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Sex Determination through Morphological and Histological Assessment of Short-finned eel (*Anguilla bicolor*)

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ABSTRACT

The short-finned eel (*Anguilla bicolor*) is an important tropical aquaculture species in Southeast Asia, but its expansion is limited by insufficient knowledge of its reproductive biology, which constrains seed production efforts. This study examined sex-differentiated morphological and reproductive characteristics in *A. bicolor* by rearing 80 individuals (120 – 1340 g; 44.6 – 86.6 cm) for 24 months in a recirculating aquaculture system. Clear sexual dimorphism was observed, with females exhibiting larger and rounder while males were smaller with elongated mandibles. Females achieved significantly greater growth, with total length ranging from 80.2 – 83.7 cm and body weight from 1035.0 – 1205.0 g, compared to males which ranged from 45.7 – 50.7 cm and 215.0 – 330.0 g. Relative condition factors were 1.15 ± 0.04 for females and 1.24 ± 0.12 for males. Histological analysis confirmed the presence of previtellogenic and vitellogenic oocytes in females, whereas males displayed only undifferentiated spermatogonia, indicating limited reproductive advancement under the culture conditions. Length – weight regressions showed positive allometry overall, but sex-specific analyses revealed weakened correlations at the end of culture, with females showing moderate association (R^2 = 0.4402) and males lower association (R^2 = 0.3118). Organosomatic indices indicated no significant differences in gonadosomatic index between sexes, although males exhibited a significantly higher hepatosomatic index (1.10 ± 0.17%), with liver weight positively correlated with total length (R^2 = 0.6508, P = 0.0108). These findings provide essential baseline information on morphological and reproductive differentiation in *A. bicolor*, supporting improved broodstock selection and management strategies for enhanced eel seed production.

Keywords: Anguilla bicolor; Sexual dimorphism; Reproductive biology; Morphological identification; Organosomatic index.

INTRODUCTION

Sex determination in teleost is regulated through interactions among genetic, hormonal, and environmental factors, making fish a diverse model for studying vertebrate sexual differentiation. Many species display flexible systems, including environmental sex determination (ESD) and genetic sex determination (GSD), or a combination of both (Kitano et al. 2024; Abd Mohmin et al. 2025). The short-finned eel (*Anguilla bicolor*), a commercially important catadromous species in Southeast Asia, remains challenging to culture due to difficulties in identifying sex at early stages, as external sexual dimorphism only becomes evident near maturation (Arai and Abdul Kadir 2017; Sukardi et al. 2018). This limitation complicates broodstock selection and reduces breeding efficiency (Palaiokostas et al. 2020).

A. bicolor shares life-cycle characteristics with other anguillids, including oceanic spawning, larval drift, and freshwater growth phases (Arai and Abdul Kadir 2017). During culture, the lack of visible gonadal structures prevents reliable sexing, yet accurate identification is essential for managing sex ratios, scheduling hormone induction, and optimizing growth performance (Rajendiran et al. 2021). In anguillids, females typically grow larger and for longer periods than males, a trend observed across species and relevant to market value (Rachmawati et al. 2023).

Researchers have therefore examined internal and morphometric indicators to support sex identification. Traits such as total length, body weight, and condition factor (Kn) often show sex-related patterns, with females typically exhibiting stronger BW – TL correlations

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(Samuel et al. 2024). Organosomatic indices including gonadosomatic index (GSI), hepatosomatic index (HSI), and eye index (EI) also reflect reproductive development and physiological status (Rachmawati et al. 2023). However, these indices alone cannot definitively determine sex, especially in immature individuals. Histology remains the most reliable method, enabling direct observation of gonadal cells and developmental stages (Horiuchi et al. 2022).

Environmental factors such as stocking density and temperature may also influence sex ratios, with high densities promoting male development in related eel species (Van Thong et al. 2025). This sensitivity underscores the need for diagnostic markers that are not dependent on environmental conditions. Accurate sex identification also enhances hormone-induced maturation protocols, where treatments such as HCG, CPE, and synthetic gonadotropins show varying effectiveness depending on developmental stage (Attia et al. 2025). Recent studies highlight the role of steroid hormones and fatty acid profiles as additional biomarkers, especially 11-ketotestosterone, estradiol, and reproductive PUFAs (Damsteegt et al. 2020). It is hypothesized that an integrated morphological, histological, and endocrinological approach improves the accuracy of sex determination in *A. bicolor*. Accordingly, this study aims to evaluate the effectiveness of this integrated approach.

MATERIALS AND METHODS

Experiment design and rearing condition

This experiment utilized 80 individuals of *A. bicolor* eels cultured over a 24-month period in captivity. Initially all *A. bicolor* ranging in weight from 130.0 to 1340.0 g, were imported from Indonesia specifically for research purposes at the Universiti Malaysia Sabah (UMS) Fish Hatchery. This study was approved by ethical committee the Researcher's Guidelines of the Code of Practice for the Care and Use of Animals for Scientific Purpose Universiti Malaysia Sabah (AEC 0003/2023).

A. bicolor broodstock were maintained in seawater within a 150-tonne recirculating aquaculture system (RAS) tank. Meanwhile, fish body weight and total length measurements were recorded initially and continued at three-month intervals. Throughout the 24-months of culturing period, the broodstock were fed squid to satiation, with each squid enriched by inserting a fish oil tablet to enhance nutritional intake. Water quality was closely monitored twice daily, at 0930hrs and 1430hrs with dissolved oxygen (6.70-7.23 mg/L), temperature (28-29^{0}C), pH (7-7.8) and bottom cleaning and water renewal performed as needed to maintain optimal conditions.

