Short Communication

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

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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 it is 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 m³. 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 cm³, 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).
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 (Sirskey 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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex Status</th>
<th>Indifferent</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL (cm) Range</td>
<td></td>
<td>9.00 – 28.50</td>
<td>22.00 – 42.70</td>
<td>24.00 – 81.00</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>17.3514 ± 4.2799</td>
<td>28.2919 ± 5.16340</td>
<td>47.4259 ± 19.1525</td>
</tr>
<tr>
<td>BW (g) Range</td>
<td></td>
<td>0.59 – 29.00</td>
<td>13.00 – 157.00</td>
<td>20.29 – 1062.00</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>9.7018 ± 7.9443</td>
<td>38.4646 ± 34.9282</td>
<td>289.2168 ± 289.8995</td>
</tr>
<tr>
<td>EI Range</td>
<td></td>
<td>0.12 – 7.58</td>
<td>0.16 – 15.34</td>
<td>0.57 – 18.28</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>1.4658 ± 1.2207</td>
<td>3.5847 ± 3.5678</td>
<td>5.5193 ± 2.9787</td>
</tr>
<tr>
<td>FI Range</td>
<td></td>
<td>1.74 – 6.51</td>
<td>1.60 – 6.84</td>
<td>2.88 – 6.53</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>3.2939 ± 0.8721</td>
<td>4.4802 ± 1.2599</td>
<td>4.4380 ± 0.8750</td>
</tr>
<tr>
<td>GSI (%) Range</td>
<td></td>
<td>0.03 – 4.2</td>
<td>0.03 – 0.81</td>
<td>0.03 – 4.37</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>0.3617 ± 0.6121</td>
<td>0.2796 ± 0.1980</td>
<td>1.1538 ± 1.0573</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>147</td>
<td>74</td>
<td>88</td>
</tr>
</tbody>
</table>

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.

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**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.
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