Journal of Tropical Biodiversity and Biotechnology
Volume 08, Issue 02 (2023): jtbb74702
DOI: 10.22146/jtbb.74702

Research Article

Differences in Swim Bladder Histology of *Anguilla bicolor bicolor* at Various Stages of Sexual Maturity

Nur Indah Septriani[1]*, Muhammad Ardillah Rusydan[1], Gizela Aulia Agustin[1], Nareta Defiani[1], Fajar Sofyantoro[1], Ariel Hananya[2], Dwi Eny Djoko Setyono[3]

1)Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281
2)PT. Delos Teknologi Maritim, Jl. Salak No.38, RT.6/RW.2, Guntur, Kecamatan Setiabudi, Kota Jakarta Selatan Daerah Khusus Ibukota Jakarta 12980
3)National Research and Innovation Agency (BRIN) Gunungkidul, Yogyakarta, Indonesia 55861
* Corresponding author, email: nurindahseptriani@ugm.ac.id

Keywords:
silver eel
yellow eel
gas gland
mucosa
submucosa
Submitted:
17 May 2022
Accepted:
19 January 2023
Published:
09 June 2023
Editor:
Ardaning Nuriliani

ABSTRACT

The current study observed the histological differences of the swim bladder of the tropical eel, *Anguilla bicolor bicolor*, as an adaptation resulting from hydrostatic change. A total of 15 eels were collected from Pasir Puncu, Keburuhan, Purworejo and Segara Anakan, Cilacap, Indonesia in June 2017, September 2020, and April 2021. The eels were grouped into 4 stages based on the silvering stage and sex, namely: yellow undifferentiated, yellow female, silver male, and silver female. The average length and body weight of yellow undifferentiated eels were 255.07 ± 45.91 mm and 13.66 ± 8.5 g, respectively; for yellow female, the values were 374.35 ± 41.51 mm and 56.5 ± 12.02 g; for silver male, the values were 432.43 ± 15.15 mm and 140.29 ± 13.85 g; and for silver female were 702 ± 0.00 mm and 545 ± 11.31 g. The present study successfully recorded the histological structure of the swim bladder of *A. bicolor bicolor* in silver male and silver female stages. Silver males and females displayed a greater significant development of the swim bladder than yellow stages in the gas gland, mucosa, and submucosa layers. These results suggest that an increase in the gas gland thickness allows a greater contribution from gas to gas secretion, the mucosa exerts a mechanical effect on the newly formed gas bubbles, and the submucosa thickness reduces gas conductivity from the swim bladder wall.

INTRODUCTION

Eels (Anguillidae, *Anguilla*) are a facultative catadromous fish, with juveniles living in estuaries, rivers, and lakes, while the adult stage migrates to marine environments for reproduction (Tesch 2003). The life cycle of freshwater eels is divided into 5 stages, namely leptocephalus larvae, glass eel post-larvae, elver young eel, non-mature adult yellow eel, and migratory adult silver eel (Bertin 1956; Cresci 2020; Hatakeyama et al. 2022; Van Wichelen et al. 2022). Upon sexual maturation, the eels undergo physiological and morphological changes to help them undertake the long and difficult oceanic migration to offshore spawning sites. Eels usually display changes in morphological and physiological characters that include an increase in eye diameter (Pankhurst 1982; Beullens et al. 1997), changes in the integumentary structure and colour (Pankhurst & Lythgoe 1982), changes in retinal sensitivity to adapt to dark environments (Pankhurst & Lythgoe 1983; Bowmaker et al. 2008), lateral line

differentiation (Zacchei & Tavolaro 1988), gastrointestinal degeneration (Pankhurst & Sorensen 2011), changes in osmoregulatory processes (Righton et al. 2012), and swim bladder modification (Yamada et al. 2001) used as a volume gas exchange regulator during swimming (Smith & Croll 2011).

The swim bladder of *Anguilla* is classified as physostomous in the glass eel stage and is supplied and discharged through the pneumatic duct (Prem et al. 2000). However, the swim bladder behaves more like physoclistous fish during the metamorphosis process from yellow to silver phase (Pelster 2013). Research on changes in swim bladder at the developmental stage of *Anguilla rostrata* has been previously reported, revealing that the concentration of crystal guanine deposition within the swim bladder in American eels increased 1.5 times with gonadal maturity (Kleckner 1980a; Kleckner 1980b) and increasing acid secretion in gas gland during silvering process (Drechsel et al. 2022). Another study revealed that the length and luminal diameter of the rete mirabile capillaries in the silver eel stage of *A. rostrata* are higher than that of the yellow stage (Yamada et al. 2001). In addition, the GSI value of *A. rostrata* is lower than 3.5 and producing 5 times more gas compared to yellow eels (Yamada et al. 2001), allowing *A. rostrata* to migrate and lay eggs in deeper waters (Schneebauer et al. 2021). Swim bladders of Japanese eels *Anguilla japonica* start to develop in rivers or in shallow seawater before entering the open sea, once they are in the open sea, the swim bladder characteristics become stable (Tesch 2003). The previous study also assumed that swim bladder function would be improved compared to the yellow eels during their spawning migration experience in high hydrostatic pressure (Pelster 2015; Schneebauer et al. 2021; Drechsel et al. 2022).

Research on swim bladders in tropical eels is very limited, and given the theory that tropical eels have closer spawning grounds than temperate eels (Aoyama et al. 2003), it's possible that changes in swim bladder characteristics are less obvious. The current study aimed to observe the histological structure of the swim bladder of the tropical eel *Anguilla bicolor bicolor* as the consequence of adaptation to environmental hydrostatic change to spawn in the open sea. The results of this study are expected to provide the baseline information for sustainable conservation of tropical eels by differentiating the histological structure of the swim bladder between the yellow and silver stages, as well as males and females.