At the end of the experiment, a total of 20 eels were examined, comprising 10 males and 10 females. Due to the lack of reliable external sexual dimorphism in Anguilla spp., sex could not be determined at the beginning of the experiment. Therefore, sex identification was conducted at the end of the trial through abdominal dissection based on the presence of ovaries or testes, followed by histological confirmation of gonadal tissues. Testes and ovaries were preserved in 10% buffered formalin for histological analysis, while liver samples were stored at -20°C to enable detailed biochemical assessments.

Laboratory and data analysis

Regression analysis was applied to determine the allometric growth of female and male *A. bicolor*. The raw data for regression analysis consist of total length (cm), body weight (g), gonad weight (g), and liver weight (g) using was initially computed using Microsoft Excel 2010. The length-weight relationships were expressed through the power logarithmic equation $W = aL^b$ (Ricker, 1975), where W represents body weight (g), L is total length (mm), "a" is the intercept, and "b" is the allometric coefficient. The relationship was considered isometric when the "b" coefficient was approximately equal to three (Phillips et al. 2018).

The parameters "a" and "b" were log-transformed into the equation $\log_{10}W = \log_{10}a+b\log_{10}L$ and a regression line was plotted. The relationships and allometric growth rates were compared among hormone-treated *A. bicolor* groups by the "b" slope values. The relationship between gonad and liver weight and the total length of male *A. bicolor* was also determined through regression analysis. The reproductive pattern was assessed through reproductive indices such as the gonadosomatic index (GSI), hepatosomatic index (HSI), and relative condition factor (RCF). Fish samples were dissected after measuring total length (cm) and body weight (g). Gonad weight (g) was measured using an analytical balance with precision to 0.0001g.

The relative condition factor (RCF) was calculated according to Mohmin et al. (2022) using the equation:
Relative condition factor (Kn) = W (Observed weight, g) / W' (Expected weight, g)
Where: W' - the expected weight of the fish individual (aLb with the earlier estimated b value; Kn - 1 or more is an indication that the fish has achieved the expected growth.

The GSI was calculated to examine the relationship between gonad weight and body weight in relation to reproduction using the formula (Li et al. 2018):

$$\text{GSI} = 100 \text{ x } \frac{\text{Gonad weight (g)}}{\text{Total body weight (g)}}$$

Similarly, liver weight (g) was recorded, and the HSI was calculated to assess changes in liver weight in relation to body weight using the following formula (Li et al. 2018):

$$\text{HSI} = 100 \text{ x } \frac{\text{Liver weight (g)}}{\text{Total body weight (g)}}$$

Histograms of GSI, HSI and RCF were generated based on mean values according to mid-length to compare patterns between male *A. bicolor*.

The eye index (EI) of males and females *A. bicolor* were calculated using the formula (Sundin et al. 2022) as follows:

$$\text{EI} = \frac{\text{HED} + \text{VED}}{4 \text{ x TL}} \text{ X } 100$$

HED : Horizontal eye diameter (mm)
VED : Vertical eye diameter (mm)
TL : Total length of the fish (mm)

Histology analysis

The testes were excised following blood sample collection for hormonal analysis. Histological examination of the male gonads was performed using standard procedures, whereby tissues were fixed in 10% neutral buffered formalin for 24 h, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections were cut at a thickness of 6 µm, stained with hematoxylin and eosin (H&E), and examined under a light microscope at 100× magnification. Microscopic criteria for classifying gonadal developmental stages were adapted and modified based on characteristics described by DeMartini and Howard (2016).

Statistical analysis

In this study, length – weight, gonad weight – total length, and liver weight – total length relationships were analyzed using regression analysis to evaluate scaling with body size. To compare sexes, histograms of the Gonadosomatic Index (GSI), Hepatosomatic Index (HSI), and Eye Index (EI) were generated based on mean values. Differences between males and females were tested using independent-samples t-tests ($P < 0.05$). Statistical analyses were conducted using IBM SPSS Statistics, including the Shapiro – Wilk test for normality and Levene's test for homogeneity of variances. All assumptions were verified prior to hypothesis testing to ensure valid statistical interpretation.

RESULTS

Comparison on body morphology and reproductive organ features in male and female *A. bicolor*

A comparative morphological analysis between male and female *A. bicolor* revealed clear sexual dimorphism in body size over the 24-month culture period by naked eyes. Female *A. bicolor* consistently exhibited significantly greater total body length and overall mass (1095.74 ± 45.74g) compared to the male (258.15 ± 29.58g), despite being reared under identical environmental and nutritional conditions (Fig. 1).

Fig. 1: Female attained bigger size (a) compared to male *A. bicolor* (b) in the same group ages within 24 months culture period; scale bar: 5 cm.

Fig. 2 shows the female (Fig. 2a) exhibited a noticeably rounder and more robust body with the body round (19.7 ± 0.96 cm) compared to males (9.5 ± 1.08 cm) (Fig. 2b). This roundness was particularly evident in the abdominal region, where females appeared heavier and more laterally expanded. In contrast, males displayed a relatively slender and elongated body form with less abdominal distension. These suggesting a consistent pattern of sexual dimorphism linked to growth and maturation. The data indicate that female *A. bicolor* attained substantially larger sizes (group "a"), while males remained in the smaller size group (group "b"), highlighting inherent physiological and growth rate differences between the sexes.

Fig. 2: (a) Female has rounder bodied at a given length compared to male *A. bicolor* (b) in the same group ages within 24 months culture period; scale bar: 3 cm.

Female *A. bicolor* (Fig. 3a) exhibited a less protruding lower jaw, resulting in a comparatively smoother and more streamlined cranial profile, as highlighted by the red line. These features provided a more rounded head contour, indicating reduced mandibular extension. Contrastingly, male *A. bicolor* (Fig. 3b) displayed a more prominent and elongated mandible, which produced a noticeably protruding snout, as illustrated by the green line. The contrast between the streamlined female head profile and the pronounced male mandibular projection suggests a consistent morphological differentiation between sexes, further reinforcing the role of craniofacial traits in distinguishing male and female *A. bicolor* within the same age group under culture conditions.

