Comparison of Reproductive Aspects of the Tropical Eel *Anguilla bicolor* (McClelland 1884) in Freshwater and Estuarine Habitats

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Abstract

The tropical anguillid eel, Anguilla bicolor McClelland, experiences significant growth up to the adult size in both freshwater and estuarine ecosystems, encountering salinity gradients that may impact their reproduction. Therefore, this study aimed to investigate the reproductive aspects of A. bicolor collected from Serayu River (freshwater) and Segara Anakan (estuary), Cilacap, Central Java, during July 2020 field surveys. The examined parameters included eye index (EI), fin index (FI), gonadosomatic index (GSI), hepatosomatic index (HSI), and sex ratio of eel specimens in freshwater and estuary. Furthermore, the total length (TL) and body weight (BW) ranged from 260 - 630 mm and 18 - 419 g in freshwater, and 260 - 630 mm and 18 - 447 g in estuary. Significant differences (P<0.01) were observed in the reproductive characteristics between the two habitats (P<0.01), with higher eye index, fin index, gonadosomatic index, and hepatosomatic index in freshwater-caught eels than the values obtained in estuary inhabitants. The mean of these four parameters were 6.99 ± 2.98%, 4.08 ± 0.71%, 1.70 ± 1.13%, and 1.92% ± 1.07 in freshwater, while 3.48 ± 1.60%, 3.45 ± 0.479%, 1.12 ± 0.23%, and 0.28 ± 0.19% were found in estuary. The sex ratio was significantly affected by habitat, with female eels dominating in freshwater (62.5%) compared to estuary (32%). These results suggested that eels in freshwater habitats might exhibit early gonadal maturation compared to those in estuary.

Keywords: *Eel, migratory, reproduction, sex ratio*

Introduction

Anguilla spp. are catadromous species that traverse various aquatic habitats during their life cycle (Yokouchi *et al*., 2014). The eel life cycle comprises five stages, namely leptocephalus, glass eel, elver, yellow eel, and silver eel. Leptocephalus inhabits the sea and metamorphoses into glass eels, which then migrate from seawater to freshwater for growth and maturation into silver eels. They subsequently return to the sea to spawn (Arai *et al*., 2006; Tsukamoto *et al*., 2011; Lin *et al*., 2012; Churcher *et al*., 2014; Nowosad *et al*., 2014; Cresci *et al*., 2017; Chai and Arai, 2018). Successful reproduction in *A. bicolor* requires the presence of both male and female broodstocks. However, obtaining male eels in their natural environment remains challenging and problematic (Rachmawati and Susilo, 2011; Rachmawati *et al*., 2017; Arai and Kadir, 2017b). Male *A. bicolor* is rarely found in freshwater compared to females (Rachmawati and Susilo, 2011; Rachmawati *et al*., 2017). Sudo *et al*. (2013) reported that male *A. rostrata* and *A. japonica* live in both saline and freshwater environments, while only females thrive in freshwater. *A. bicolor bicolor* lives is commonly found in freshwater during its growth phase (= yellow eel), while some individuals persist in brackish water ecosystems. Those living in brackish water ecosystems tend to have smaller body sizes than FW inhabitants (Arai and Chino, 2019). The provided information is crucial for understanding the maturation phase of eels in both habitats, particularly for *A. bicolor.*

Eel sex can be distinguished based on their body length (BL), with males measuring <40 cm and females reaching >40 cm (Tesch, 2008; Arai and Kadir, 2017a). Additionally, eels with the same size but different morphological skin colors can be used as a basis for determining sex (Rachmawati *et al*., 2022). In general, female silver eels usually have a body length above 40 cm, while the size of their male counterparts is below 40 cm (Rachmawati *et al*., 2022). Earlier studies reported on the reproductive aspects of *A. bicolor bicolor* in different environments (freshwater, estuary, and marine) during migration (Arai and Chino, 2022). Some discovered variations in the migratory history of the tropical catadromous eels *A. bicolor bicolor* and *A. bicolor pacifica* (Arai and Chino, 2019). The reproductive aspects of *A. bicolor* in FW and ES environments are not yet determined. Therefore, this study aimed to compare the

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reproductive parameters of *A. bicolor* in two habitats to investigate possible differences existing in their reproduction strategy. The metrics used for this evaluation process were eye index, fin index, gonadosomatic Index, and hepatosomatic index (Nowosad *et al*., 2014; Gong *et a*l., 2017).