MATERIALS AND METHODS

Sampling Location and Staging

Live eels were collected with *wuwu* (traditional baited trap) from Segara Anakan, Cilacap, Indonesia, (Figure 1A) in June 2017 and with fishing gear (Maguro, Lieyuwang 5000, Damyil, and Deco) from Pasir Puncu, Kaburuhan, Purworejo, Indonesia, (Figure 1B) in September 2020 and April 2021. The map figures were constructed using QGIS software. Individuals were identified through morphological characters (Ege 1939) and grouped into four stages based on gonad maturity and sex (Melia et al. 2006; Sugeha, et al. 2009; Geffroy & Bardonnet 2016; Arai & Abdul Kadir 2017). The four stages are yellow undifferentiated, yellow female, silver male, and silver female. Yellow eels present partial melanization at the tip of the pectoral fins and yellow color on the belly, while silver eels present full melanization at the tip of the pectoral fins and metallic hue on the belly (Okamura et al. 2007).

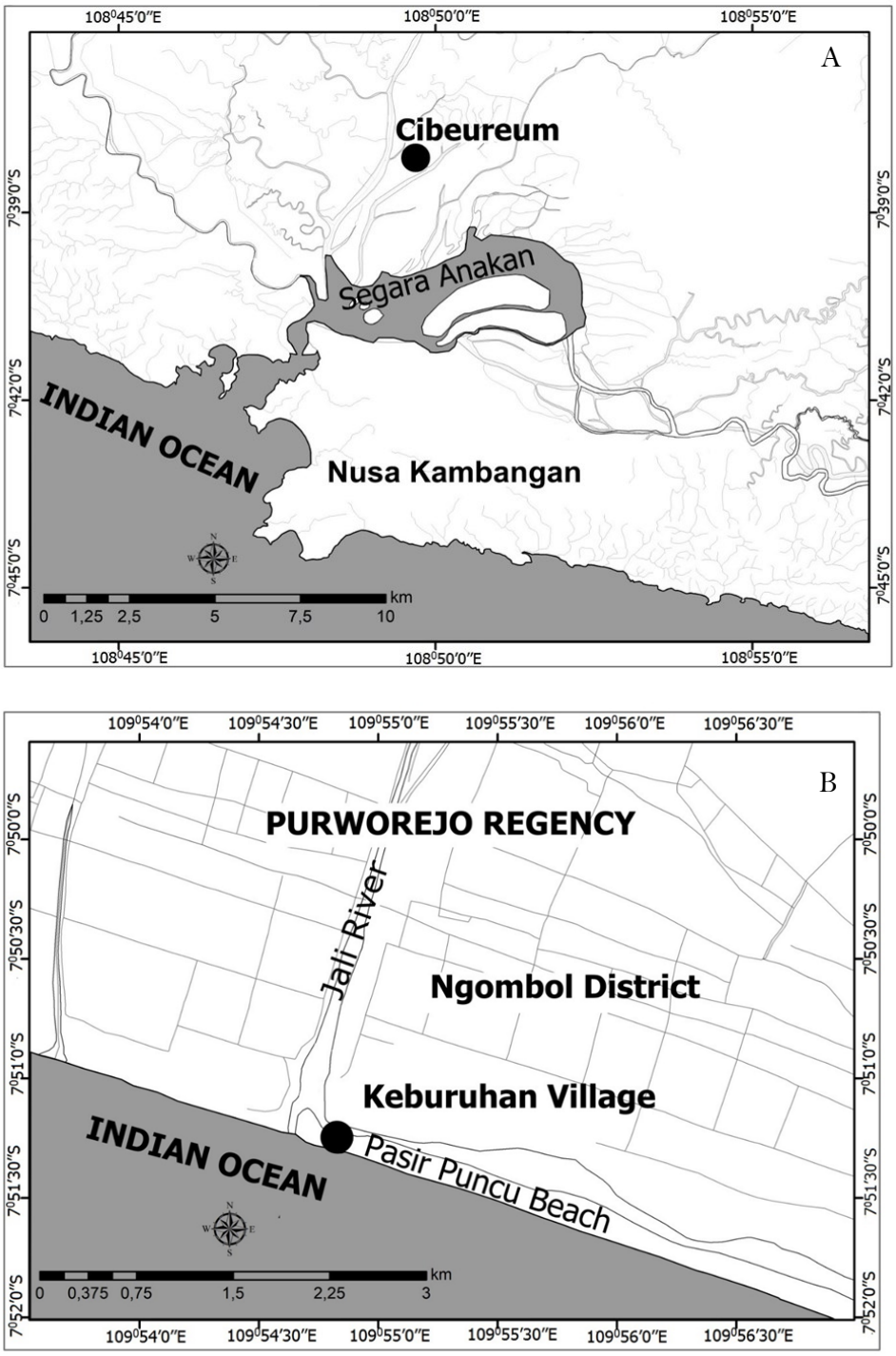

Figure 1. The map shows the study area in (A) Segara Anakan and (B) Pasir Puncu, Indonesia.

Morphometric Measurements

Live eels were transported to the Laboratory of Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada, Indonesia, where they were anaesthetized and euthanized with 100 ppm MS222, following animal welfare policies of The Integrated Research and Testing Laboratory (No. 00117/04/LPPT/II/2017), to acquire their external and internal biometrics.