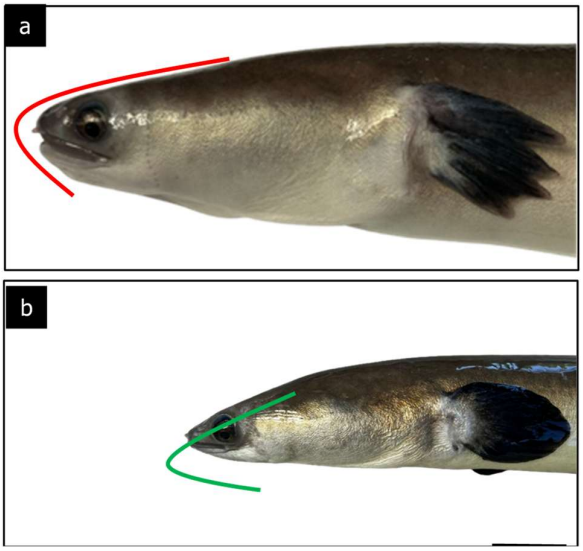

Fig. 3: (a) Females showed a less protruding jaw with a streamlined head profile (red line), while (b) males exhibited a more protruding mandible (green line) in *A. bicolor* of the same age group 24-month culture; scale bar: 2 cm.

Gonad observation and histology analysis between male and female showed in Fig. 4a and 4b. In female specimens, the gonads appeared morphologically mature, displaying a well-developed, curtain-like ovarian structure characteristic of advanced reproductive stages (Fig. 4a). In contrast, male gonadal tissue exhibited a markedly less developmental stage (Fig. 4b). Gonad morphology showed underdeveloped testicular lobes.

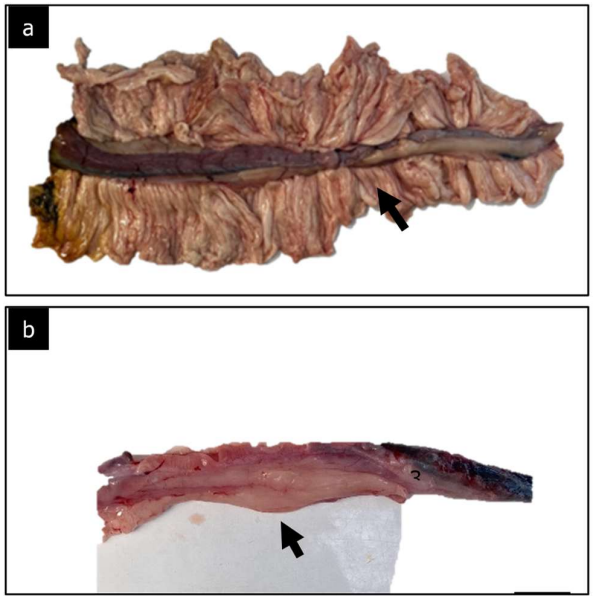

Fig. 4: (a) Female *A. bicolor* exhibited a gonad morphology with a distinct *curtain-like* ovarian structure and (b) the male displayed an underdeveloped testicular lobe; scale bar: 2 cm.

Macroscopic gonad observations, complemented by detailed histological analyses, revealed clear sex-specific differences in gonadal development between male and female *A. bicolor*. Histological examination confirmed this maturity by the presence of oocytes at distinct developmental phases. Specifically, vitellogenic oocytes (VO), indicated by orange arrows, were identified by their dense yolk accumulation, suggesting active vitellogenesis. Additionally, previtellogenic oocytes (PVO), highlighted with red arrows, were observed in the chromatin-nucleolus phase, characterized by a prominent central nucleolus and limited cytoplasmic development Fig. 5a.

While in male specimens, histological sections revealed the presence of primarily undifferentiated spermatogonia (Sg), with no evidence of active spermatogenesis or more advanced germ cell types (Fig. 5b). This indicates that the male was in an early stage of testicular development, likely pre-spermatogenic, and had not yet initiated the cellular processes associated with sexual maturation.

As shown in the Fig. 6, PVOs were the most abundant stage, with a mean density of 23.2 ± 5.49 cells/mm², indicating active early-phase oogenesis across most individuals. VO density was lower, averaging 10.9 ± 4.84 cells/mm², suggesting the presence of maturing oocytes but at a less advanced frequency. PO density was the least among the three stages, with a mean value of 3.75 ± 1.28 cells/mm², reflecting the transition of oocytes from early to more advanced stages of development.

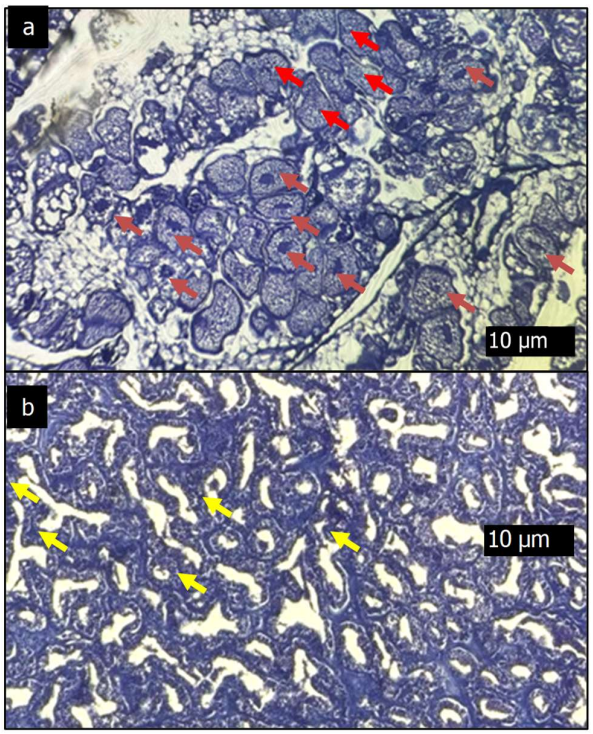

Fig. 5: (a) Female developed ovary confirmed by histological analysis; the orange arrow indicates vitellogenic oocytes (VO), while the red arrow highlights previtellogenic oocytes (PVO), while (b) the male containing undifferentiated spermatogonia (Sg); yellow arrow.

