Int. J. Aquat. Biol. (2025) 13(6): 10-18
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Growth patterns, sex maturation stages, and fatty acid profiling of hatchery-cultured male short-finned eel (*Anguilla Bicolor*) in relation to the reproduction index under different hormonal treatments

Hanis Binti Abd Mohmin[1], Shigeharu Senoo[2], Fui Fui Ching[*1]

[1]Higher Institution Centre of Excellence, Borneo Marine Research Institute, University Malaysia Sabah, 88400 Kota Kinabalu, Malaysia.
[2]Aquaculture Technology and Production Center, Kindai University, Shirahama, Wakayama, 649-2211, Japan.

Abstract: This study investigated the effects of different hormonal treatments on growth performance, maturation, and fatty acid composition in hatchery-cultured male short-finned eel (*Anguilla bicolor*). A total of 12 individuals were subjected to four treatments — control (no hormone), Human Chorionic Gonadotropin (HCG), pituitary gland extract (PG), and Ovaprim (OVP) — over 100 days. Growth patterns were assessed through morphometric measurements and allometric analysis, while reproductive development was evaluated using gonadosomatic index (GSI), hepatosomatic index (HSI), histological characteristics, and steroid hormone profiling (11-ketotestosterone and estradiol). Fatty acid composition was determined using gas chromatography (GC). The HCG treatment significantly enhanced gonadal development (GSI = 4.36±0.21, $P<0.001$) and elevated 11-KT levels, promoting advanced spermatogenesis, as further evidenced by histological analysis. However, the HCG-injected eel showed lower body weight gain and a weaker length-weight correlation, indicating a shift in energy allocation toward reproduction. Meanwhile, the OVP-injected eel exhibited balanced growth with strong weight-length relationships ($R^2 = 0.94$) and the highest monounsaturated fatty acid (MUFA) levels. PG-injected eel, however, demonstrated moderate effects on reproductive and growth indices. Fatty acid profiling revealed that HCG increased polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA), which corresponded with higher spermatozoa abundance. These results show that HCG can effectively induce sexual maturation in male *short-finned eels, although it may reduce* somatic growth. The varied responses to hormone treatments suggest the potential for enhancing breeding strategies in eel aquaculture through more precise hormonal management.

Article history:
Received 24 May June 2025
Accepted 10 October 2025
Available online 25 December 2025

Keywords:
Growth patterns
Sex maturation stages
Fatty acid profiling
Hormone treatment

Introduction

The short-finned eel, *Anguilla bicolor*, is a species of significant economic value in aquaculture, driven by its high demand in global markets. Particularly prized in culinary traditions across Asia, it is sought after for its rich flavor and nutritional benefits, making it a staple in many regional cuisines (Arai, 2022). The species' growing popularity has intensified interest in aquaculture to meet global demand while reducing pressure on wild populations. The International Union for Conservation of Nature (IUCN) has classified *A. bicolor* as "Near Threatened," highlighting the need for sustainable farming practices to safeguard the species from further decline (Williamsom et al., 2024).

Recent advancements in aquaculture technology offer a promising tool for cultivating *A. bicolor*, with a focus on sustainable practices that balance economic viability with ecological preservation. This is crucial as wild populations face various threats, including overfishing, habitat degradation, and climate change (Shanmughan et al., 2022). Effective management strategies are therefore essential, not only to support the commercial interests of the aquaculture industry but also to ensure the long-term conservation of this important species (FAO, 2022). These strategies include improvements in hatchery techniques, selective breeding, and optimized feeding regimes, all

[*]Correspondence: Fui Fui Ching
E-mail: cfuifui@ums.edu.my
DOI: https://doi.org/10.22034/ijab.v13i6.2528

aimed at enhancing production efficiency while reducing the environmental impact of eel farming.

Hormonal treatments are widely used in aquaculture to induce gonadal maturation and spawning in fish species (Pankhurst, 2016). In *A. bicolor*, various hormones have been tested to enhance reproductive performance in captivity, and among them is Human Chorionic Gonadotropin (HCG), known to stimulate gonadal development and has been shown to increase the gonadosomatic index (GSI) in males significantly. Studies indicate that HCG increases sperm production and improves gamete quality, which correlates with an increased reproductive index in the European eel, *A. anguilla* (Palstra et al., 2023). Meanwhile, the pituitary gland (PG) hormone, which contains endogenous gonadotropins, has also been shown to promote maturation in eels (Rousseau et al., 2013). While Ovaprim (OVP) is a synthetic hormone that combines elements of salmon gonadotropin and domperidone, effectively inducing spawning in various fish species (Ashraf et al., 2023), it has not been reported to be used in eels.

Allometric growth refers to the differential growth rates of various body parts during development, which are closely linked to sexual maturation and reproductive fitness. Research indicates that understanding these growth dynamics will lead to better breeding management by identifying optimal conditions for maturation (Martinez et al., 2016). For instance, variations in growth rates between somatic and gonadal tissues can indicate readiness for spawning, which is critical for successful aquaculture operations (Peña et al., 2023).

