Reproduction Characteristics of Tropical Eel *Anguilla bicolor* McClelland in Different Developmental Stage

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**Abstract.** The reproductive characters provide information about the gonad maturity of eel. The prepubertal stage occurs in the yellow eel, and the pubertal stage achieves in the silver eel. This study aimed to evaluate the reproductive characters of tropical eel, *Anguilla bicolor* McClelland in the different developmental stages. Eels with $33 - 81$ cm $\pm 13.54$ length, and weight of $98 - 1062$ g $\pm 262.99$ were used to determine their reproductive characters. The variables observed were the total length, body weight, Gonadosomatic Index (GSI), eye index and estradiol levels. The results showed that total length, body weight and GSI of silver eels were higher than yellow eels ($P < 0.01$). Total length, body weight, and GSI were $66.09$ cm $\pm 9.34$, $556.83$ g $\pm 236.24$, and $2.12\% \pm 1.88$ respectively. The eye index and plasma estradiol level of yellow eel, pre-silver, and silver eel were similar ($P > 0.05$). In conclusion, there are many differences in reproduction characteristics between a yellow and silver eels. The gonad of silver eel is more mature than that of a yellow eel.

1. **Introduction**

*Anguilla bicolor* McClelland is a Catadromous species with interesting life cycle. It starts from a leptocephalus which changes to glass eel, then migrates to fresh water from seawater to grow up to its puberty. The developmental stage of eel can be divided into three categories, i.e., yellow eel, pre-silver, and silver [1]. Metamorphose of the eel from yellow to silver eel occurs in freshwater. The fish, then prepares to migrate to seawater for spawning.

During migration, the eels complete their gonad maturity by changing their reproductive characters such as increasing gonad weight, eye diameter, fin, and steroid hormone concentration. This study has been done in *Anguilla anguilla* [2, 3]. The process of reproductive character change before migrating is called silvering [4]. Silvering is identical to the maturation of gonad [5]. The important parameters to observe gonad maturity of an eel are eye index, fin index, GSI, body coloring, and steroid concentration.

Information on the change of eel reproductive characters during its developmental stage is lacking. This information is essential to understand gonad maturity in every developmental stage of the fish. This study aimed to evaluate the reproductive characters of tropical eel, *Anguilla bicolor* McClelland, in different developmental stages; and gonad maturity.

2. **Methods**

The research was conducted at the Animal Physiology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman. The eels were obtained from a fisherman in the Pesanggrahan village, Cilacap.
The fish were acclimated for about a day in the fiberglass aquarium (100 x 100 x 130 cm³) filled with 300 l water to eliminate stress during transportation. After the acclimation, they were grouped based on their developmental stage, i.e., yellow eel, pre-silver eel, and silver eel. The fish were anesthetized with clove oil 5 ppm for about 30 minutes [6]. Then, they were weighed, and their blood was drawn by syringe. Their body length was measured with a ruler; eye diameter and pectoral fin length were measured using a caliper. The fish abdomen was dissected from the anus to pectoral for gonad isolation. Gonad was weighed using an analytical scale (Explorer OHAUS) for measuring the GSI (Gonadosomatic Index). The observed variables were Eye Index, Fin Index, Gonadosomatic Index (GSI) and estradiol serum concentration.

The fish blood was transferred into Eppendorf tubes and allowed to room temperature for 30 minutes. The tubes were stored for ±12 hours at 4º C refrigerator up to centrifugation in Eppendorf Centrifuge 5415 for 15 minutes at 3500 rpm to obtain the serum. The serum was transferred into new Eppendorf tubes and stored in Glacier Ultralow Temperature Freezers (Plymouth, USA), for estradiol measurement (Kit 17 β-estradiol REF 30 330-Bio Marieux Inc., France).

The horizontal (A) and vertical diameter (B) of the orbital eye were measured with a caliper to get eye diameter. Eye Index is \( [(A+B)/4] \times \pi \times \text{body length (mm)} \times 100 \) [7]. Measurement of pectoral fin length with capiler was done by measuring the fin length (FL) from base to tip. Fin Index is \( \text{FL (mm)/body length (mm)} \times 100 \) [7].

