Journal of Tropical Biodiversity and Biotechnology
Volume 07, Issue 02 (2022): jtbb68824
DOI: 10.22146/jtbb.68824

Short Communication

Anatomical and Histological Characteristics of Gonad of Tropical Eel *Anguilla bicolor* McClelland, 1844 in Different Length Body Size

Farida Nur Rachmawati[1]*, Ridwan Affandi[2], Yulia Sistina[1]

1) Faculty of Biology, Universitas Jenderal Soedirman, Jl. Dr Soeparno No 63 Karangwangkal Purwokerto 53122, Central Java, Indonesia. Tel./Fax. +62-281-637894
2) Fisheries Faculty Bogor Agricultural Institute. Jl. Raya Dramaga Kampus IPB Dramaga Bogor 16680 West Java, Indonesia
* Corresponding author, email: farida.rachmawati@unsoed.ac.id

Keywords:
anatomical and histological characters
Anguilla bicolor
determination sex status
eel
gonad
Submitted:
01 September 2021
Accepted:
21 April 2022
Published:
24 June 2022
Editor:
Ardaning Nuriliani

ABSTRACT
The sex status of *Anguilla bicolor* McClelland is difficult to be distinguished between males and females. Thus, we evaluated 309 fishes from The Serayu River, with length and weight range around 9 - 81 cm and 0.59 - 1260 g respectively to access the anatomical and histological characteristics of their gonads for each sex. Parameters assessed were body sizes, eye diameter, fin length, and gonad weight. The results showed that body length, weight, eye index, and gonadosomatic index of the male were significantly smaller (p<0.01) than those of the females. Fins index was not significantly different (p>0.05) between males and females. The histological structure of gonad showed that the eel gonads can be classified as either indifferent gonads, testis, or ovary based on the length of body size. Collecting all the data together it can be concluded that *A. bicolor* is a gonochoric.

Copyright: © 2022, J. Tropical Biodiversity Biotechnology (CC BY-SA 4.0)

Anatomical and physiological parameters are essential factors in determining the maturity of eel gonads (Arai & Abdul Kadir, 2017a; Ismail et al. 2017; Ching et al. 2019). Mature male eels *Anguilla bicolor* are rarely found in freshwater (Rachmawati & Susilo 2011; Arai & Abdul Kadir 2017b; Rachmawati et al. 2017) because they prefer to live in brackish water or seawater. The immature ones, soon migrate to the sea to complete the phase of spermatogenesis (Aida et al. 2003; Tesch 2008). The length of *Anguilla japonica* males is found in fresh water and saltwater ranged between 42.5 - 63.4 cm, whereas the length of female eels is ranged between 46.1 and 85.6 cm (Sudo et al. 2013). Body length is a sex-determining factor (Sugeha et al. 2009; Arai & Abdul Kadir 2017), but body length always overlaps in different sexual statuses, thus is it challenging to distinguish the male and female eels. Previous studies reported that male eel is shorter in length of body size than that of female eel (Frisch 2004; Bark et al.2007; Tesch 2008; Côté et al. 2015). Observations of *A. bicolor's* body length was varied among researchers. Rovara et al. (2008) studied that the length of females is over 50 cm, while the lengths of indifferent gonads or interstitial gonads were under 50 cm. Whereas, Sugeha et al. (2009) reported that the body length of male *A. bicolor* in Segara Anakan were 24.5 to 54.5 cm, while the body length of females were 33.5 to 77.8 cm. So, it is interesting to observe the anatomical characteristics of eels at various lengths body sizes in relation to their sex.