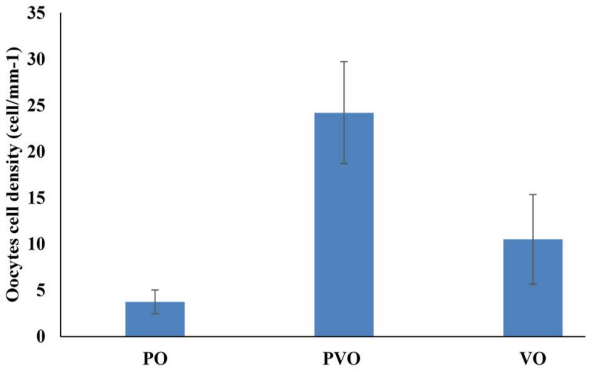

Fig. 6: Oocytes cell density (cell/ mm^{-1}) containing PO, PVO and VO.

These results highlight a predominance of mid-phase oocytes (PVO) in the sampled females, suggesting that the population was generally undergoing synchronized early vitellogenesis, with some individuals advancing toward maturation. The presence of VO confirms progression into later stages of oocyte development, though at a reduced density. This pattern supports the histological observations of ovarian development, indicating reproductive readiness in cultured female *A. bicolor* under the current rearing conditions. These results not only reflect the asynchronous gonadal maturation patterns between sexes in *A. bicolor*, but also provide an information into sex-specific reproductive timelines, which are important for effective broodstock management and hormonal induction protocols in aquaculture settings.

Allometric index

The length – weight relationship illustrated in Fig. 7 depicts the association between body weight (BW) and total length (TL) of *A. bicolor* at the initial culture period. During this stage, 80 individuals were measured, exhibiting a TL range of 44.6 – 86.6 cm and a BW range of 120.0 – 1340.0g. The regression analysis revealed a strong positive correlation between BW and TL, described by the equation $y = 0.0004x^{3.4479}$ with a high coefficient of determination (R2 = 0.9768). This indicates that more than 97% of the variation in body weight can be explained by total length, suggesting a highly consistent growth pattern across the sampled population (n = 80). The exponent value of 3.4479 exceeds 3.0, indicating positive allometric growth, whereby body weight increased at a relatively faster rate than length.

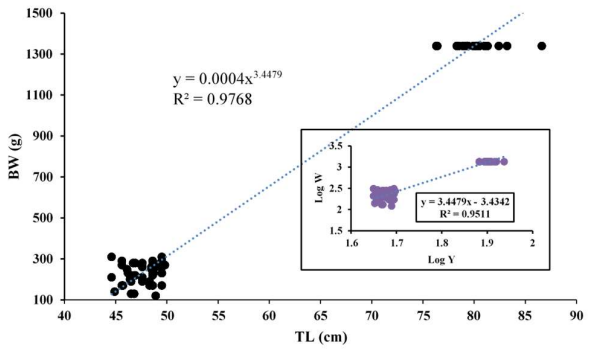

Fig. 7: Relationship between BW (body weight) and TL (total length) of *A. bicolor* at the initial culture period; n = 80.

The log-transformed regression further supported this relationship, showing a strong linear association between log-transformed BW and TL, with the equation y = 3.4479x−3.4342 and an R^2 value of 0.9511. The clustering of data points within both the raw and log-transformed plots highlights minimal variation among individuals, reinforcing the robustness of the growth model. These results collectively demonstrate that *A. bicolor* exhibited strong length – weight dependence at the onset of culture, with growth patterns favoring weight accumulation relative to length.

Fig. 8 presents the relationship between body weight (BW) and total length (TL) of *A. bicolor* at the end of the culture period, revealing clear sexual dimorphism. Male individuals were comparatively smaller, with a TL range of 45.7 – 50.7 cm and a BW range of 215.0 – 330.0 g, whereas females attained substantially greater sizes, with TL ranging from 80.2 – 83.7 cm and BW from 1035.0 – 1205.0 g. This marked difference underscores the pronounced disparity in growth performance between sexes under the same culture conditions. For males (n = 46), the relationship is defined by the equation $y = 0.0205x^{2.4277}$, with an R^2 value of 0.3244, indicating a weak correlation between BW and TL. The log-transformed regression yields $y = 2.4277x - 1.6873$ with an R^2 of 0.3118, reinforcing the weak association (Fig. 4.8a).

In females (n = 28), the relationship is described by $y = 0.0288x^{2.3952}$, with an R^2 of 0.2851, also indicating a weak correlation. However, the log-transformed model for females shows a stronger relationship, given by $y = 2.3967x - 1.545$ with an R^2 of 0.4402 (Fig. 4.8b). This suggests that the correlation between body weight and total length is moderately stronger in females than in males at the end of the culture period.

