLE I: Results of Hatching of Olive Ridley (L.olivacea) Turtle Eggs in Artificial Nests in TCEC, Serangan, during the period of 1 April - 31 May 2019.

No.	Origin	Depth (cm)	Incubation period (days)	Hatching Success (%)
1	Klungkung	35	47	50,0
2	Jumpai	36	47	48,8
3	Klungkung	30	47	59,6
4	Klungkung	35	47	60,2
5	Serangan	35	46	76,7
6	Klungkung	35	46	50,8
7	Jumpai	35	49	13,9
8	Klungkung	40	49	47,9
9	Petitenget	35	48	22,9
10	Petitenget	35	50	13,0
11	Klungkung	40	48	32,1
12	Petitenget	40	52	31,8
13	Serangan	40	49	70,9
Average		36.2	48.1	44,51
Std. Deviation		2.98	1.71	20,45

Remarks: Hatching numbers are calculated from the total number of hatchlings that have arisen (Emerged), hatchlings that have hatched but still live in the nest (Live in Nest), and hatchlings that have hatched but died in the nest (Dead in Nest); then the total hatchlings that have been calculated will be divided by the total number of eggs in the nest.

Factors Causing Failure

Of the 13 olive ridley turtle (*L.olivacea*) nests that were sampled in this study, the hatchery failure rates ranged between 23.3-87%, with an average of 55.51 ± 20.45%. The lowest hatch failure rate is equal to (23.3%), which occurs in nests originating from Pantai Serangan. While the highest (87%) was found in nests originating from Petitenget Beach. The full summary is shown in Table II.

Table II: Results of Observation on Failure Rate of Olive Ridley (*L.olivacea*) Turtle Egg Hatching in Artificial Nests in TCEC, Serangan, during the period of 1 April - 31 May 2019.

NO.	ORIGIN	DEPTH (CM)	INCUBATION PERIOD (days)	FAILURE RATE (%)
1	Klungkung	35	47	50,0
2	Jumpai	36	47	51,3
3	Klungkung	30	47	40,4
4	Klungkung	35	47	39,8
5	Serangan	35	46	23,3
6	Klungkung	35	46	49,2
7	Jumpai	35	49	86,1
8	Klungkung	40	49	52,1
9	Petitenget	35	48	77,1
10	Petitenget	35	50	87,0
11	Klungkung	40	48	67,9
12	Petitenget	40	52	68,2
13	Serangan	40	49	29,1
Average		36,23	48,08	55,51
Std. Deviation		2,98	1,71	20,45

Remarks: The hatch failure rate is calculated from 100% minus hatching success.

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As explained earlier, the rate of hatch failure obtained in this study was 55.51 (± 20.45)%. More careful observations show that eggs that are not hatched are due to: eggs that did not develop/Undeveloped eggs (38.39%), followed by eggs with incomplete and dead / Unhatched embryos (8.50%), eggs with a fully developed and deadly embryo / Unhatched Term (1.96%), and a hatchling that will hatch but die / Dead Hatching in Pipped (0.92%). As well as live hatchlings that could not hatch perfectly / Live Hatching in Pipped Egg (5.04%).

Egg samples from Jumpai Beach, had the highest number of categories for undeveloped eggs (93 eggs) at nest depths of 35cm and 49 days of incubation, eggs with embryos that were not fully developed and dead / unhatched (54 eggs) and live hatchlings which cannot hatch perfectly / Live Hatching in Pipped Egg (28 eggs) at a nest depth of 36cm and incubation time of 47 days. Eggs translocated from Klungkung Beach, have the highest number for the egg category with fully developed but dead embryos / Unhatched Term (12 eggs) at nest depth 35cm and incubation time of 47 days, and hatchlings that will hatch perfectly and die / Dead Hatching in Pipped (5 eggs) at nest depth 30-35cm and incubation time of 47 days. The full summary is shown in Table 4.3.

Table III: Eggs samples that have been categorized

Origin	Depth (cm)	Incubation Period (days)	Egg Category								
			E	LIN	DIN	LPE	DPE	UD	UH	UHT	TOTAL
Klungkung	35	47	0	60	0	7	5	0	36	12	120
Jumpai	36	47	0	78	0	28	0	0	54	0	160
Klungkung	30	47	6	78	0	21	5	2	25	4	141
Klungkung	35	47	1	60	1	1	1	28	5	6	103
Serangan	35	46	1	90	1	4	3	21	0	0	120
Klungkung	35	46	0	62	0	0	0	60	0	0	122
Jumpai	35	49	0	15	0	0	0	93	0	0	108
Klungkung	40	49	0	56	0	0	0	61	0	0	117
Petitenget	35	48	0	24	0	4	0	77	0	0	105
Petitenget	35	50	0	14	0	2	0	91	1	0	108
Klungkung	40	48	0	36	0	4	0	72	0	0	112
Petitenget	40	52	0	35	0	2	0	70	3	0	110
Serangan	40	49	0	73	0	4	0	12	6	8	103
	Percentage (%)		0,52	44,54	0,13	5,04	0,92	38,39	8,50	1,96	

