Morphometric and histological studies of the gonads of male and female hatchlings of olive ridley turtles (*Lepidochelys olivacea*)

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Abstract. Sex determination in the neonatal phase is difficult because gonadal dimorphism is found in turtle hatchlings. This study aimed to confirm gonad dimorphism in olive ridley turtles (*Lepidochelys olivacea*) using morphometric and histological studies. Samples were collected from Boom Beach, Banyuwangi in turtle nests based on the number of nests, densities and habits. The dead turtle hatchlings were collected and dissected for gonad determination. We observed the morphometry of the gonad shape and size. We used H&E staining for both sexes to determine differences in the histological structure of the gonads. All data are expressed in means ± SD then analyzed using two-sample t-test (p<0.05) for significant statistical analysis. The gonads were found in the dorsal part of the body cavity, posterior to the lungs, the ventral base of the kidneys, and the walls of the peritoneum. Gonadal cortex thickness, lumen diameter of the paramesonephric duct and germinal epithelium were significantly greater in females than males. In conclusion, there were significant differences in gonads morphometry. We revealed that the structure of the gonadal cortex, the diameter of the lumen of the paramesonephric duct and the germinal epithelium can determine the sex of olive ridley turtle hatchlings.

1. Introduction

Current information regarding the sex ratio of sea turtles is needed in determining population dynamics. The sex ratio of hatchlings is allegedly different from the sex ratio as adults. Comparison between the sex ratios of hatchlings and adult turtles can inform important parameters such as mortality, migration and differences in distribution between sexes [1].

The sex ratio of hatched turtles is influenced by several factors during the egg incubation process [2]. Environmental temperature is one of the factors that influence the embryonic development process. Hatching of olive ridley turtle eggs in Brazil shows that embryos incubated at high temperature (31.2-33.1°C) can hatch as females in a higher proportion than males [3]. The eggs in the nest with cooler temperatures (26.3-30.4°C) resulted in a larger proportion of males. It is believed that
global climate change factors can affect the existence of turtle populations in the future. Climate change is the main focus in determining the sex of sea turtles [4]. Increased temperature results in an imbalance in the sex ratio of the turtle [5-7]. Incubation of turtle eggs with high temperatures in recent years has resulted in the hatching ratio of turtles with higher female sex [8,9]. The morphometric differences between male and female turtles are evident as adults. On the other hand, hatchlings did not show a clear dimorphism to identify sex easily through external observation. This study aimed to prove the differences between male and female hatchlings based on morphometry and histology of the gonad glands.

2. Material and Methods
2.1. Ethical approval
This study approved by Animal Ethics and Use Committee Universitas Airlangga in certificate number 368/HRECC.FODM/VIII/2020.

2.2. Collecting samples
This study was conducted during the turtle nesting season from May to July 2020 at Boom Beach, Banyuwangi. Patrols along the coastline were conducted at night to mark hatching turtle nests. A total of 15 dead hatchlings on the surface of the nest were collected as samples. The gonadal organ dissection was performed for analysis using H&E staining.

2.3. Statistical Analysis
Data represented in mean ± SD. Data were analyzed using two-sample t-test (p<0.05).

3. Results and Discussion
The morphometric results showed that there were significant differences in the following variables, i.e. gonadal cortex thickness, lumen diameter of the paramesonephric duct and germinal epithelium (Table 1). Compared to male hatchlings, the gonad glands of female hatchlings were larger in all variables (Figure 1).

| Aspects                     | Male       | Female     |
|-----------------------------|------------|------------|
| Gonad                       | Cortex thickness | 4.84 ± 0.16 | 26.59 ± 0.96** |
| Paramesonephric duct        | Lumen diameter | 28.63 ± 1.41 | 40.96 ± 0.89** |
|                             | Germinal epithelium thickness | 9.96 ± 0.63 | 11.14 ± 0.64* |

* p<0.05 compared to corresponding value for male  
** p<0.01 compared to corresponding value for male

Figure 1. Difference in lumen size of paramesonephric duct in male (left) and female (right)
The gonads were found in the dorsal cavity of the body, posterior to the lungs, ventral base of the kidneys, and in the walls of the peritoneum. The gonadal structure was found to be attached precisely to the kidney and enclosed in a peritoneal membrane. The remaining yolk in the embryonic phase can be found attached to the gonads [10]. Testosterone levels in the chorioalantois membrane can be measured as supporting information to determine gender [11]. Morphometry is a method of measurement in units of length with a ratio of the size of the body parts. Geometry morphometry is used as an alternative method in determining the sex of animals [12]. The analysis of morphometric geometry was able to identify the dimorphism between the sexes and was used to estimate the sex of hatchlings in non-invasive measures with high accuracy [13]. The histological characteristics of the gonads in hatchlings were carried out to determine the quantitative difference between male and female hatchlings. Sexual differentiation of the gonads into testes or ovaries occurs due to the presence of cells and fibrous connective tissue.

4. Conclusion
In conclusion, there were significant morphometric differences in the structure of the gonadal cortex, paramesonephric duct lumen diameter, and germinal epithelium in females compared to male hatchlings. Gonadal morphometry can determine the sex of hatchlings of olive ridley turtles (*Lepidochelys olivacea*).

5. References
[1] Janzen F J and Phillips P C 2006 *J. Evol. Biol.* 19 1775-1784
[2] Santos K C, Livesey M, Fish M and Lorences A C 2017 *Mitig Adapt. Strategy Glob. Change* 22 121-135
[3] Castheloge V D, dos Santos M R D and de Castilhos J C 2018 *Herpetol. Conserv. Biol.* 13 488-496
[4] Mitchell N J and Janzen F J 2010 *Sex Develop.* 4 129-140
[5] Hays G C, Broderick A C, Glen F and Godley B J 2003 *Glob. Change Biol.* 9 642-646
[6] Fuentes M M P B, Limpus C J and Hamann M 2011 *Glob. Change Biol.* 17 140-153
[7] Fuentes M M P B, Hamann M and Limpus C J 2010 *J. Exp. Marine Biol. Ecol.* 383 56-64
[8] Hawkes L A, Broderick A C, Godfrey M H and Godley B J 2007 *Glob. Change Biol.* 13 923-923
[9] Witt M J, Hawkes L A, Godfrey M H, Godley B J and Broderick A C 2010 *J. Exp. Biol.* 213 901-911
[10] Hamid I S, Ekowati J and Purnama M T E 2019 *Indian Vet. J.* 96 80-82
[11] Hamid I S, Aksono E B, Sukmanardi M and Purnama M T E 2018 *Eur. J. Oncol. Pharm.* 1 e00007
[12] Ceballos C P and Valenzuela N 2011 *Evol. Biol.* 38 163–181
[13] Ceballos C P, Hernández O E and Valenzuela N 2014 *Evol. Biol.* 41 81-98
[14] Wyneken J, Epperly S P, Crowder L B, Vaughan J and Blair Esper K 2007 *Herpetol.* 63 19-30
[15] De Solla S R, Martin P A, Fernie K J, Park B J and Mayne G 2006 *Toxicol. Chem.* 25 520-526

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