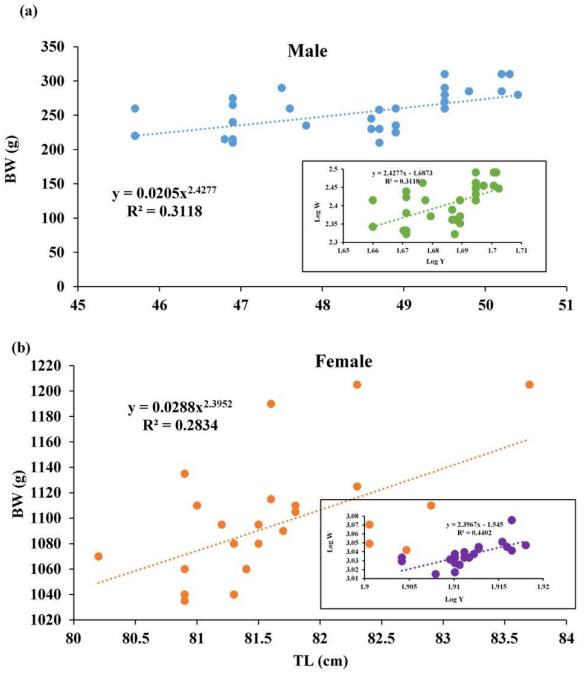

Fig. 8: Relationship between BW (body weight) and TL (total length) of (a) male and (b) female *A. bicolor* at the end culture period; Male (n = 46), Female (n = 28).

Table 1 Provides the observed and expected body weights, along with relative condition factors (Kn) for both sexes at the final culture periods. Males (n = 46) showed an observed body weight of 258.15 ± 29.58g and an expected body weight of 208.11 ± 13.03g, with a Kn of 1.24 ± 0.12. Females (n = 28) exhibited an observed body weight of 1095.74 ± 45.74g, an expected body weight of 948.42 ± 20.83 g, and a Kn of 1.15 ± 0.04. The analysis revealed a statistically significant difference in Kn between males and females (t = 4.502, df = 60.516, P < 0.001). The mean difference was 0.08635, with a 95% confidence interval ranging from 0.04799 to 0.12470.

Table 2 presents a linear regression analysis for the relationship between gonad and liver weights with total length at the end of the 24-month culture period for male and female *A. bicolor*. For the gonad weight – total length relationship, males (n = 10) show an R^2 value of 0.0251, an intercept (a) of -6.5114, and a slope (b) of 4.1434, with a standard error (S.E) of 16.4593, a t-statistic of -0.395607, a weight range of 1.3 – 8.6g, and a P-value of 0.7041, indicating no significant correlation. In females (n = 10), the R^2 value is 0.6508, with an intercept (a) of -6.0353, a slope (b) of 3.7399, a standard error of 13.7422, a t-statistic of -0.439182, a weight range of 10.4 – 20.5 g, and a P value of 0.6721, also showing no significant correlation.

For the liver weight – total length relationship, males (n = 10) have R^2 value of 0.6508, an intercept (a) of -8.8613, a slope (b) of 5.5111, a standard error of 2.5742, a t-statistic of -3.4423, a weight range of 2.1 – 3.8 g, and a P value of 0.0108, indicating a significant correlation.

Table 1: The observed, expected body weight and relatives condition factors (Mean ± SD) at the end (24 months) culture period of female and male *A. bicolor*

Sex	n	W (Observed body weight, g)	W' (Expected body weight, g)	Kn (Relative Condition Factor)
Male	46	258.15 ± 29.58	208.11 ± 13.03	1.24 ± 0.12[a]
Female	28	1095.74 ± 45.74	948.42 ± 20.83	1.15 ± 0.04[b]

Notes: A significant difference was detected (t = 4.502, df = 60.516, P < 0.001; Cohen's d = 0.884)

Table 2: Linear regression analysis for gonad and liver weight relationship with total length at the end (24 months) of culture period female and male A. bicolor

Items	n	R^2	Intercept (a)	Slope (b)	S.E	t-stat	Weight range (g)	P-values
Gonad weight – Total length relationship								
Male	10	0.0251	-6.5114	4.1434	16.4593	-0.395607	1.3 – 8.6	0.7041
Female	10	0.6508	-6.0353	3.7399	13.7422	-0.439182	10.4 – 20.5	0.6721
Liver weight – Total length relationship								
Male	10	0.6508	-8.8613	5.5111	2.5742	-3.4423	2.1 – 3.8	0.0108*
Female	10	0.0377	-3.2523	2.0740	7.0911	-0.4586	4.3 – 6.2	0.6587

Notes: Statistical significance was determined at P < 0.05.

In females (n = 10), the R^2 value is 0.0377, with an intercept (a) of -3.2523, a slope (b) of 2.0740, a standard error of 7.0911, a t-statistic of -0.4586, a weight range of 4.3 – 6.2 g, and a p value of 0.6587, showing no significant correlation.

At the end of a 24-month culture period, organosomatic indices specifically the gonadosomatic index (GSI) and hepatosomatic index (HSI) were assessed in male and female *A. bicolor* in Fig. 9. The average body weight of female *A. bicolor* (1095.74 ± 45.74 g) was significantly higher than that of males (257.5 ± 37.73 g), reflecting sexual size dimorphism common in anguillid species.

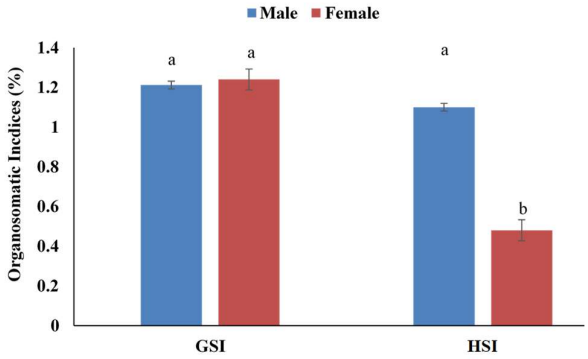

Fig. 9: Organosomatic indices of *A. bicolor* male and female during final (24 months) culture period.