This survey was carried out from September 2015 to December 2016. *Anguilla bicolor* samples were caught from The Serayu River. Total of 309 eels with a length range between 9 - 81 cm and weight range between 0.59 - 1260 g were reared in a fibre aquarium with the size 2 x 1 x 1 m^3. We assessed some parameters such as body sizes, eye diameter, fin length, and gonad weight to evaluate the anatomical characteristics such as eye index, fin index, GSI (Gonadosomatic Index), and sex status. Prior to data collection, eels were anesthetized for about 15 minutes in water containing clove oil at the dose of 5 ppm (Rachmawati & Susilo 2011; Rachmawati et al. 2017). The eel was weighed with a technical scale and measured the body length with a ruler. The body length was measured using standard scale from the head to the tail. Eye index was calculated based on eye diameter measurement, using a calliper by measuring the horizontal and vertical diameter of the orbital of the eye (Yokouchi et al. 2009). Fin index was calculated based on Yokouchi et al. (2009) by measuring the length of the fin from the base to the tip of the pectoral fin. Gonads were collected from the anus to the pectoral, and weighed using an analytical scale for the measurement of GSI (Gonadosomatic Index), according to Rupia et al. (2014). Histological observation of gonads follows standard procedures: gonad was sliced 1 cm^3, fixed in NBF 10% for tissues processed with routine paraffin embedding, sliced approximately 6 μm in transverse sections, and stained with Haematoxylin-Eosin for histological assessment under a light microscope. Data were analyzed using one-way ANOVA, with 95% or 99% significance and qualitative data was analyzed descriptively (De Smith 2018).

The results showed that anatomically, eel gonads with a body length of 9 to 28.5 cm, looks like a thin thread that are milky white (Figure 1). They were called as indifferent gonads. Moreover, histological structure of those indifferent gonads was dominated by primordial germ cells with a large nucleus (Figure 2).

The gonad fish with length range of 22 to 42.70 cm showed anatomical structure of a lobular transparent with a round shape along the ligament and clear white like jelly appearance (Figure 3). This structure under histological assessment were showing presence of tubular containing spermatogonia (Figure 4), so they were testicles (male).

Anatomical structure of the gonad's length of eel range 24 to 81 cm was showed structure like a ribbon/lamella, milky white, folded along sides both (the right and left sides) of the abdomen (Figure 5). Histological structure of the gonad showed the structure of follicles (Figure 6), indicating ovary (female).

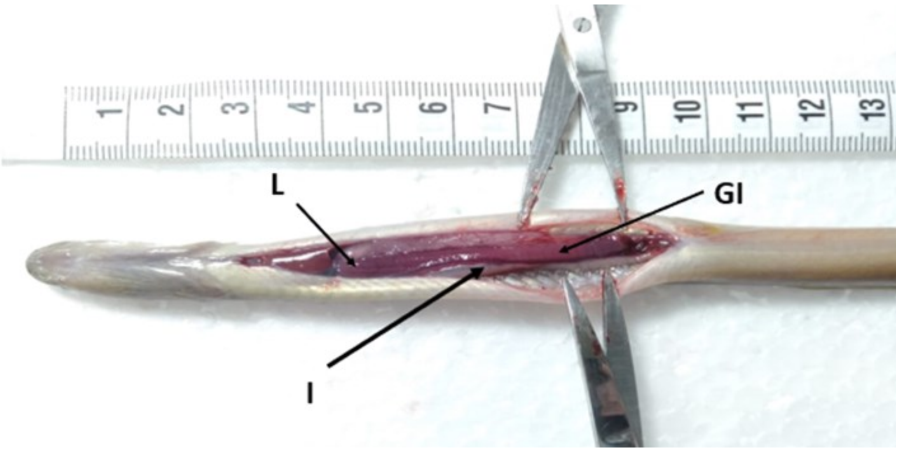

Figure 1. Anatomical Structure of Indifferent Gonad of Tropical Eel *Anguilla bicolor* McClelland (I: indifferent gonad; L: liver; GI: gastrointestinal).