Description: E: Emerged (appears on the surface); LIN: live in Nest (hatch and live in a nest); DIN: Dead in Nest (hatch and die in the nest); LPE: Live Hatching in Pipped Egg (will hatch and live); DPE: Dead Hatching in Pipped (will hatch and die); UD: Undeveloped (not developing); UH: Unhatched (the embryo is not fully developed and dead); UHT: Unhatched Term (embryo develops perfectly and dies).

Sex Ratio

Based on anatomical and histological studies, the sex of 65 cutaneous hatchlings was found as many as 16 males and 49 females. With a sex ratio of 32.65% ≈ 33%, it can be said that in 100 female hatchlings there are 32 male hatchlings. With five relocation nests with the highest number of females (5 animals), each nest comes from Jumpai Beach (36cm), Klungkung (35cm), Serangan (35cm), Petitenget (40cm). Three nests were relocated with 4 female and 1 female hatchling from Klungkung Beach (35cm and 30cm) and Petitenget (35cm). Three nests were relocated with 3 female and 2 male hatchlings from Klungkung Beach (35cm), Petitenget (35cm), and Serangan (40cm). One nest relocated with 2 female hatchlings and 3 male males from Klungkung Beach (40cm). One nest is relocated with 1 female and 4 male hatchlings from Jumpai Beach (35cm). The full summary is shown in Table 4.4.

Gonads of the hatchlings are identified by looking at the white strip located on the ventral surface of the kidney. The male reproductive system consists of paired testes, epididymis, vas deferens, and penis, while the female reproductive system consists of a pair of ovaries, mesenteries, oviducts, and clitoris. The morphology of male and female gonads is shown as seen by the eye (Figure 4.1).

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TABLE IV: Gender Observation Results of Olive Ridley (*L.olivacea*) Tetasan Turtle Turtle Relocation Results in TCEC, Serangan, during the period 1 April - 31 May 2019.

NO.	ORIGIN	DEPTH (CM)	SEX	
			MALE	FEMALE
1	Klungkung	35	1	4
2	Jumpai	36	0	5
3	Klungkung	30	1	4
4	Klungkung	35	0	5
5	Serangan	35	0	5
6	Klungkung	35	2	3
7	Jumpai	35	4	1
8	Klungkung	40	0	5
9	Petitenget	35	2	3
10	Petitenget	35	1	4
11	Klungkung	40	3	2
12	Petitenget	40	0	5
13	Serangan	40	2	3
		Sex Ratio (%)		32,65

Note: The sex ratio is calculated from the total male hatchlings divided by the total number of female hatchlings then multiplied by 100%.

The best technique to find the position of the newly hatched gonad of the hill is to first identify a kidney that is shaped like a bean and find a white structure on the ventral side of the kidney (Figure 1). In plain view, the thickness of the ovary looks uneven, white, crescent-shaped, its tip tapered, the mesovarium is partially visible and has two wavy edges, and the oviduct is clearly visible on the abdominal wall (Figure 1.b). Conversely, males have a pair of testicles that are smooth, long, white, while the epididymis is yellow, leaf-shaped, and is located outside the testis (Figure 1.a). It should be noted that, because of its small size, the ovary and testis are sometimes difficult to distinguish from hatching.