Morphological measurements included total length (TL), horizontal eye diameter (HED), vertical eye diameter (VED), and pectoral fin length (PFL). These measurements were carried out using a digital caliper (Taffware SH 20) with an accuracy of 0.01 mm. Body weight (BW) was determined using a digital weighing scale to the nearest 0.01 g. BW

of less than 100 g, swim bladder weight (SBW), and gonadal weight (GW) were determined using a digital jewelry scale (8028-series) to the nearest 0.001 g. TL, EDV, EDH, GW, BW, and PFL values were used to measure the following:

Gonadosomatic Index (GSI) = 100 GW BW^{-1}

Eye Index (EI) = 100π TL^{-1} [0.25 (HED+VED)] ^{2} (Pankhurst 1982)

Pectoral fin index (PFI) = 100 PFL TL^{-1} (Hagihara et al. 2012; Huyen et al. 2022)

Swim bladder Index (SBI) = SBW BW^{-1} (Hagihara et al. 2012; Huyen et al. 2022)

Histological Preparation

The swim bladders of *A. bicolor bicolor* were prepared following the standard paraffin method (Layton et al. 2018). Initially, the swim bladders were extracted from the body, fixed with Bouin's solution overnight, and washed with 70% alcohol. The next step was tissue dehydration using graded ethanol followed by immersion in toluene or xylene EMSURE® solution to purify the tissue (Wolfe 2019).

When the tissue was ready, it was infiltrated with paraffin (Leica Surgipath®, Leica Biosystem Richmond, Inc. USA). Sections were trimmed into 6 slides from every sample with a thickness of 5 µm using a microtome (MICROM HM310®). Afterwards, the tissue was stained with hematoxylin eosin (HE) dye consisting of hematoxylin Ehrlich, eosin 2%; and Mallory acid fuchsin (MAF) dye consisting of Mallory (aniline blue and orange blue), phosphomolybdic acid (PMA) 1%, and acid fuchsin. Tissue observation was carried out using a Leica ICC50® microscope to observe the gas glands, the mucosa, and submucosa layers with 10 x 10 and 40 x 10 magnifications. The thickness of the gas gland, mucosa, and submucosa layers were measured to the nearest µm using the Image J software, and the data are presented as Gas Gland Index (GGI), Mucosa Index (MI) and Submucosa Index (SMI) (Schneider et al. 2012).

Histological Measurements

To differentiate the maturation levels and adaptation to hydrostatic environment, histological differences were examined. The histological structures of the swim bladders were analyzed descriptively, qualitatively, and quantitatively by comparing the swim bladder index. Histological observations were carried out based on the thickness of gas gland cells (GG), mucosa (M), and submucosa (SM) layers of the undifferentiated yellow, yellow eels, silver males, and silver females. The GGI, MI, and SMI were measured as in Yamada et al. (2004) as follows:

Gas Gland Index (GGI) = 100 GG TL^{-1}

Mucosa Index (MI) = 100 M TL^{-1}

Submucosa Index (SMI) = 100 SM TL^{-1}

GGI, MI, and SMI were calculated from 1-6 slides for each sample, and every slide was measured from 5 directions as replications.

Data Analysis

The collected data were analysed using the Kruskal-Wallis test and the pairwise comparison test with a confidence level of 95%. All statistical analyses were conducted using the SPSS software version 24.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

As shown in Table 1, eels obtained from Pasir Puncu (n=8) belonged to yellow undifferentiated, yellow female, and silver male. Meanwhile, the

Segara Anakan eels (n=7) only consisted of the silver male and female. In this study, the total length and body weight of female silver is higher than male silver eel. This also supports previous research that mature female *A. bicolor bicolor* is larger in size than male *A. bicolor bicolor* (Arai & Abdul Kadir 2017).

The sizes (total length and body weight) of silver stage (male and female) were significantly larger ($p < 0.05$) than those of yellow undifferentiated. Meanwhile, the sizes of yellow undifferentiated and yellow female were not significantly different. Additionally, the total length and body weight of the silver stages were not significantly different from those of the yellow stage of females (Figure 2).

The statistical analyses results showed that GSI, EI, and PFI of silver males were significantly higher than those of yellow undifferentiated, while SBI did not show significant differences among the four categories (Figure 3).

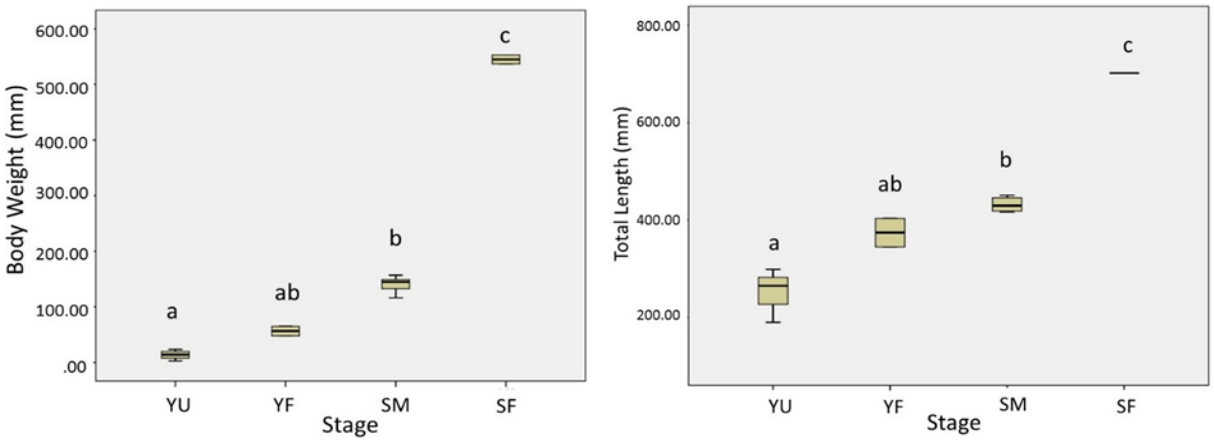

Figure 2. Total length and body weight of eels (*A. bicolor bicolor*); YU - yellow undifferentiated; YF - yellow females; SM - silver males; SF - silver female; different letters on the bar indicate significant differences between stages.