Materials and Methods

Experimental fish

A. bicolor specimens were collected with the assistance of local fishers, from Serayu River, Banyumas Regency (an (FW) site) and Segara Anakan, Cilacap, Central Java, Indonesia (an (ES) site). The Serayu River, one of the largest rivers on Java Island, has a catchment area of 4,375 km^2 and a length of 18 km with 11 tributaries. Segara Anakan is a large lagoon located on the south coast of Java Island, bordering the West and Central Java Provinces. Additionally, it is a preparation site for the migration of silver eels. Total length (TL), body weight (BW), eye diameter, fin length, and gonad weight were measured.

The specimens were sedated using 5 ppm eugenol for 15 mins or until immobilized (Rachmawati *et al.,* 2017). Total length was measured using a digital balance with an accuracy of 0.1 g, while body weight, eye diameter, and pectoral fin length were estimated with a digital caliper at an accuracy of 0.1 mm.The stages of eel maturity were determined based on body coloration in fresh samples, specifically in the abdominal region, where the most prominent coloration was observed (Tesch, 2008).

The horizontal and vertical orbital diameters were measured to determine the eye index (EI) using the following equation (Gong *et al*., 2017):

$$EI = [(A + B)/4]\ 2 \times \pi/TL\ (mm) \times 100$$

Note: *A* = the horizontal orbital diameter (mm); *B* = the vertical orbital diameter (mm); *TL* = body length (mm)

The fin index (FI) was calculated based on the measured length of pectoral fins from the basal area to the tip, as presented below (Gong *et al*., 2017):

$$FI = Lp\ (mm)/TL\ (mm) \times 100$$

Note: *Lp* = the pectoral fin length, *TL* = the body length

Eels collected from both sites were dissected from the anus to the abdomen to expose and separate the gonads and liver (Rachmawati *et al*., 2022). The excised gonads and liver tissues were weighed using a digital balance (Explorer O-HAUS) with an accuracy of 0.01 g. GSI and HSI indices were calculated according to the formula from Rupia *et al.* (2013):

$$GSI = (\text{gonad weight}/\text{body weight}) \times 100\%$$

$$HSI = (\text{Liver weight}/\text{body weight}) \times 100\%.$$

The sex of each specimen was determined based on gonad morphology (Rachmawati *et al*., 2022). Three morphological parameters were employed to differentiate sexes, namely thin thread (undifferentiated sex), lobule (testicles), and Lamela with transverse folds (ovaries). The number of specimens exhibiting these morphological characteristics was counted to estimate the sex ratio (SR) (Mahmood *et al*. 2011) using the following formula:

$$S.R. = NF/NM$$

Note: S.R.= Sex ratio; NF= Number of females; NM= Number of males

Data analysis

The data obtained were presented as mean ± standard deviation. The sex ratio of eels in FW and ES was analyzed in descriptive terms (De Smith, 2018). The data on eye index, gonadosomatic index, and hepatosomatic index between the two habitats were compared using an independent sample t-test (SPSS 18.0 version of Windows software).

Result and Discussion

In this study, a total of 47 eel specimens were collected from the Freshwater and estuary study sites, comprising 16 eels from freshwater and 31 eels from estuary. Among these, a total of 33 were yellow and 14 were found to be silver colored. The yellow eels, characterized by a yellowish abdomen as presented in Figure 1, were immature and had a total length of approximately 35 cm. Meanwhile, the mature silver eels (with >40 cm total length had silvery bellies as shown in Figure 2. According to Figure 3, specimens collected between the two habitats exhibited wide ranges of total length (260-630 mm in freshwater and 243-660 mm in estuary) and body weight (18-419 g in freshwater and 18-447 g in estuary), indicating that growing and adult phase eels were present in both environments. These results aligned with a previous study which identified two life history patterns of *A. bicolor,* including (1) constant living in brackish or marine waters without entering freshwater and (2) habitat transitions from freshwater to brackish water or the sea (Arai and Chino, 2010). For *A. japonica*, three types have been