The GSI calculation referred to Rupia et al. [8]. GSI equals to (gonad weight/body weight) x 100. The gonads were fixed in NBF solution for paraffin. The analyses of gonadal histology structure to evaluate sex and gonad maturity status referred to Arai and Kadir [9]. The data including body length, body weight, eye index, fin index, GSI and estradiol concentration were analyzed by one-way ANOVA [10].

3. Results

There were three developmental stages of *A. bicolor* based on their abdomen color. Yellow eel has a yellow abdomen, pre-silver has silver abdomen but not entirely, and silver eel shows entirely silver abdomen color. The eye index and estradiol concentration of the eels in the different developmental stage were similar. GSI of pre-silver (2.03%) and silver (2.14%) were similar and higher than the GSI of yellow eel (0.88%) (Figure 3).

**Figure 1.** The body length average (cm) of *Anguilla bicolor* McClelland in three different developmental stages
Figure 2. The body weight average (g) of *Anguilla bicolor* McClelland in three different developmental stages.

Figure 3. The average of GSI (%) and estradiol concentration of *Anguilla bicolor* in three different developmental stages.

Figure 4. The average of Eye Index of *Anguilla bicolor* in three different developmental stage.
The body length of yellow eel, pre-silver, and silver eel were 44.10±10.36, 52.54±13.08 and 66.09±9.55 (cm) respectively. The body length and weight tended to increase from yellow eel to silver eel (Figure 1). Whereas the eye index and estradiol concentration of the given eels in the different developmental stages were similar, i.e., 9.38±4.48 (Figure 3), 9.24±4.58 and 7.15±2.74 for Eye Index (Figure 4). Yellow eels are the juvenile stage with a body length of 20–45 cm. Estradiol concentration of the given eels in the different developmental stage were also similar as in the Eye Index with the amount of 0.17±0.23, 0.12±0.12 and 0.30±0.3391 (ng/ml).

4. Discussion

The reproductive characters of the eels in three different developmental stages in this research were similar except the GSI which increased in the puberty phase (pre-silver and silver eel). Generally, GSI, eye index and steroid concentration of eel increase during silverying and migration [3]. The eel GSI increases before migration and reaches the maximum when spawning. Before migrating, GSI reached 40% in female and 10% in male [11]. The GSI of pre-silver and silver *Anguilla bicolor* before migrating or during their prepubertal stage was 0-3% in females and 1 – 9% in males [12,13,6].

Estradiol concentration in yellow, pre-silver and silver eel of *A. bicolor* was low (0.12 – 0.30 ng/ml). It increases during silverying or migration [3], but before migration concentration of this hormone was low (< 0.5 ng/ml) [6]. During maturation, the estradiol concentration of *A. japonica* is about 5.82 ng/ml. This concentration is higher than prepubertal stage [16].

Yellow, pre-silver and silver eels of *A. bicolor* showed similar reproductive characters except for the size and GSI. It indicates that *A. bicolor* has the smallest size during the yellow stage, whereas the most significant size was during the silver stage. These characters can determine the stage of their development.

Eye Index demonstrated an increase during silverying/migration. The eel eyes enlarge because of exposure to a high salinity environment. Eye index not only show gonad maturity but also increase size similarly to eye diameter during metamorphosis from yellow to silver [14]. At puberty stage (silver), eels have an eye index less than 7 [15]. In this research, the eye index was similar in all developmental stages (>7). In general, the yellow eel has an eye index less than 7, whereas silver eel is more than 7. In this research, the yellow eel of *A. bicolor* showed a body length of 44.1 cm ± 10.36 and eye index more than 7. Yellow eel is a juvenile stage of the eel life cycle with body length about 20 – 45 cm. The eye index of yellow eel in this research reached more than 7. In general, eye index of yellow eel with a body length of 20-30 cm was less than 7.