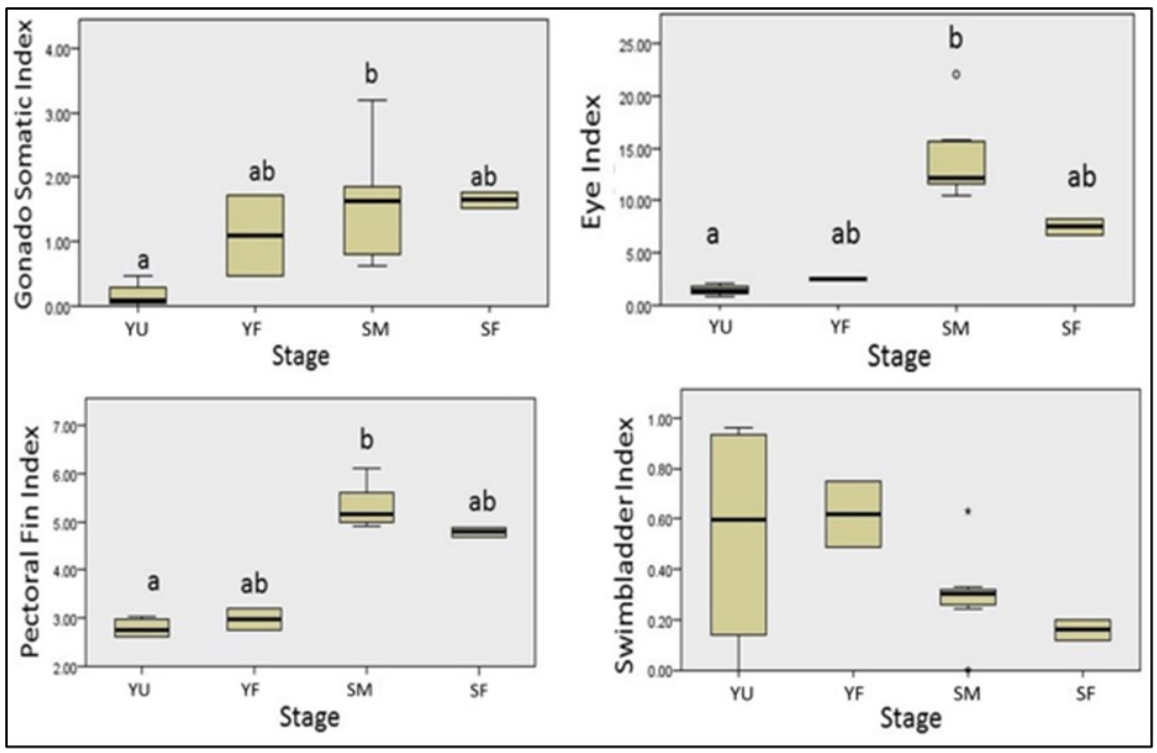

Figure 3. Gonadosomatic index (GSI), eye index (EI), pectoral fin index (PFI), swim bladder index (SBI) of *A. bicolor bicolor*; YU - yellow undifferentiated; YF - yellow female; SM - silver male; SF - silver female; different letters on the bar indicate significant differences between stages.

Table 1. Total length, body weight, stage, and sex of *A. bicolor bicolor* collected from Segara Anakan and Pasir Puncu.

Specimen No.	*Total length (mm)*	*Weight (g)*	*Sampling location*	*Sampling date*	*Stage and sex*	*TL ± SD and W ± SD*
1	264.49	23.4	Pasir Puncu	April 2021	yellow undifferentiated	255.07 ± 45.91 mm and 13.66 ± 8.50 g
2	266.1	12.45				
3	299.18	15.88				
4	190.52	2.92				
5	345	48	Pasir Puncu	September 2020	yellow female	374.35 ± 41.51 mm and 56.5 ± 12.02 g
6	403.7	65				
7	430	157	Pasir Puncu	September 2020	silver male	432.43 ± 15.15 mm and 140.29 ± 13.85 g
8	418	150				
9	450	145	Segara Anakan	June 2017		
10	451	148.23				
11	442	133.75				
12	419	131.76				
13	417	116.27				
14	702	537	Segara Anakan	June 2017	silver female	702 ± 0.00 mm and 545 ± 11.31 g
15	702	553				

As shown in Figure 4 a-h, the swim bladder consists of 3 layers, 1) the gas gland layer (GG) containing cuboidal epithelium, gas gland cells, and blood vessels, 2) the mucosa layer (M) in the form of smooth muscle, and 3) the submucosa layer comprising dense connective tissue. MAF staining shows collagen fibers (CF) as blue, while blood vessels in *rete mirabile* (RM) and GG (Figure 4e and h) were stained as purple. The epithelium of gas gland in the yellow undifferentiated stage is cuboidal in shape and not folded, whereas in the yellow female and silver stages, the epithelium is folded. The epithelial cells size in the silver stage are longer. There are blood vessels (BV) in the lamina propria located in the basal of the gas gland layer at the yellow stage, while at the silver stage, blood vessels are found abundantly in between the gas gland cells. Higher developmental stage results in thicker mucosa and submucosa layers, which consists of connective tissue that regulate gas permeability (Morris & Albright 1975).