Figure 1. Immature yellow eel of *Anguilla bicolor* McClelland

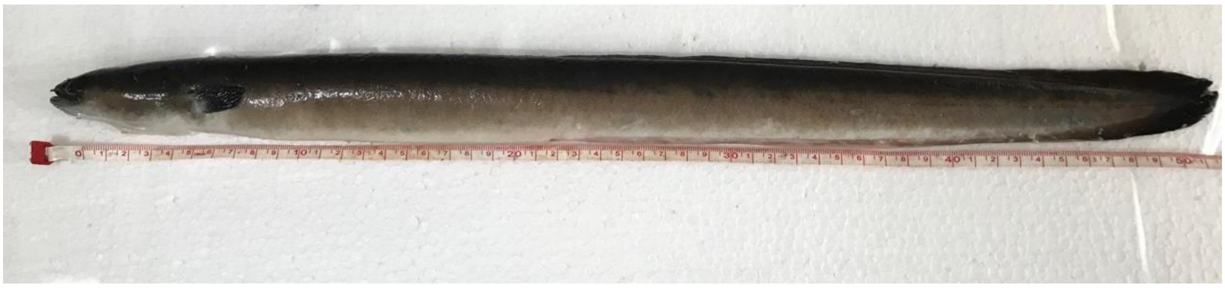

Figure 2. Sexually mature silver eel of *Anguilla bicolor* McClelland

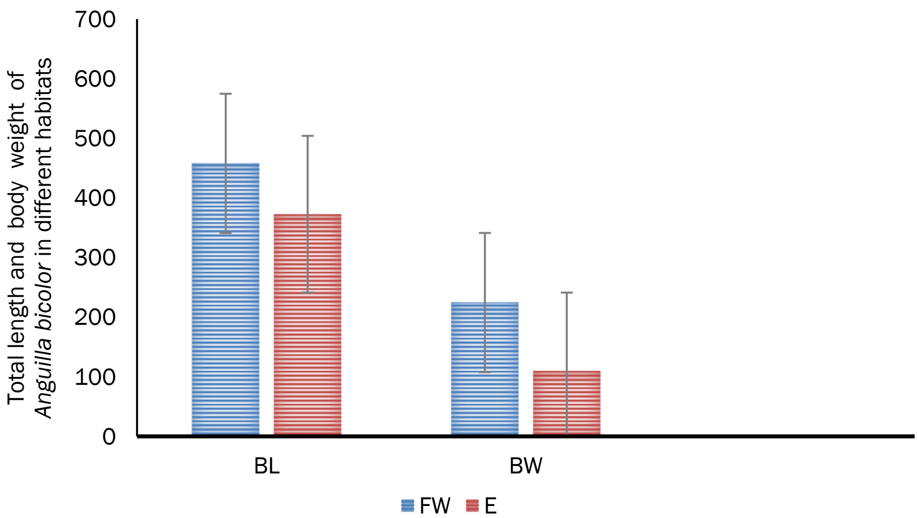

Figure 3. Mean total length (TL) and body weight (BW) of *Anguilla bicolor* collected in freshwater (FW) and estuarine (ES) habitats.

identified, namely seawater eels (40%; without a life history of migration to freshwater), estuary eels (43%; living in estuary or between freshwater and marine habitats), and freshwater or river eels (17%; with unique catadromous migration) (Kotake *et al*., 2005; Arai and Chino, 2019).

The comparison of morphometric indicators revealed significant differences between the freshwater and estuary habitats. Freshwater -caught eels had significantly higher eye index values than those obtained in estuary ($P<0.01$). Eye index ranged between 4.06 and 12.90 in freshwater, while it varied from 0.59 to 6.27 in estuary as presented in Figure 4. Conversely, fin index did not differ significantly between the two habitats ($P>0.01$), with values of 2.58-5.61 and 2.89-4.34 recorded in freshwater and estuary sites, respectively. Some specimens collected in freshwater had eye index of >7, indicating that they were already at the silver eel stage (Nowosad et al., 2014; Rachmawati *et al*., 2017; Rachmawati and Sistina, 2020b). Those obtained in estuary had an eye index of <7 and are considered yellow eels. A higher eye index and fin index in migratory eels compared to non-migratory have been reported (Hagihara *et al*., 2020), as these metrics were associated with the silvering process known to occur during migration.