Reproductive hormones play an essential role in regulating gonadal development and overall reproductive function in many teleost species. These hormones act as chemical signals that coordinate various physiological processes critical for sexual maturation, gametogenesis, and eventual spawning (Geffroy and Bardonnet, 2016). Among the most significant hormones involved in these reproductive pathways are estradiol and 11-ketotestosterone, both of which have been extensively studied for their roles in regulating key aspects of gonadal development (Kumar et al., 2021). Manipulating these hormone levels through exogenous treatments can significantly improve reproductive outcomes, making hormone therapies an essential aspect of hatchery management and breeding programs (Damsteegt et al., 2020).

Fatty acids, particularly saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), are essential for optimal reproductive performance in fish (Kacar et al., 2016). Various studies have highlighted the significant role of nutrients, particularly lipids, in these reproductive processes. For instance, research has shown that lipid profiles, analyzed through fatty acid composition, provide key insights into reproductive health and development (Kacar et al., 2016; Babatunde et al., 2017; Simat et al., 2020). Therefore, incorporating appropriate fatty acid profiles into broodstock diets can significantly affect the success of breeding programs. Hence, this study investigated the effects of different hormonal treatments of HCG, PG, and OVP on growth performance, maturation, and fatty acid composition in hatchery-cultured male short-finned eel.

Materials and Methods

Experiment design and rearing conditions: This experiment was conducted at the fish hatchery of the Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah (UMS), Malaysia. The experiment was conducted over 100 days using 12 male *A. bicolor* initially weighing 244.5±16.9 g. All experimental eels were cultured in the 700-L HDPE black tank, each equipped with a recirculating aquaculture system (RAS) that provided a continuous flow rate of 10-15 L/min, along with a chiller to maintain a constant seawater temperature of 20°C. The tanks were maintained under low-intensity lighting (~20 lx) with a 12-hour light:12-hour dark photoperiod (Politis et al., 2014). Fish were fed to satiation with squid, and water quality was monitored twice daily (at 0930 and 1430), with bottom cleaning and water renewal conducted as needed.

Four treatments were tested: Control (no hormone),

HCG, PG, and OVP, with each treatment prepared in triplicate. Each *A. bicolor* was tagged near the dorsal muscle with a universal microchip for easy identification. Body weight and total length were recorded at the start and subsequently at five-day intervals throughout the experiment. Blood samples were collected every 5 days after hormone injection. At the end of the experiment, gonad and liver samples were collected from each group for further analysis.

Growth pattern: The allometric growth index of male *A. bicolor* was evaluated by analyzing the relationships between the total length, body weight, gonad weight, and liver weight. Using Microsoft Excel 2010, regression analysis was applied, with length-weight relationships 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 3 (Froese, 2006).

Reproductive indices, including the gonadosomatic index (GSI), hepatosomatic index (HSI), and relative condition factor (RCF), were calculated to assess reproductive patterns. After post-blood collection, testis samples were obtained at the end of the experiment for histological analysis. Standard histological procedures were employed, including fixation in 10% buffered formalin, paraffin embedding, and hematoxylin-eosin staining, to classify gonad maturation stages. Microscopic criteria from Abdel-Aziz et al. (2022) and Brown-Peterson et al. (2011) were applied for gonadal staging. GSI was determined by relating gonad weight to total body weight, while HSI was calculated by comparing liver weight to body weight. The RCF was calculated following Le Cren (1951) to assess the fish's growth condition, with values close to or exceeding 1 indicating expected growth.

Hormone steroid level: To evaluate reproductive hormone levels, blood was collected from each fish near the caudal fin area every 5 days of hormone injection, and the hormones 11-ketotestosterone (11-KT) and estradiol (E2) were measured using ELISA kits from Qayee-Bio, with procedures following the manufacturer's protocol.

Fatty acid profiling: Liver samples from male *A. bicolor* were collected, weighed at the end of the experiment for hepatosomatic index (HSI) calculation, and stored at -20°C. Before fatty acid analysis, the samples were freeze-dried for 24 hours and then sorted by maturation phase to minimize variation in their fatty acid profiles. Fatty acids were extracted and esterified from male liver samples based on Abdulkadir and Tsuchiya (2008) one-step method, with 200-300 mg samples processed in three replicates. Samples were mixed with hexane, an internal standard solution, and 14% BF_3 in methanol, then heated to 100°C for 120 minutes. After cooling, fatty acid methyl esters (FAMEs) were isolated, transferred to vials, and stored at -20°C for gas chromatography-flame ionization detection (GC-FID) analysis. Fatty acid concentrations (CFA) were calculated by comparing peak areas of the sample and internal standard, using the formula of CFA = AS/AIS x CIS/WS, where AS = peak area of fatty acid in the sample in the chromatogram, AIS = peak area of internal standard in chromatogram, CIS = concentration of internal standard (mg), and WS = weight of sample (g). Fatty acids were identified by comparing their retention times with those of a standard mixture containing all fatty acids identified in this study. Each fatty acid was quantified by calculating its peak area relative to the total peak area. These values are referred to as fatty acid content (% weight of total fatty acids).