The GGI range was between 0.45 ± 0.08 and 1.18 ± 0.23 in yellow undifferentiated eels, between 0.96 ± 0.15 and 1.12 ± 0.3 in yellow females, between 1.05 ± 0.27 and 2.92 ± 0.67 in silver males, and from 2.06 ± 0.36 to 2.12 ± 0.79 in silver females (Table 2). The highest GGI (2.37 ± 0.77) was observed in silver male, while the lowest (0.73 ± 0.32) was found in the yellow undifferentiated (Table 2). These results are lower compared to those of Japanese eel, in which the GGI can reach up to 10 (Yamada et al. 2004).

The GGI and SMI of yellow stages (undifferentiated and female) were significantly lower than those of silver stages (male and female), but not between the same color stages. While the MI was significantly different between the first three stages, this was not the case between silver stages (Figure 5).

The silvering process of freshwater eels has been reported to be accompanied by increased body size and GSI to support egg ripening and increased swimming capabilities to prepare for oceanic migrations to spawning locations (Durif et al. 2006). Compared to the males, female eels tend to maximize their body weight and length to maximize fecundity (Wenner & Musick 1974; Vøllestad & Jonsson 1986; Helfman et al. 1987). The EI of *A. bicolor bicolor* in this study also showed 3 folds growing trend in females with increasing sexual maturity from yellow to sil-

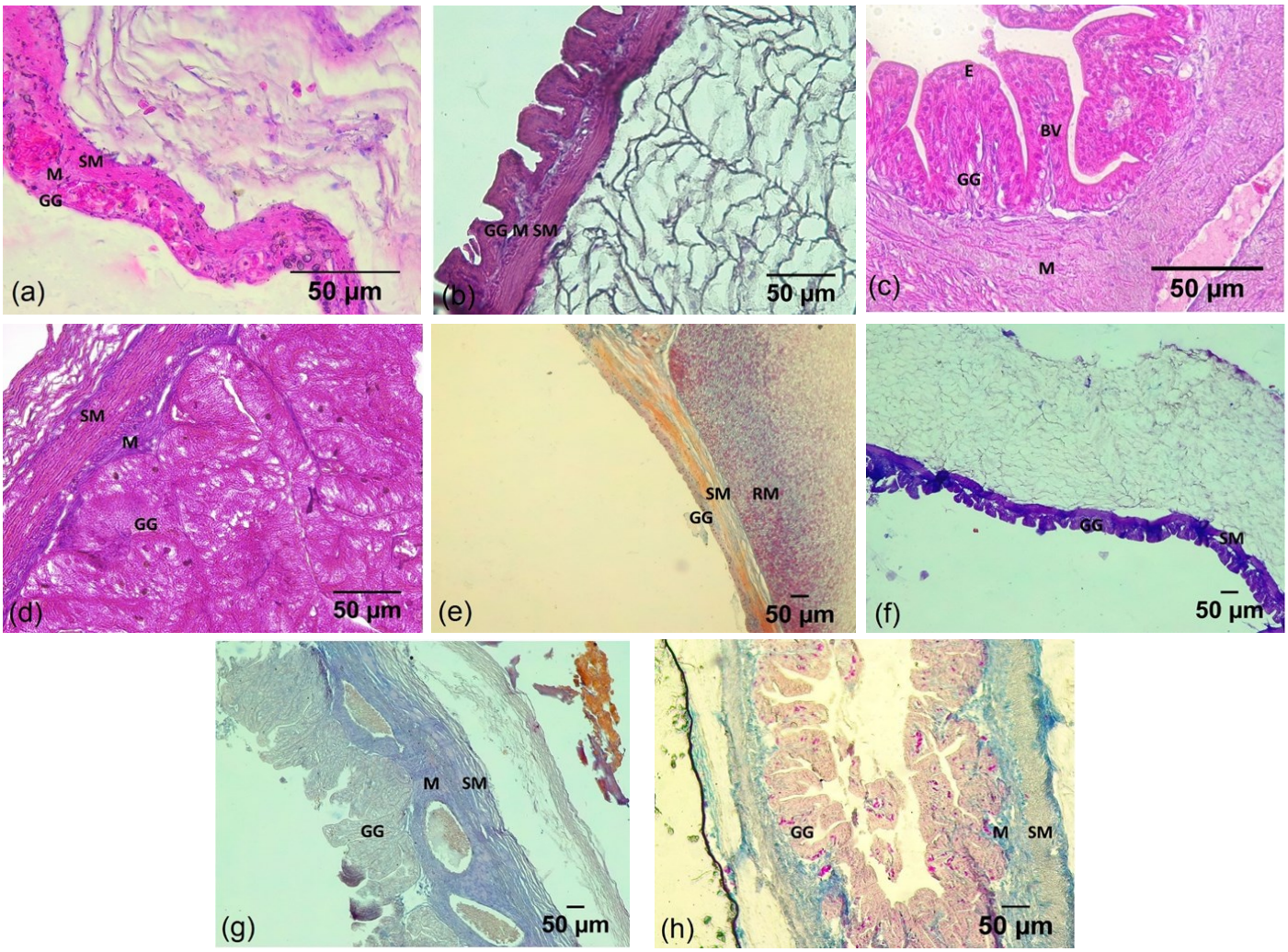

Figure 4. Histology of the swim bladder of *A. bicolor bicolor*: (a) yellow undifferentiated; (b) yellow female; (c) silver male; (d) silver female, (a-d with HE staining, 400x magnification); (e) yellow undifferentiated; (f) yellow female; (g) silver male; (h) silver female (e-h with MAF staining 100x magnification); BV - blood vessel; E – epithelium; GG - gas gland; M - mucosa; SBL - swim bladder lumen; SM - submucosa; RM - rete mirabile.