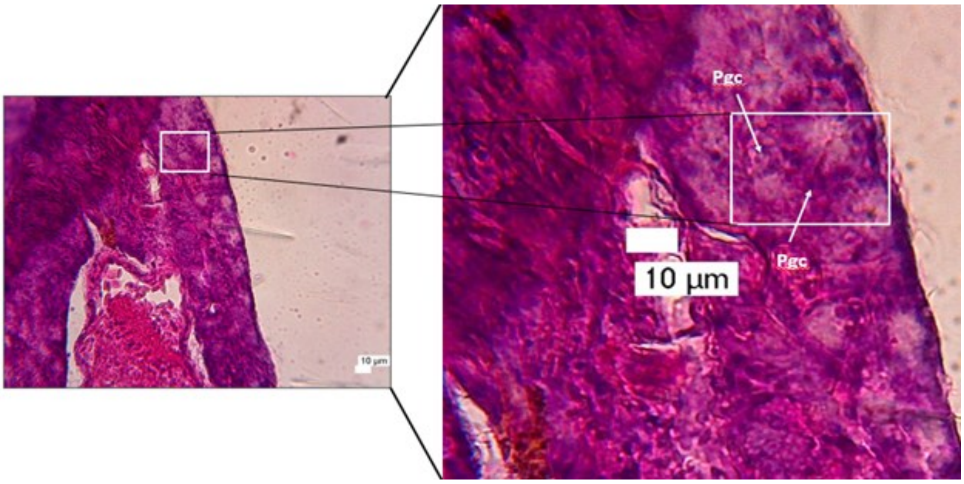

Figure 2. Histological Structure of Indifferent Gonad of Tropical Eel *Anguilla bicolor* McClelland (Pgc: primordial germ cell). Haematoxylin-Eosin Staining. Scale bar: 10 mm.

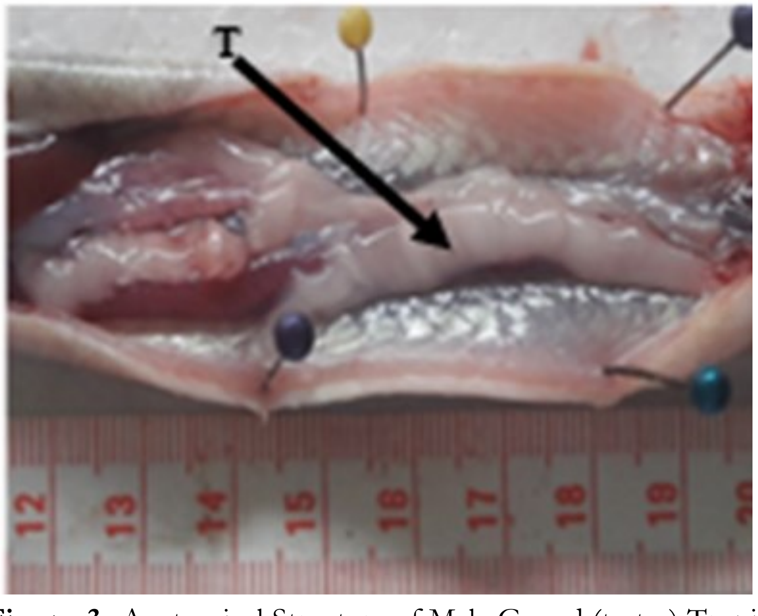

Figure 3. Anatomical Structure of Male Gonad (testes) Tropical Eel *Anguilla bicolor* McClelland (T: testes).