Eye index and fin index) are morphometric indicators of gonad maturation (Yokouchi *et al*., 2009). Eels developed into silver eels with associated physiological and morphological changes as they migrate from freshwater to the sea in preparation for spawning (Clevestam *et al*., 2011). Changes in body color and weight including head shape and eye diameter, are indications of gonad maturity (Arai *et al*., 2006).

Interestingly, the gonadosomatic index (GSI)of eels in estuary were significantly lower than those in freshwater (P<0.05). The values varied between 0.03 and 0.90% in estuary while ranging from 0.68 to 3.25% in freshwater. See Figure 4. These results were consistent with an earlier study that reported a lower GSI of *A. bicolor* collected in an estuary environment (Hagihara *et al*., 2020). Other sources stated GSI values ranging from 1.04 to 2.96%, 0.1 to 2.88%

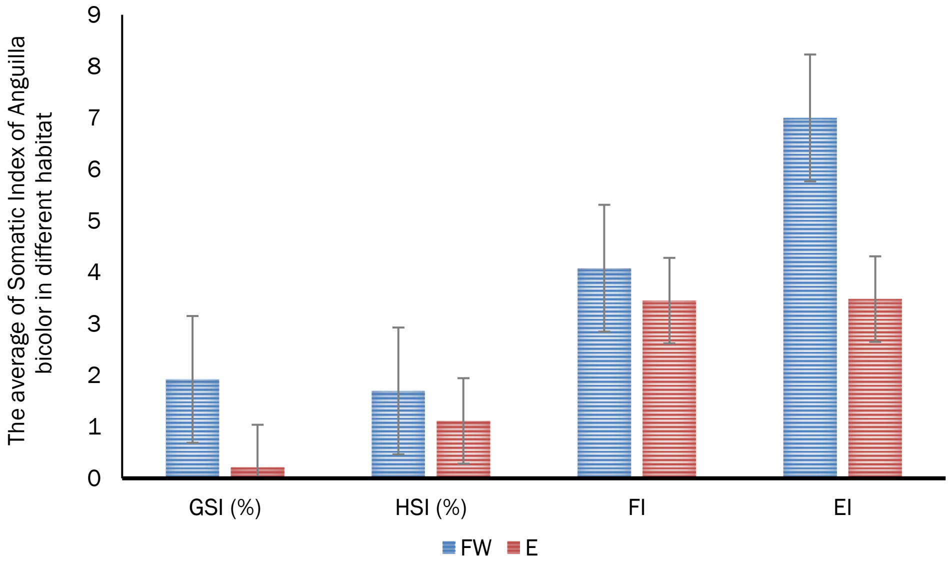

Figure 4. Mean gonadosomatic (GSI), hepatosomatic (HSI), eye (EI), and fin (FI) indices of *Anguilla bicolor* in freshwater and estuarine habitats.