The GSI values did not differ significantly between sexes, with males exhibiting a GSI of 1.52 ± 1.03% and females 1.24 ± 0.23% (P > 0.05), suggesting comparable levels of gonadal development despite the difference in body mass. This is consistent with the lack of significant differences indicated by shared letter notations ("a") in Fig. 9.

In contrast, a highly significant difference (P < 0.00001) was observed in HSI between the sexes. Male *A. bicolor* showed a significantly higher HSI value (1.10 ± 0.17%) compared to females (0.48 ± 0.05%). The higher liver-somatic allocation in males suggests elevated energy reserves or metabolic activity, potentially linked to hormonal status or differential lipid mobilization strategies during maturation.

The eye index (EI) and total length of male and female *A. bicolor* were evaluated at the start (1st month) and end (24th month) of a 24-month culture period to assess sex-related morphometric differences.

At the initial stage, males exhibited a significantly higher eye index (0.88 ± 0.08) than females (0.60 ± 0.04), with a highly significant difference (P = 0.0001). This trend persisted through the final stage, where males maintained an eye index of 0.88 ± 0.04, while females remained significantly lower at 0.62 ± 0.04 ($P = 1.33 \times 10^{-11}$). These differences were also clearly depicted in Fig. 10, where distinct groupings indicated statistical separation between male and female eye indices at both time points (male = "a"; female = "b").

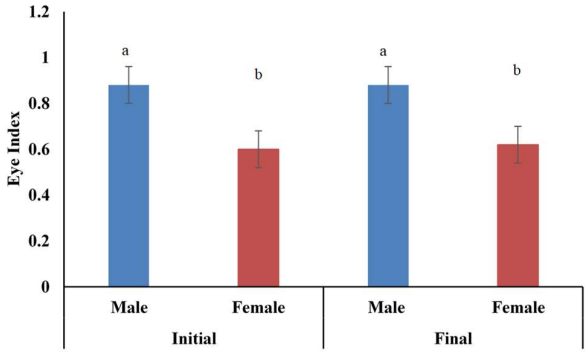

Fig. 10: Eye index values of *A. bicolor* females and males during initial and final culture period.

Regarding total body length, females were significantly larger than males throughout the study. At the start, females averaged 79.74 ± 1.83 cm in length compared to 47.2 ± 1.75 cm in males. After 24 months, females reached an average of 81.61 ± 0.76 cm, while males measured 48.7 ± 1.39 cm. These consistent differences in both length and eye index underscore the sexually dimorphic growth and morphometric traits of *A. bicolor*, with males exhibiting proportionally larger eyes and females achieving greater somatic growth.

DISCUSSION

Body morphology

Evidence from this study indicates that female *A. bicolor* attained significantly larger body sizes compared to

males, with a mean body weight of 1095.74 ± 45.74 g in females compared to 258.15 ± 29.58 g in males, representing approximately a 4.2-fold difference in body mass. Retnoaji et al. (2023) and Irwan et al. (2009) similarly reported that wild *A. bicolor* populations in Malaysia exhibited female body weights ranging between 800 – 1200 g, whereas males typically ranged between 200 – 400 g, demonstrating a comparable magnitude of sexual size dimorphism. This finding aligns with the female-biased growth pattern reported in other anguillid species such as *A. anguilla* (European eel) and *A. japonica* (Japanese eel), where females may attain body sizes 2 – 5 times larger than males due to extended somatic growth periods prior to maturation (Geffroy & Bardonnet 2016). Similarly, Ditya et al. (2025) documented that more than 70% of individuals exceeding 900 g in Indonesian shortfin eel populations were females, indicating that larger size classes are predominantly female. Comparable patterns have also been recorded across different geographical regions and environmental conditions (Mulyadi et al. 2025), reinforcing that the female-biased body size observed in the present study is quantitatively consistent with previously reported ranges rather than being site-specific.

Given the limited prior study of sexual dimorphism in eels, the present findings offer a primary yet practical data for understanding the sex differentiation based on craniofacial characteristic differences (Arai et al. 2011). Significant differences were observed in jaw and snout morphology, with males exhibiting greater mandibular protrusion and increased jaw extension relative to head length, whereas females displayed a comparatively streamlined cranial profile. The measurable divergence in craniofacial proportions suggests functional differentiation rather than incidental morphological variation. Hsu et al. (2023) reported similar structural divergence in anguillids, proposing that enhanced mandibular projection in males may improve prey capture efficiency, while streamlined cranial morphology in females may reduce hydrodynamic drag, thereby optimizing energetic efficiency during migration and reproductive preparation.

Comparable sexually dimorphic craniofacial traits have been documented in other teleosts. For example, female cichlids exhibit significantly larger buccal cavity volumes than males, a difference directly linked to mouthbrooding behaviour and quantified differences in cranial dimensions (Balolia 2025). Such functional morphological scaling demonstrates that craniofacial dimorphism is frequently associated with reproductive or ecological specialization rather than size disparity. In tropical eels, morphological specialization has similarly been linked to reproductive strategies and habitat adaptation (Rachmawati et al. 2024).

In many teleost fishes, craniofacial traits also serve as secondary sexual characteristics influencing mate choice, competitive interactions, and dominance hierarchies (Hsu et al. 2023). Therefore, the observed craniofacial differentiation in *A. bicolor* may represent an external indicator of reproductive status or fitness, particularly if correlated with gonadal maturation or endocrine activation**.** However, behavioural validation and hormonal correlation analyses are required to confirm whether these morphological traits function as reproductive signals or are solely ecologically adaptive structures.

Non-invasive morphological sexing techniques are practically useful for aquaculture breeding and management purposes (Ching et al. 2019). The distinct craniofacial dimorphism reported in the present study, including mandibular projection and snout shape can be used as a practical tool for early sex identification of *A. bicolor*. Similar morphological charateristics have proven useful in fish aquaculture and could enhance selective breeding programs in other eel species if validated across populations and developmental stages (Hsu et al. 2023).