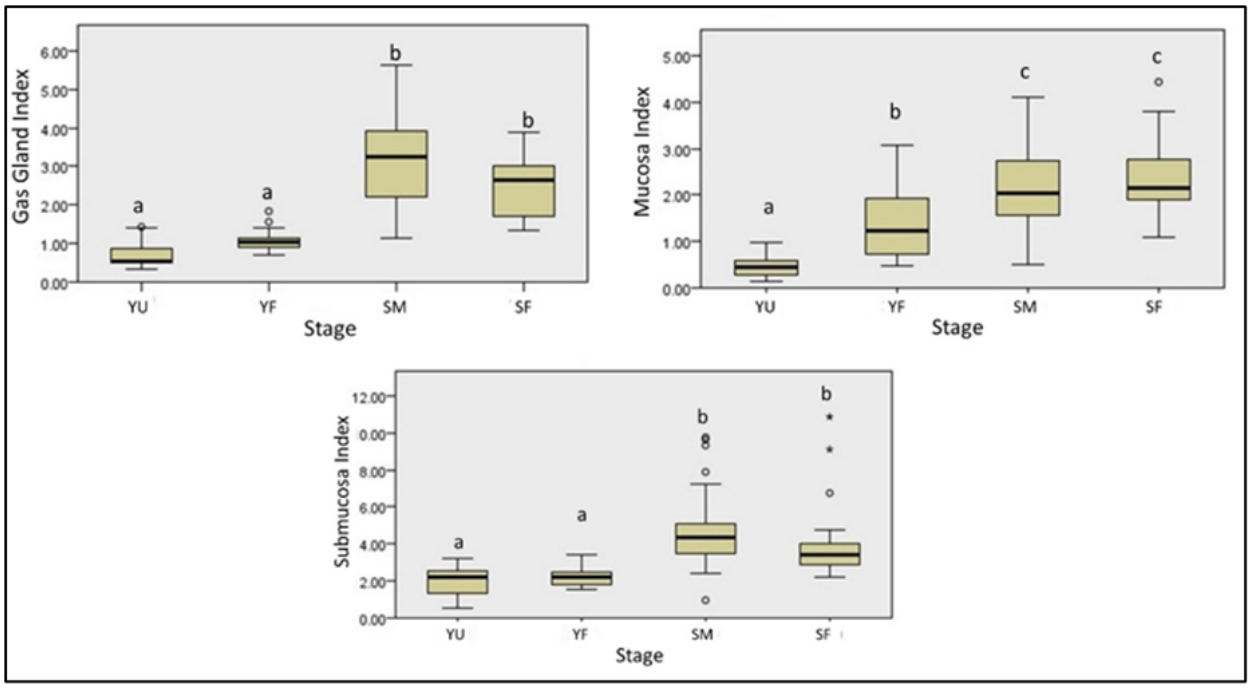

Figure 5. The gas gland, mucosa, and submucosa indices of the swim bladder of *A. bicolor bicolor*. YU - yellow undifferentiated; YF - yellow female; SM - silver male; SF - silver female; different letters on the bar indicate significant differences between stages.

Table 2. Gonadosomatic index (GSI), eye index (EI), pectoral fin index (PFI), swim bladder index (SBI), gas gland index (GGI), mucosa index (MI), and submucosa index (SMI) of *A. bicolor bicolor* collected from Segara Anakan and Pasir Puncu, Indonesia.

Stage	Mean ±SD						
	GSI	*EI*	*PFI*	*SBI*	*GGI*	*SMI*	*MI*
Yellow undifferentiated (n=4)	0.16 ± 0.21	1.42 ± 0.53	2.79 ± 0.22	0.72 ± 0.38	0.73 ± 0.32	0.45 ± 0.23	1.72 ± 0.74
Yellow female (n=2)	1.09 ± 0.88	2.50 ± 0.23	2.97 ± 0.31	0.62 ± 0.18	1.04 ± 0.11	1.11 ± 0.81	2.21 ± 0.41
Silver male (n=7)	0.70 ± 0.91	14.14 ± 4.04	5.34 ± 0.45	0.35 ± 0.14	2.37 ± 0.77	1.76 ± 0.80	4.16 ± 1.35
Silver female (n=2)	1.65 ± 0.18	7.48 ± 1.06	4.79 ± 0.13	0.16 ± 0.06	2.09 ± 0.04	2.18 ± 0.02	4.04 ± 0.10

ver stages. The increased diameter of the eyes in the silver stage also indicates an enhanced ability to adapt to dark environments such as their deep spawning grounds. An increase in PFI might also indicate that pectoral fins function as power stabilizers to avoid predators (Hagihara et al. 2012).

Based on histological observations, silver stages (male and females) had significantly thicker layers of swim bladder than other stages as shown by the histological result (Figure 3) and measurements (Figure 5). These data suggested that an increase in the thickness of the gas gland allows a greater contribution of gas to gas secretion, and that the submucosa thickness reduces gas conductivity from swim bladder wall (Yamada et al. 2001; Yamada et al. 2004). Mucosa layer contains bundle sheets of smooth muscles that play an important role in the mechanical movement of the swim bladder and are closely associated with gas glands (Fange 1958).

The results of the current study are in line with the previous study (Marshall 1972), demonstrating that deep-sea fish swim bladders have a gas gland thickness larger than that of shallow sea fish, allowing increased gas secretion. Our study also showed that GGI and SMI of Indonesian eels were lower than those of temperate eels, indicating that temperate eels might migrate deeper than tropical eels.