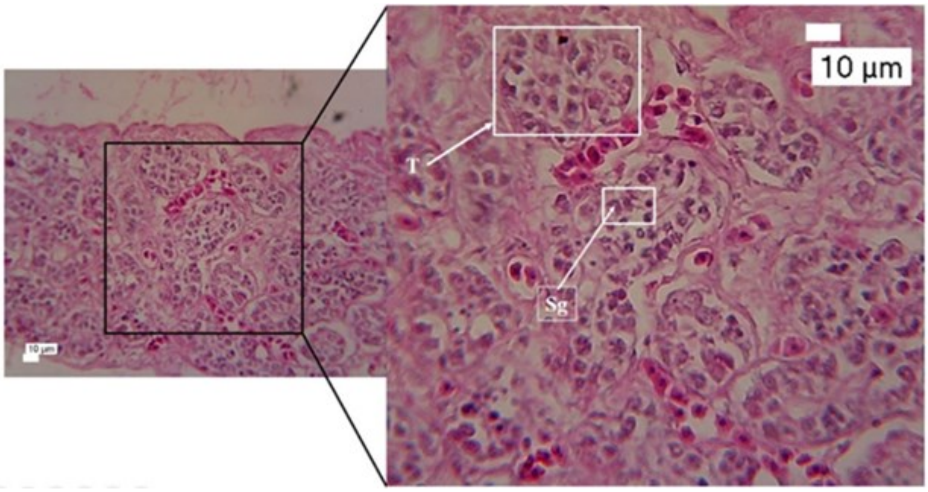

Figure 4. Histological Structure of Testes of Tropical Eel *Anguilla bicolor* McClelland (T: tubules; Sg: spermatogonium). Haematoxylin-Eosin Staining. Scale Bar: 10 µm.

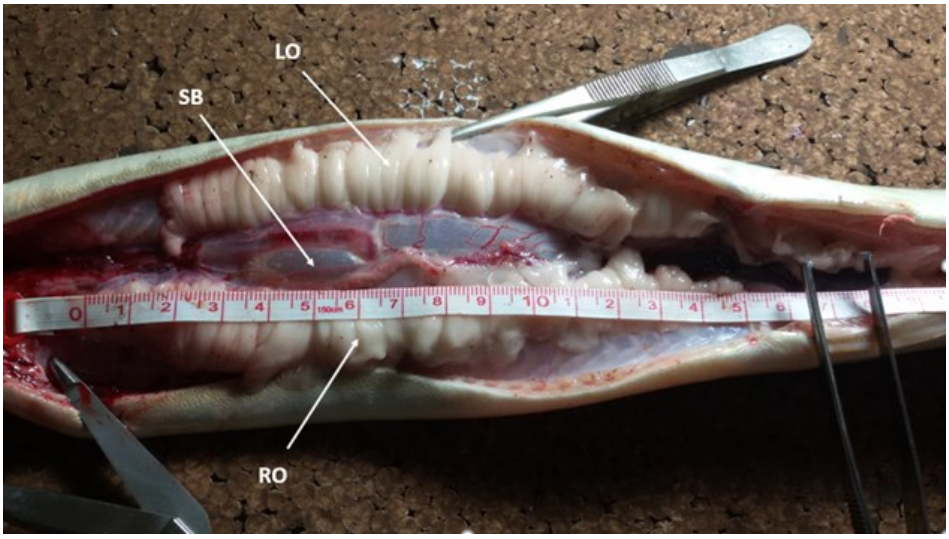

Figure 5. Anatomical Structure of Female Gonads (Ovaries) of Tropical Eel *Anguilla bicolor* McClelland (LO: left ovary; RO: right ovary; SB: swim's bladder).