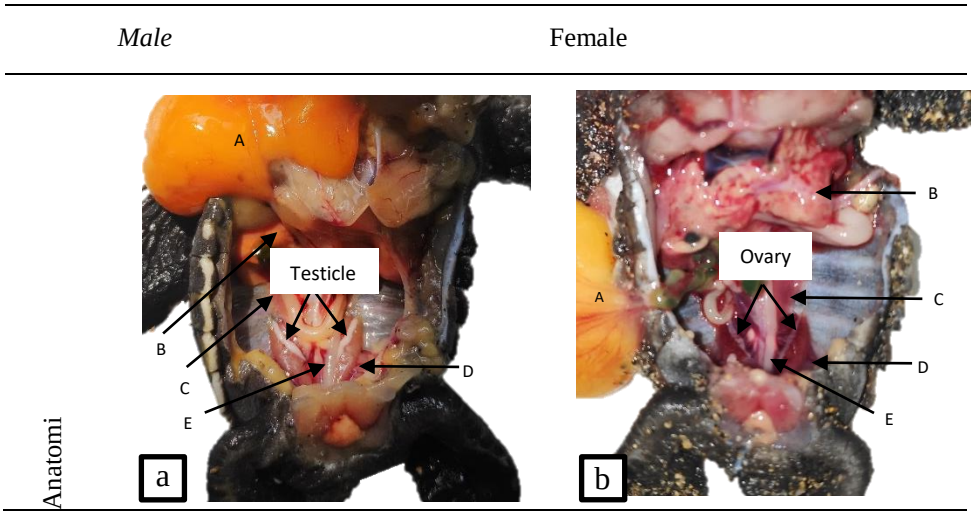

Fig. 1: Observation of Gonad Anatomy Male and female hatchlings (Remarks: A = egg yolk; B = heart; C = lung; D = kidney; E = colon)

In this case, the preparation of histological specimens can be used as a supporting tool to determine the sex of the hatchlings (Figure 2). Observations were carried out using a microscope with 100x and 400x magnification, male and female gonads consisting of cortex and medulla. The ovaries have thick cortices, where there are many different immature oocytes and have relatively few cavities in the medulla. The testis, on the other hand, has a thinner cortex and medulla with more cavities (Figure 2).

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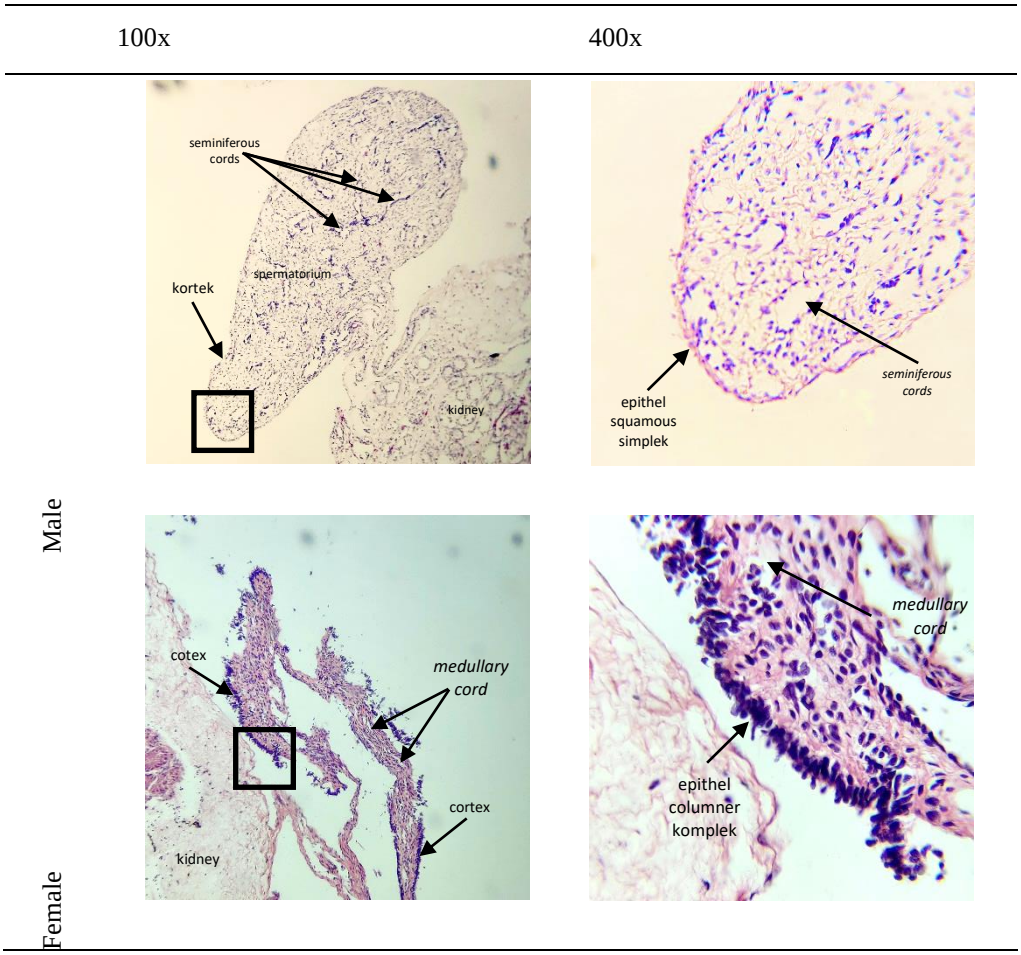

Figure 2: L.olivacea Gonad which is 2 weeks old; top: testicles; bottom: ovary

IV. DISCUSSION

The results of observation of hatching success in 13 relocated nests in TCEC showed a relatively low percentage of hatching (<70%). The hatching success of good turtle eggs in a nest is at least 70%.[6] This happens due to the technique of relocating turtle eggs carried out by TCEC staff not yet fulfilling the nest relocation code of conduct which includes relocation time, transportation media, and the technique of replanting eggs into artificial nests in TCEC. [6]Observations revealed that the trend of turtle egg removal is generally carried out more than 1 day. The transfer of turtle eggs should be done before the implantation process begins to occur at the age of 6 hours after oviposition. In addition, the transfer of turtle eggs from natural nests to artificial nests does not use appropriate transportation media (without sand provided) so that the chance position changes significantly.