In the present study, a significant and sudden increase of the gas gland thickness and mucosa thickness were observed at silver stages. It is hypothesized that these changes would allow for a shift in the behavior from a benthic, shallow water life in riverine environments to a deep oceanic migration and distant spawning locations. Similar changes were also evident in silver Japanese eels with GSI > 3.5 (Yamada et al. 2001). The early stage silver eels completed the development of swim bladders while still residing in riverine environments prior to commencement of their spawning migrations (Yamada et al. 2001).

CONCLUSIONS

The current study highlighted the difference in the histological structure of the *A. bicolor bicolor* swim bladder from four sexual maturity developmental stages: yellow undifferentiated, yellow female, silver male, and silver female. Similar to temperate eels such as *A. japonica* and *A. rostrata*, the tropical species *A. bicolor bicolor* also displayed thickening of the gas gland, mucosa, and submucosa layers at the mature silver stage. Furthermore, silver females and males showed no significant differences in the

thickness of the swim bladders, suggesting a similar spawning ground habitat.

AUTHOR CONTRIBUTION

N.I.S. and D.E.D.S. designed the research. M.A.R., G.A.A., N.D., and A.H. collected the data. N.I.S. and F.S. analyzed the data and wrote the manuscript. N.I.S. and D.E.D.S. supervised all the processes.

ACKNOWLEDGEMENTS

The authors would like to thank Chinthaka Anushka Hewavitarane Ph.D. and Dr. Senny Helmiati for comments on the manuscript and to the Faculty of Biology, Universitas Gadjah Mada for supporting this research through the Merdeka Belajar Kampus Merdeka (MBKM) 2021 grant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Aoyama et al., 2003. Short-Distance spawning migration of tropical freshwater eels. *Biological Bulletin*, 204, pp.104 –108. doi: 10.2307/1543500

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

Bertin, L., 1956. *Eels, a biological study*, London: Cleaver-Hume Press Ltd.

Beullens, K. et al., 1997. Sex differentiation, changes in length, weight and eye size before and after metamorphosis of European eel (*Anguilla anguilla* L.) maintained in captivity. *Aquaculture*, 153(1-2), pp.151–162. doi: 10.1016/S0044-8486(97)00019-7

Bowmaker, J.K. et al., 2008. Eel visual pigments revisited: The fate of retinal cones during metamorphosis. *Visual Neuroscience*, 25(3), pp.249 –255. doi: 10.1017/S0952523808080152.

Cresci, A., 2020. A comprehensive hypothesis on the migration of European glass eels (*Anguilla anguilla*). *Biological Reviews*. 95(5), pp.1273–1286. doi:10.1111/brv.12609.

Drechsel, V. et al., 2022. Aquaporin expression and cholesterol content in eel swim bladder tissue. *Journal of Fish Biology*, 100, pp.609-618. doi: 10.1111/jfb.14973

Durif, C. et al., 2006. Impact of silvering stage, age, body size and condition on reproductive potential of the European eel. *Marine Ecology Progress Series*, 327, pp.171–181. doi: 10.3354/meps327171.

Ege, V., 1939. *A revision of the genus Anguilla Shaw, a systematic, phylogenetic and geographical study (Dana-Report No. 16).*, Carlsberg Foundation; C.A. Reitzels Forlag; Oxford University Press; Bianco Luno.

Fange, R., 1958. Structure and function of the gas bladder in *Argentina silus*. *Quarterly Journal of Microscopical Science*, 99(1), pp.95–96. doi: 10.1242/jcs.s3-99.45.95

Geffroy, B. & Bardonnet, A., 2016. Sex differentiation and sex determination in eels: consequences for management. *Fish and Fisheries*, 17(2), pp.375–398. doi: 10.1111/faf.12113.

Hagihara, S. et al., 2012. Morphological and physiological changes of female tropical eels, *Anguilla celebesensis* and *Anguilla marmorata*, in relation to downstream migration. *Journal of Fish Biology*, 81(2), pp.408–426. doi: 10.1111/j.1095-8649.2012.03332.x.

Hatakeyama, R. et al., 2022. Developmental features of Japanese eels, *Anguilla japonica*, from the late leptocephalus to the yellow eel stages: an early metamorphosis to the eel-like form and a prolonged transition to the juvenile. *Journal of Fish Biology*, 100(2), pp.454–473. doi:10.1111/jfb.14956.

Helfman, G. et al., 1987. Reproductive ecology of the American eel. In *Common strategies of anadromous and catadromous fishes*. American Fisheries Society, Symposium 1, Bethesda, Maryland, pp.42–56. ISBN 0-913235-42-3

Huyen, K.T. et al. 2022. Morphological characteristics and population structure of Marbled Eel (*Anguilla marmorata)* in Thua Thien Hue, Vietnam. *Journal of Applied Animal Research*, 50, pp.54-60. doi: 10.1080/09712119.2021.2018326

Kleckner, R.C., 1980a. Swim bladder volume maintenance related to initial oceanic migratory depth in silver-phase *Anguilla rostrata*. *Science*, 208(4451), pp.1481–1482. doi: 10.1126/science.7384792.

Kleckner, R.C., 1980b. Swimbladder wall guanine enhancement related to migratory depth in silver phase *Anguilla rostrata*. *Comparative Biochemistry and Physiology Part A: Physiology*, 65(3), pp.351–354. doi: 10.1016/0300-9629(80)90041-9.

Layton, C., Bancroft, J. & Suvarna, S., 2018. Fixation of tissues. In *Bancroft's Theory and Practice of Histological Techniques E-Book*. Amsterdam: Elsevier, p. 40.