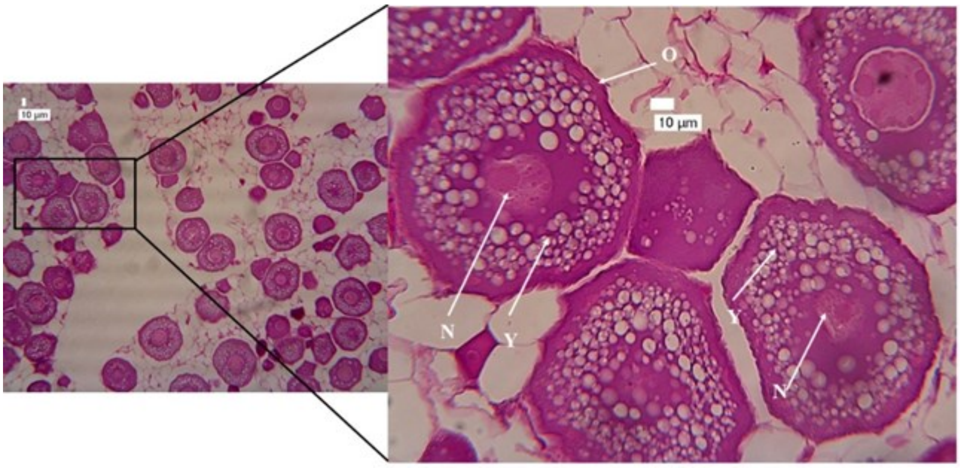

Figure 6. Histological structure Follicles of Female Gonad (Ovaries) of Tropical Eel *Anguilla bicolor* McClelland (O: follicle cells; N: nucleus; Y: Yolk). Haematoxylin - Eosin Staining. Scale Bar: 10 µm.

These results proved that gonads of *A. bicolor* are similar with that of *A. marmorata* (Wakiya et al. 2019), composed of three sexual characteristics:

indifferent gonad, testes, and ovary. This study differs from previous report in *A. anguilla*, namely (1) indifferent gonad, (2) interstitial gonad, (3) male gonad, and (4) female gonad (Sugeha et al. 2009). Geffroy et al. (2013) reported that indifferent gonad of *A. rostrata* developed from interstitial stages (Sirsky organs) or directly from ovaries. The Sirsky organ was an intersexual organ histologically dominated by male structures but contains a small number of oocytes (Geffroy et al. 2013). Such structure was not found in this study. Based on anatomical observations of the gonad, it is easier to distinguish testicles from ovaries on samples longer than 30 cm, however below that size, the gonad is difficult to be determined.

Histologically, gonad of eel with the length range of 9.00 to 28.50 cm (17.3514 ± 4.2799 cm) composed of cells with large nuclei (Figure 2). Thus, type of gonads was called indifferent gonad. The eel with total body length ranges between 22.00 and 42.70 cm (28.2919 ± 5.1640 cm), consists of testis tubules (Figure 4), so they were male. Whereas the eel with total body length range of 24.00 to 81 cm (47.4259 ± 19.1525 cm) was composed of follicles ovaries, thus they were females (Figure 6). Overall, the quantitative data on eye index (EI), fin index (FI), GSI, as well as body length (BL) and body weight (BW) with sex status showed that their anatomical data were overlapping not as a discrete condition (Table 1).

Table 1. Mean (± SD) of anatomical parameter of *Anguilla bicolor* McClelland in each sex status (indifferent, male, and female)

Parameter		Sex Status		
		Indifferent	**Male**	**Female**
BL (cm)	Range	9.00 – 28,50	22.00 -42,70	24.00 – 81,00
	Mean ± SD	17.3514 ± 4.2799	28.2919±5.16340	47.4259±19.1525
BW (g)	Range	0.59 – 29,00	13.00 – 157,00	20.29 – 1062,00
	Mean ± SD	9.7018 ±7.9443	38.4646±34.9282	289.2168±289.8995
EI	Range	0.12 – 7,58	0.16 – 15,34	0.57 – 18,28
	Mean ± SD	1.4658±1.2207	3.5847±3.5678	5,5193±2,9787
FI	Range	1.74 – 6,51	1.60 – 6,84	2.88 – 6,53
	Mean ± SD	3.2939±0.8721	4.4802±1.2599	4.4380±0,8750
GSI (%)	Range	0.03 – 4,21	0.03 – 0,81	0.03- 4,37
	Mean ± SD	0.3617±0.6121	0.2796±0.1980	1,1538±1,0573
N		147	74	88

Notes:
BL: body length
BW: body weight
EI: eye index
FI: fin index
GSI: gonadosomatic index

The average of eye index values of an indifferent gonad, male, and female were 1.4658, 3.8067, and 5.3617, respectively (Table 1). The mean of eye index of eel overlaps between each sex ($p < 0.01$).