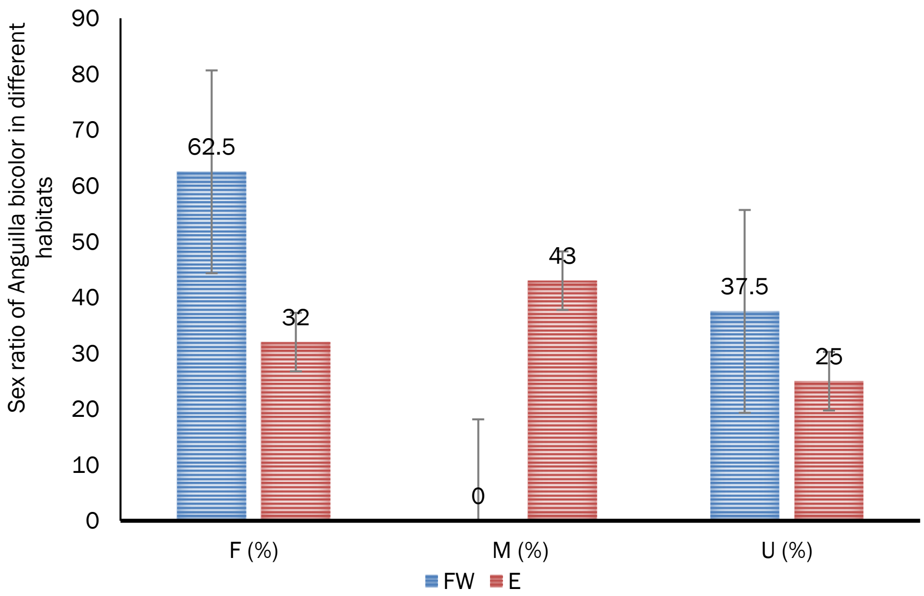

Figure 5. Percentage composition (%) of female (F), male (M), and undifferentiated gonads (U) of *Anguilla bicolor* collected in freshwater and estuarine habitats.

(Rachmawati and Susilo, 2009; 2011), and 0.03 to 4.37% (Rachmawati and Sistina, 2020) for similar species, which were comparable to the values obtained in this current study. Lower GSI values suggested that *A. bicolor* gonads were still in the immature stage, as opposed to *A. japonica*, found with a GSI of 4.3% at the pubertal stage (Kotake *et al.*, 2005). Generally, adult female eels have been shown to contain a GSI of >20%, while <20% were detected in their male counterparts (Rachmawati *et al.*, 2020b).

Hepatosomatic Index (HSI) was examined as an essential physiological index for determining gonad maturity, specifically in females. In the current study, HSI values in freshwater (0.83-3.68%) were higher than those of estuary -caught eels (0.78-1.63%) as seen in Figure 4. These results were consistent with higher HSI reported in migratory tropical anguillid eels, such as *A. celebensis* and *A. marmorata* (Hagihara *et al.*, 2012). In females, an increase in HSI before the spawning season suggested a higher level of energy reserves in the form of glycogen, while a significant decrease after spawning corresponded to the loss of hepatic glycogen (Duta and Banerjee, 2016).

The sex ratio was also affected by habitat, with female eels being more dominant in freshwater than in estuary as indicated in Figure 5. In freshwater, females accounted for 62.5% of the total specimens collected, while eels with undifferentiated gonads comprised 37.5%. Male eels were not found in fresh water in this investigation (Figure 5.). These results suggested that male eels, with reported lower GSI than females, would complete the process of gametogenesis in seawater, leading to being uncommon in freshwater (Tesch, 2008). These results were consistent with previous studies that reported female *A. bicolor* to be more prevalent in freshwater than in estuary (Rachmawati and Susilo, 2011; Arai and Kadir, 2017a). Another source found *A. bicolor* to be entirely female in Segara Anakan (Rachmawati and Susilo, 2011). In Peninsular Malaysia, 73% of eel specimens were females, while 13% were males, and the remaining 14% had undifferentiated gonads (Arai and Kadir, 2017a). Similarly, female *A. japonica* appeared dominant in freshwater, comprising 79.6% (Yokouchi *et al.*, 2014). On the other hand, males in estuary accounted for 43% of the total eel specimens, which was 11% higher than females, and the remaining 25% had undifferentiated gonads, supporting previous data (Cote *et al.,* 2017). Observations of various sizes of *A. bicolor* collected from diverse environments revealed that male eels had smaller body sizes than females (Rachmawati and Sistina, 2020a). Moreover, freshwater eels generally exhibit larger body sizes and faster gonad maturation than those from estuary. Females marked by a shift in silver body color, with EI and GSI levels above 7% as well as a 20% increase in body fat, will migrate to the sea for eventual spawning (Nowosad *et al*., 2014).

Conclusion

The tropical eel *A. bicolor* exhibited sexual size dimorphism in response to habitat use or preference. Female eels were predominantly abundant in freshwater, while males were more common in estuary habitats. Eels found in freshwater showed early gonad development and sex differentiation compared to those in estuary. Overall, the results shed light on the reproductive aspects of *A. bicolor* in different habitats, highlighting the significance of environmental factors in shaping the reproductive strategy of this species.

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