Non-invasive sex differentiation using reliable morphological features, particularly craniofacial traits, represents a practical approach for hatchery management. Early sex identification facilitates selective rearing strategies that optimize growth performance and broodstock management by preferentially isolating females due to their superior growth rates and reproductive contributions (Mandelli 2016). Given the economic significance of *A. bicolor* in tropical eel aquaculture, integrating these morphological features into hatchery protocols may help overcome persistent challenges in deciding sex of eel (Yuan et al. 2022).

Reproductive organ

The morphometric and histological observations revealed clear sex-specific differences in gonadal development under captive conditions. Female ovaries exhibited advanced reproductive features, characterized by the presence of both previtellogenic oocytes (PVO) and vitellogenic oocytes (VO), with VO constituting a visibly distinct proportion of the oocyte population, indicating progression beyond early primary growth stages. Numerous studies have demonstrated that the simultaneous occurrence of PVO and VO is indicative of active vitellogenesis and advancement toward reproductive maturation (Reading et al. 2018; Nguyen et al. 2024). In the present study, the co-occurrence of these stages across examined females suggests coordinated ovarian activation rather than isolated individual variation, with several individuals approaching the threshold for final maturation (Hagihara et al. 2018).

These findings are quantitatively consistent with reports in cultured *A. japonica* and *A. australis*, where vitellogenic oocytes were detected in hormonally or environmentally conditioned females, whereas males under comparable conditions remained predominantly at early spermatogenic stages (Lokman et al. 2015). In contrast to females, male gonads in the present study did not exhibit equivalent advancement toward late spermatogenic phases, reinforcing sex-specific differences in reproductive responsiveness under captivity. Importantly, the detection of multiple oocyte developmental stages in captive *A. bicolor* females highlights their physiological capacity to initiate vitellogenesis, a critical reproductive milestone that demonstrates measurable endocrine activation and supports the biological feasibility of developing controlled breeding protocols in captivity (Ganias 2013; Jéhannet et al. 2023).

In contrast, male gonadal development progressed significantly more slowly than females, with histological sections showing testes composed predominantly of spermatogonia and lacking advanced spermatogenic stages

such as spermatocytes or spermatids, indicating the absence of active spermatogenesis (Mazzeo et al. 2016). The predominance of early germ cells without progression to later spermatogenic phases quantitatively demonstrates incomplete testicular maturation under captive conditions. This pattern is consistent with findings in *A. anguilla* and *A. japonica*, where captive males typically remain at early spermatogenic stages and require hormonal induction to achieve full spermatogenesis (Divers et al. 2022). Divers et al. (2022) reported that untreated captive males remained arrested at the spermatogonial stage, whereas hormonally treated individuals showed rapid progression to spermiation within weeks, highlighting the endocrine dependency of male maturation. The reproductive divergence observed between sexes presents a practical challenge for broodstock management, as females demonstrate inherent capacity to initiate vitellogenesis, whereas males often require exogenous hormonal stimulation to trigger spermatogenic progression and achieve reproductive synchrony necessary for successful fertilization (Divers et al. 2022).

From the aquaculture perspective, it certainly complicates gamete availably, as the absence of mature spermatozoa at the time of female reach peak ovulation reduces fertilization chances. Therefore, the present study highlighted particularly on male *A. bicolor* with aim to establish rearing protocol of mature male in the captivity to enable synchronizing gamete release between sexes. Taken together, these findings have further highlighted the importance of the current study in sex-specific reproductive strategies in eel aquaculture.

These combined morphological and histological findings enrich the understanding of reproductive biology of *A. bicolor* in captivity and provide a science-based prove for management strategies to be optimized. For example, selecting larger females could improve egg availability and quality, while early intervention with hormonal treatments in males might accelerate spermatogenic development and promote synchronization between sexes. These approaches find precedent in *A. japonica* aquaculture, where sex-specific hormonal regimens and conditioning protocols have been critical to advancing captive breeding success (Okamura et al. 2014). Moreover, the elucidation of sexual dimorphism in both growth and gonadal development in *A. bicolor* features prominently in recent studies advocating for tailored husbandry practices that important for sex-based physiological variance to maximize reproductive output (Rachmawati et al. 2023).

The findings of this study are consistent with the report by Arai and Abdul Kadir (2017) and Nguyen et al (2020) who demonstrated that female *A. bicolor* exhibit significantly higher gonadosomatic index (GSI) values and progress through multiple oocyte maturation stages, whereas males predominantly remain at early spermatogenic phases. In the present study, females displayed histological evidence of vitellogenic progression, contrasting with the comparatively limited advancement observed in males, thereby reinforcing previously documented patterns of sex-specific reproductive development. Arai and Abdul Kadir (2017) further reported that females undergo continuous oocyte recruitment throughout the year, reflecting an opportunistic reproductive strategy, while male maturation appears more temporally constrained. A comparable sex-biased maturation trajectory was observed in this study under captive conditions, suggesting that environmental control alone may be insufficient to stimulate full spermatogenic progression in males (Wylie et al. 2025) These parallel supports the interpretation that captive environments may suppress or delay male reproductive activation, thereby highlighting the potential necessity of hormonal induction to achieve reproductive synchrony and optimize controlled breeding protocols.

This investigation confirms that *A. bicolor* females can initiate and progress through early vitellogenic development in captivity, whereas males tend to remain in early, undifferentiated gonadal stages absent hormonal induction. The differences in growth and maturation between male and female *A. bicolor* highlight the need for hatchery management to adopt sex-specific approaches in broodstock handling, feeding regimes, and hormonal induction to improve spawning outcomes. In aquaculture, selective rearing and nutritional optimization for females alongside hormonal stimulation protocols for males could maximize reproductive conditioning and improve fertilization success rates (Kumar et al. 2026).