Value of the fin index at each stage of gonad corresponds to the sex status ($p < 0.05$). The average of fin index among the sex status is 3.2939 ±

0.8721 (indifferent gonad); 4.4802 ± 1.2599 (male), and 4.4380 ± 0.8750 (female). The values were similar between males and females (Table 1), thus cannot be used to determine the sex. Previous report shown that FI was not related to the sex status but rather to the degree of maturity of the gonad (Durif et al. 2009; Van den Thillart & Dufour 2009; Nowosad et al. 2014). The increasing of swimming activity during the migration from freshwater to seawater can increase the fin index value (Durif et al. 2009; Van den Thillart & Dufour 2009; Nowosad et al. 2014).

The GSI value was range between 0.03 and 4.37% (Table 1). The mean of GSI value (%) in indifferent gonad, male, and female was 0.3617 ± 0.6121; 0.2796 ± 0.1980; and 1.1538 ± 1.0573 respectively (Table 1). The highest GSI value indicates female individual as compared to other sex gonadal ($p <$ 0.01).

Our study showed that anatomical and histological characteristics of *Anguilla bicolor* McClelland's, such as length, body weight, eye index, and GSI can be used as a sex-determining factor for tropical eel *A. bicolor.* Based on the results, it can be concluded that *A. bicolor* has a gonochoric not hermaphrodite, with three different sex statuses which are indifferent gonad, male, and female.

Data analysis showed that anatomical parameter indices in *A. bicolor* can be used as a sex determinant (p<0.01). Data measurement of this study found that length of indifferent gonad was range from < 21 cm up to 33 cm, the length of male was range from 21.1- 60 cm, whereas female had body length range from > 21 cm (Figure 7). These results also proved that sex status was overlapping in *A. bicolor* (Figure 7). The results of this study confirmed previous studies in *A. japonica, A. bicolor* (Sugeha et al. 2009), and *A. rostrata* (Krueger & Oliviera 1999; Jessop 2010) which proved that the body length can be used to determine sex status.

AUTHORS CONTRIBUTION

F.N.R. collected and analysed the data and wrote the manuscript. R.A. designed the research and supervised all the processes. Y.S. managed all the processes and the English translation.

ACKNOWLEDGMENTS

We would like to thank the Jenderal Soedirman University for funding this research (2017/UN23.14/PN/2016).

CONFLICT OF INTEREST

We declared that we have no known competing for financial interests or personal relationships that could have influenced the work reported in this paper.

REFERENCES

Aida, K., Tsukamoto, K. & Yamauchi, K. (Eds.). 2012. Eel biology. Springer Science & Business Media.

Arai, T. & Abdul Kadir, S. R., 2017. Opportunistic spawning of tropical anguillid eels *Anguilla bicolor bicolor* and *A. bengalensis bengalensis*. *Scientific Reports*, 7(November 2016), *1–17*. doi: 10.1038/srep41649

Bark, A. et al., 2007. Current status and temporal trends in stocks of European eel in England and Wales. ICES *Journal of Marine Science*, 64, pp.1368 – 1378.

Ching, F. et al., 2019. Histological investigation on gonad maturation of cultured shortfin eel, *Anguilla bicolor* (McClelland, 1844) in captivity. *Journal of Physics: Conference Series*, 1358, pp. 12–16. doi: 10.1088/1742-6596/1358/1/012016.

Côté, C. L. et al., 2015. Growth, Female Size, and Sex Ratio Variability in American Eel of Different Origins in Both Controlled Conditions and the Wild: Implications for Stocking Programs. *Transactions of the American Fisheries Society*, 144(2), pp. 246–257. doi: 10.1080/00028487.2014.975841.

De Smith, M.J., 2018. A Comprehensive Handbook of Statistical Concept, Techniques and Software.

Durif, C.M.F. et al., 2009. Seasonal evolution and individual differences in silvering eels from a different location. In: G. Van den Thillart, J. Rankin & S. Dufour (eds) Spawning Migration of the European Eel. Springer, Netherland. Pp.13 – 38. Tools. The Winchelsea Press, Drumlin Security Ltd, Edinburgh. 660 pp.

Frisch, A., 2004. Sex-change and gonadal steroids in sequentially hermaphroditic teleost fish. *Reviews in Fish Biology and Fisheries*, 14, pp. 481 – 499.