Marshall, N., 1972. Swim bladder organization and depth ranges of deep-sea teleosts. *Symposium of the Society of Experimental Biology*, 26, pp.261–272.

Melia, P. et al., 2006. Age and growth of *Anguilla anguilla* in the Camargue lagoons. *Journal of Fish Biology*, 68(3), pp.876–890. doi: 10.1111/j.0022-1112.2006.00975.x.

Morris, S.M. & Albright, J.T., 1975. The ultrastructure of the swimbladder of the toadfish, *Opsanus tau* L. *Cell and Tissue Research*, 164(1). doi: 10.1007/BF00221697.

Okamura, A. et al., 2007. A silvering index for the Japanese eel *Anguilla japonica*. *Environmental Biology of Fishes*, 80(1), pp.77–89. doi: 10.1007/s10641-006-9121-5.

Pankhurst, N.W., 1982. Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* (L.). *Journal of Fish Biology*, 21(2), pp.127–140. doi: 10.1111/j.1095-8649.1982.tb03994.x.

Pankhurst, N. W., & Lythgoe, J. N. 1982. Structure and colour of the integument of the European eel *Anguilla anguilla* (L.). *Journal of Fish Biology*, 21(3), pp. 279–296. doi:10.1111/j.1095-8649.1982.tb02833.x

Pankhurst, N.W. & Lythgoe, J.N., 1983. Changes in vision and olfaction during sexual maturation in the European eel *Anguilla anguilla* (L.). *Journal of Fish Biology*, 23(2), pp.229–240. doi: 10.1111/j.1095-8649.1983.tb02898.x.

Pankhurst, N.W. & Sorensen, P. W., 2011. Degeneration of the alimentary tract in sexually maturing European *Anguilla anguilla* (L.) and American eels *Anguilla rostrata* (LeSueur). *Canadian Journal of Zoology*, 62(6), pp.1143-1149. doi: 10.1139/z84-165

Pelster, B., 2013. The swimbladder. In *Eel Physiology*. Enfield: CRCPress), pp. 44–67.

Pelster, B., 2015. Swimbladder function and the spawning migration of the European eel *Anguilla anguilla*. *Frontiers in Physiology*, 5, 486. doi: 10.3389/fphys.2014.00486.

Prem, C. et al., 2000. Swim bladder gas gland cells produce surfactant: in vivo and in culture. *American Journal Physiologicy Regulatory Integrative Comparative Physiology*, 279(6), R2336–R2343. doi: 10.1152/ajpregu.2000.279.6.R2336

Righton, D.A., Aarestrup, K., & Jellyman, D., 2012. The *Anguilla* spp. migration problem: 40 million years of evolution and two millennia of speculation. *Journal of Fish Biology*, 81, pp.365–386

Schneebauer, G. et al. 2021. Expression of transport proteins in the rete mirabile of european silver and yellow eel. *BMC Genomic.*86 (822): 1 -15. doi: 10.1186/s12864-021-08180-2

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), pp.671–675. doi: 10.1038/nmeth.2089.

Smith, F.M. & Croll, R.P., 2011. Autonomic control of the swimbladder. *Autonomic Neuroscience*, 165(1), pp.140–148. doi: 10.1016/j.autneu.2010.08.002.

Sugeha, H.Y., Jatmiko, I., & Muhammad, S., 2009. Sexual development of the tropical short-finned eel *Anguilla bicolor bicolor* of the Segara Anakan Waters, Central Java, Indonesia. *Jurnal Perikanan Universitas Gadjah Mada*, 11(1), pp.108-123. doi: 10.22146/jfs.2988

Tesch, F.W., 2003. *The Eel, 3rd edn. Ed. by J. E. Thorpe*, Oxford: Blackwell Science.

Van Wichelen, J. et al., 2022. Glass eel (*Anguilla anguilla* L. 1758) feeding behaviour during upstream migration in an artificial waterway. *Journal of Fish Biology*, 101(4), pp.1047-1057. doi: 10.1111/jfb.15171.

Vøllestad, L.A. & Jonsson, B., 1986. Life-history characteristics of the European Eel *Anguilla anguilla* in the Imsa River, Norway. *Transactions of the American Fisheries Society*, 115(6), pp.864–871. doi: 10.1577/1548-8659(1986)115<864:LCOTEE>2.0.CO;2.

Wenner, C.A. & Musick, J.A., 1974. Fecundity and gonad observations of the American Eel, *Anguilla rostrata* , migrating from Chesapeake Bay, Virginia. *Journal of the Fisheries Research Board of Canada*, 31 (8), pp.1387–1391. doi: 10.1139/f74-164.

Wolfe, D., 2019. Tissue processing. In *Suvarna SK, Layton C, Bancroft JD (eds) Bancroft's theory and practice of histological techniques.* London: Elsevier, pp. 73–83. doi: 10.1016/B978-0-7020-6864-5.00006-2.

Yamada, Y. et al., 2001. Morphological and histological changes in the swim bladder during maturation of the Japanese eel. *Journal of Fish Biology*, 58(3), pp.804–814. doi: 10.1111/j.1095-8649.2001.tb00532.x.

Yamada, Y. et al., 2004. Monthly changes in the swim bladder morphology of the female Japanese eel *Anguilla japonica* in the coastal waters of Mikawa Bay, Japan. *Ichthyological Research*, 51(1), pp.52–56. doi: 10.1007/s10228-003-0195-1.

Zacchei, A.M. & Tavolaro, P., 1988. Lateral line system during the life cycle of *Anguilla anguilla* (L.). *Bolletino di zoologia*, 55(1–4), pp.145–153. doi: 10.1080/11250008809386611.