5. Conclusion

It was concluded that there are many differences in reproduction characteristics between a yellow eel and silver eel. Silver eel has more mature gonad than a yellow eel.

References

[1] Han Y S I, Chio Liao, Huang Y S, He J T, Chang C W and Tzeng W N 2003 Synchronous changes of morphology and gonadal development of silverying Japanese eel (Anguilla japonica) *Aquaculture* 219 783–96
[2] Palstra A P, Cohen E G H, Niemantsverdriet P R W, van Ginneken V J T and van den Thillart G E E J M 2005 Artificial maturation and reproduction of European silver eel: development of oocytes during final maturation *Aquaculture* 249 533–47
[3] Nowosad J, Kucharczyk D, Czarkowski T K and Kwasek K 2014 Changes in body weight and eye size in European eel kept in fresh and salt water *Ital. J. Anim. Sci*. 13 382–6
[4] Mordenti O, Di Biase A, Bastone G, Sirri R, Zaccaroni A and Parmeggiani A 2013 Controlled reproduction in the wild European eel (Anguilla anguilla): two populations compared *Aquacult. Int*. 2 1045–63
[5] Palstra A P and van den Thillart G E J M 2010 Swimming physiology of European silver eels (Anguilla anguilla L.): energetic costs and effects on sexual maturation and reproduction *Fish Physiol Biochem* **36** 297–322

[6] Rachmawati F N and Susilo U 2011 Hormone profile and reproductive performance of Anguilla bicolor McClelland which captured at Segara Anakan lagoon *Biota* **6** 221–6

[7] Yokouchi K, Sudo R, Kaifu K, Aoyama J and Tsukamoto K 2009 Biological characteristics of silver-phase Japanese eels, Anguilla japonica, collected from Hamana lake, Japan *Coast. Mar. Sci.* **33** 1–10

[8] Rupia E J, Shen J, Wu J, Chen W, Liu L, Dierckens K, Sorgeloos P and Lu W 2013 Effect of hormone injection frequency on the lipid content and fatty acid compositions in gonad, muscle, and liver of Anguilla japonica during artificial maturation *Aquacult. Int.* **22** 1105–20

[9] Arai T and Abdul Kadir S R 2017 Opportunistic spawning of tropical anguillid eels Anguilla bicolor bicolor and A. bengalensis bengalensis Available at: http://www.nature.com/Scientificreport/doi:10.1038/srep41649 Accessed on 30th November 2017

[10] Steel R G D and Torrie J H 1981 *Principles and Procedures of Statistical Biometrical Approach* (Singapore: Mc Graw Hill Book Company)

[11] Tsukamoto K, Chow S, Otake T, Kurogi H, Mochioka N, Miller M, Aoyama J, Kimura S, Watanabe S, Yoshinaga T, Shinoda A, Kuroki M, Oya M, Watanabe T, Hata K, Ijiri S, Kazeto Y, Nomura K and Tanaka H 2011 Oceanic spawning ecology of freshwater eels in the western north Pacific *Nat. Commun.* **2** 1–9

[12] Sugeha H Y, Arai T, Miller M J, Limbong D and Tsukamoto K 2009 Inshore migration of the tropical eels *Anguilla* spp. recruiting to the Poigar River estuary on North Sulawesi Island *Marine Ecology Progress Series* **221** 233–43

[13] Rachmawati F N and Susilo U 2009 Perkembangan gonad ikan sidat, Anguilla bicolor ditinjau dari titer hormon gonadotrophin *Sains Akuatik* **10** 148–54

[14] Tesch F W 2003 *The Eel* (Oxford, UK: Blackwell Science) pp 408

[15] Beullens K, Eding E H, Gilson P, Ollevier F, Komen J and Richter C J J 1997 Gonadal differentiation, intersexuality and sex ratios of European eel (Anguilla anguilla L) maintained in captivity *Aquaculture* **153** 135–50

[16] Kim D J, Young Bae J and Oh Kim E 2007 Change in sex steroid hormones and ovarian development during artificial maturation of female Eel, Anguilla japonica *Integrative Bioscience* **11** 117–24