Geffroy, B. et al., 2013. New insights regarding gonad development in European eel: Evidence for a direct ovarian differentiation. *Fish Physiology and Biochemistry*, 39(5), pp. 1129–1140. doi: 10.1007/s10695-013-9769-7

Ismail, M. F. et al., 2017. Length-weight relationship and associations between otolith dimension, age and somatic growth of *Anguilla bicolor bicolor* (McClelland, 1844) from Northwest of Peninsular Malaysia. *Tropical Natural History*, 17(1), pp. 59–64.

Jessop, B. M., 2010. Geographic effects on American eel (*Anguilla rostrata*) life history characteristics and strategies. *Canadian Journal of Fisheries and Aquatic Sciences*, 310, pp. 237–244.

Krueger, W. H. & Oliveira, K., 1999. Evidence for environmental sex determination in the American eel, Anguilla rostrata. *Environmental Biology of Fishes*, 55, pp. 381–389.

Nowosad, J, et al., 2014. Changes in body weight and eye size in female European eel kept in fresh and saltwater. *Italian Journal of Animal Science*, 13, pp. 382 – 386. doi: 10.4081/ijas.2014.3144.

Rachmawati, F. N. & Susilo, U., 2011. Profil Hormone dan Kinerja Reproduksi Ikan Sidat (Anguilla bicolor McClelland) yang Tertangkap di Perairan Segara Anakan Cilacap. Hormone Profile and Reproductive Performance of *Anguilla bicolor* McClelland which Captured at Segara Anakan Lagoon, Cilacap. *Biota*, 16(2), pp. 221–226.

Rachmawati, F. N., Susilo, U. & Muslih., 2017. Karakteristik reproduksi ikan sidat Anguilla bicolor McClelland, 1844 yang diinduksi GNRH-analog. *Jurnal Iktiologi Indonesia*, 17(2), pp. 155. doi: 10.32491/jii.v17i2.355.

Rovara, O. et al., 2008. Pematangan Gonad Ikan Sidat Betina (Anguilla bicolor bicolor) melalui induksi ekstrak hipofisis. *Jurnal Ilmu-ilmu Perairan dan Perikanan Indonesia* 15(1), pp. 69-76.

Rupia, E. J. et al., 2014. Effect of hormone injection frequency on the lipid content and fatty acid compositions in gonad, muscle, and liver of Anguilla japonica during artificial maturation. *Aquaculture International*, 22 (3), pp. 1105–1120. doi: 10.1007/s10499-013-9731-9.

Sudo, R. et al., 2013. Age and body size of Japanese eels, *Anguilla japonica*, at the silver stage in the Hamana Lake system, Japan. *Coastal Marine Science*, 36 (1), pp. 13 – 18.

Sugeha, H.Y. et al., 2009. Sexual development of the tropical short-finned eel Anguilla bicolor bicolor of the Segara Anakan waters, Central Java, Indonesia. *Jurnal Perikanan*, XI (1), pp. 87-99.

Tesch, 2008. The eel. In John willey and sons. 15 (380), pp. doi: 10.2307/1443633

Van den Thillart, G. & S. Dufour., 2009. How to estimate the reproductive succes of European silver eels. *In*: G. Van den Thillart, J. Rankin & S. Dufour (eds) Spawning Migration of the European Eel. (Pp. 3 – 9). Springer, Netherland.

Wakiya, R. et al., 2019. "Age, Growth, and Sex Ratios of the Giant Mottled Eel, Anguilla Marmorata, in Freshwater Habitats Near Its Northern Geographic Limit: A Comparison to Tropical Regions." Zoological Studies 58: e34. doi: 10.6620/ZS.2019.58-34.

Yokouchi, K. et al., 2009. Biological Characteristics of Silver-Phase Japanese Eels, Anguilla japonica Collected from Hamman Lake, Japan. *Coastal Marine Science*. 33 (1), pp. 1 – 10.