This is coupled with the technique of replanting eggs into an artificial nest whose position has rotated from the initial post-oviposition position. [7],[8] Turtle eggs that experience changes in position, the embryo will experience death or a disturbance that can threaten the continuity of embryo development, because turtle eggs cannot return to their original position after rotation. This proves that the low rate of turtle egg hatching in TCEC is very related to the relocation process of turtle turtles which is not quite right. Whereas, nests originating from the local Serangan coast showed fairly good hatching success (average> 70%) given shorter travel distances, and handling (± 6 hours).

Many factors cause the failure of hatching turtle eggs. [9] Handling eggs must be done carefully. Hands must be clean of all chemical residues (e.g., sunscreen, insect repellent, etc.) before handling eggs. During the observation period, handling eggs without using gloves or hand sterilization first, allowing contaminated eggs.

[6] The success of hatching is influenced by several environmental factors such as temperature, humidity, characteristics of nest sand (fraction and color), diffusion of nest air, and salinity. However, the weaknesses of this study were not observed by these environmental factors due to equipment limitations.

The average failure of *L.olivacea* hatching in artificial nests in TCEC is 55.5%. The cause of this failure can also be attributed to the fertile or not egg. In one of the nests found 91 eggs with UD (Undeveloped) or infertile conditions which made hatching numbers low at 13.0%, this could be attributed to unfertilized turtle eggs. In another nest 12 eggs were not hatched but were accompanied by signs that the embryo had been fully developed UHT (Unhatched Term) and 36 eggs with the condition of the eggs not hatched but accompanied by signs of small embryos that were not fully developed or called UH (Unhatched), Found also a nest has 54 eggs with UH (Unhatched) conditions. One of the causes of UH and UHT is due to the depletion of oxygen supply to the eggs (high rainfall and sand density).

During embryonic development, environmental factors play an important role in the formation of hatchlings, not only in hatchery numbers but also in determining the incubation period and sex. [6] The longer the incubation period will produce more male hatchlings, and vice versa the shorter the incubation period will produce more female hatchlings. In this study, there was a positive correlation between the incubation duration of relocation nests in TCEC and the ratio of hatchling sex. Where the average incubation period of relocation nests in TCEC is 48 days (fast) to produce the dominant female hatchlings (67%). This means that the female sex ratio: male in TCEC Serangan is fairly good. [10] that the recommendation of female sex ratios: males in the nest is 70%: 30%.

The sex ratio also has a relationship with the depth factor of the nest. [6] The shallower the nest, the temperature fluctuations of the nest become more extreme so that it affects the hatching success and sex ratio. [11] Said the average depth of olive ridley turtle nests was 47.3 cm, green turtles 68.1 cm, and leatherback turtles 82.2 cm. Therefore, the nest type of the olive ridley turtle relocation in TCEC is fairly shallow (<47.3 cm) so that it is one of the factors causing low nest hatching success and the gender ratio is biased towards females.

Based on the results of this study, the management of turtle conservation in TCEC still needs improvement in nest relocation systems and hatchling hatchlings. This can be seen from low hatching success and sex ratios under recommendations. This factor is caused by the lack of competency of TCEC staff in the field of turtle conservation and reproduction.

V. CONCLUSION

The success rate of hatching of turtle eggs incubated at TCEC Serangan was 44.49% (low). The causes of the failure of hatching of turtle eggs incubated in TCEC Serangan is the high number of undeveloped eggs (38.39%), eggs with embryos that are not fully developed and die / Unhatched (8.50%), eggs with fully developed and dead embryos / Unhatched Term (1.96%), hatchlings that will hatch but die / Dead Hatching in Pipped (0.92%), and live hatchlings that cannot be perfectly hatched / Live Hatching in Pipped Egg (5 , 04%).Other factors that influence are time (> 6 hours), transportation media (without sand), and techniques for handling relocation nests (not sterile, egg rotation). Ratio (ratio) of hatchlings sex incubated in TCEC Serangan is male: female = 33%: 67%

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