Such findings not only advance fundamental knowledge of *A. bicolor* growth and maturation management but also potentially supporting reliable, year-round artificial seed production. Future research is recommended to focus on optimizing environmental parameters, establishment of hormone treatment regimens, and incorporation of molecular markers to further enhance the feasibility and efficiency of captive breeding for this tropical anguillid species.

Allometric index

Sex-specific differences in morphometric scaling and physiological allocation were evident in captive *A. bicolor*, underscoring the interaction between somatic growth dynamics, organ development, and reproductive investment across the 24-month rearing period. Females exhibited a stronger log-transformed body weight – total length (BW – TL) relationship ($R^2 = 0.4402$) compared to males ($R^2 = 0.3118$), representing approximately 41% greater explanatory power in females and indicating higher predictability in somatic scaling**.** Although these coefficients are modest relative to values typically reported for wild anguillid populations (R^2 frequently exceeding 0.80), the comparatively higher explanatory power observed in females suggests reduced inter-individual growth variability and more uniform somatic progression**.** In contrast, the weaker male association implies greater heterogeneity in growth trajectories, potentially reflecting sex-specific energy allocation strategies or increased sensitivity to captive stressors (Yoshikawa 2013).

Comparative analyses across anguillid species consistently demonstrate sexual dimorphism in length – weight relationships. For instance, *A. anguilla* males display near-isometric growth ($b \approx 3.0$; $R^2 = 0.894$), whereas females typically exhibit negative allometry ($b = 2.625$; $R^2 = 0.829$) (Vaughan et al. 2021). Similarly, Mahmoud et al. (2024) reported strong sex-dependent scaling patterns across multiple anguillid taxa. The substantially lower R^2 values recorded in the present captive broodstock suggest attenuation of natural

morphometric scaling under controlled conditions, likely due to environmental homogeneity, restricted migratory behaviour, and cumulative handling stress (Syandri et al. 2023). These findings indicate that morphometric predictability in captivity may diverge quantitatively from wild growth dynamics, particularly in males.

Organ-specific regression analyses further revealed divergent physiological allocation patterns. Gonad weight was not significantly correlated with total length in either sex (males: $R^2 = 0.0251$, $P = 0.7041$; females: $R^2 = 0.6508$, $P = 0.6721$), indicating that somatic elongation did not proportionally translate into gonadal investment**.** Despite a moderate R^2 value in females, the absence of statistical significance suggests asynchronous or incomplete ovarian recruitment rather than coordinated maturation. These observations quantitatively support previous studies demonstrating that full gametogenesis in anguillids requires specific environmental stimuli or exogenous hormonal induction (Lokman et al. 2015; Ferrão et al. 2024).

In contrast, liver weight exhibited a significant positive relationship with total length in males ($R^2 = 0.6508$, $P = 0.0108$), explaining approximately 65% of the variance in hepatic mass, whereas no such association was detected in females ($R^2 = 0.0377$, $P = 0.6587$). This sex-specific hepatic scaling is biologically meaningful given the liver's central role in lipid metabolism, steroidogenesis, and reproductive precursor synthesis (Wang et al. 2020). The stronger male hepatic association suggests heightened metabolic preparation for spermatogenesis**,** whereas females may allocate energy toward early ovarian recruitment in a manner not strictly proportional to somatic length (Adeniran et al. 2017). Comparable hepatosomatic dimorphism has been documented in *A. japonica*, where males exhibited elevated hepatic indices during reproductive activation (Higuchi et al. 2019).

Evaluation of organosomatic indices reinforced these physiological distinctions (Lazarus et al. 2025) While females attained significantly greater body mass, consistent with anguillid sexual size dimorphism and fecundity-driven selection (Lokman et al. 2015), no significant sex difference in gonadosomatic index (GSI) was detected. This contrasts with wild and hormonally stimulated populations, where female GSI typically exceeds male values during advanced maturation stages (Sudo & Yada 2022), suggesting that captive conditions limited full reproductive progression. Conversely, males exhibited elevated hepatosomatic index (HSI), indicating increased hepatic energy mobilisation associated with early spermatogenic activity (Dahle et al 2003; Hatef and Unnaippan 2019).

Collectively, these findings demonstrate that morphometric parameters alone are insufficient predictors of reproductive progression under captive conditions, particularly in males. Instead, reproductive assessment in *A. bicolor* broodstock should integrate morphometric scaling, organosomatic indices, and targeted endocrine conditioning within a unified physiological framework. The comparatively reduced BW – TL predictability observed under captivity further underscores the need to refine environmental cues and hormonal protocols to better emulate natural maturation pathways (Wang et al. 2020; Rachmawati et al. 2023). Such integrative management strategies are essential for advancing controlled reproduction and sustainable aquaculture development of tropical anguillid species.

Conclusion

This study concludes that reproductive performance of short-finned eel (*A. bicolor*) in captivity can be effectively advanced through understanding of sex determination using morphology and reproductive indices, optimization of suitable hormonal induction for sperm production and application of appropriate sperm preservation techniques to support successful breeding in aquaculture *A. bicolor* exhibits clear sexual dimorphism, with females achieving greater growth and showing readiness to enter vitellogenesis, while males displayed slower and incomplete gonadal development unless hormones are administered. Morphological, histological, and physiological evidence coupled with the liver central role in energy allocation and organ function proven to improve the reproductive conditioning.

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Ethics Statement: All experiments were conducted in accordance with the researcher guidelines of the code of practice for the care and use of animals for scientific purposes, Universiti Malaysia Sabah (AEC 0003/2023).

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