Statistical analysis: Significance testing of regression coefficients was conducted using analysis of variance (ANOVA), whereas one-way ANOVA was used to assess the significance of individual coefficients in the linear regression model. One-way ANOVAs were used to compare individual regression coefficients in the linear regression model, GSI, HIS, steroid hormone level, and fatty acid profile between treatments. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was assessed using the Levene test. Data were transformed to meet assumptions of normality and

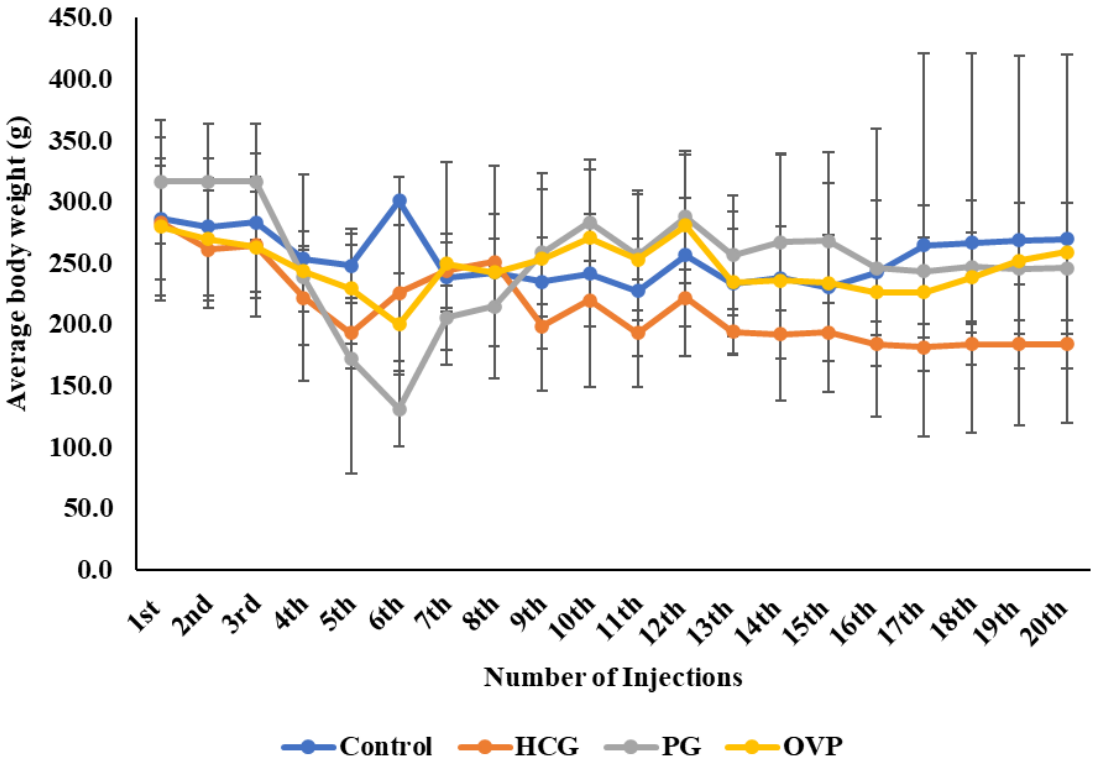

Figure 1. Effects of HCG, PG, and OVP hormone treatments compared to the control group on male eels; body weight changes (g) at 5-day intervals from the 1st to the 20th injection.

homoscedasticity when necessary. Alpha was set at 0.05 for main effects and interactions. Treatment means were contrasted using Tukey's test.

Results

Growth pattern: Figure 1 illustrates the effects of control, HCG, PG, and OVP hormone treatments on the average body weight of male *A. bicolor* over a 20-injection period with a five-day interval. The control treatment shows consistent weight gain, peaking near the 20th injection with an average weight of 270.0±149.8 g. In contrast, the hormone-treated groups (HCG, PG, and OVP) exhibit greater variability in weight changes over time. However, no significant difference was detected (P = 0.586). The HCG treatment shows initial stability, followed by a gradual decline, with the mean weight reaching approximately 184.2±19.5 g by the 20th injection. *Anguilla bicolor* injected with PG displayed a sharp decrease in weight by the 4th injection, followed by fluctuations and a slight upward trend in later injections, reaching around 246.2±53.4 g at the 20th injection. The OVP treatment maintained a relatively stable weight trajectory with moderate fluctuations, ending with a mean weight of approximately 259.2±12.5 g at the 20th injection.

Figure 2 shows the effects of control, HCG, PG, and OVP hormone treatments on total length in male *A. bicolor* over a series of 20 injections with five-day intervals. Across treatments, the control treatment shows the greatest total length, particularly after the 10th injection, with the average total length increasing steadily to a peak of 52.3±10.9 cm by the 20th injection. In contrast, the HCG, PG, and OVP treatments exhibit only minor fluctuations in total length, maintaining a relatively stable value within the specified range (approximately 47.9±2.5–49.2±1.0 cm) and showing no significant differences (P>0.05) over the experimental period. The standard deviation values remain low across all groups, suggesting consistency in the measurements. The control treatment showed an increase in total length, indicating that the other treatments (HCG, PG, and OVP) had minimal effects on total length relative to

Table 1. The length-weight relationship parameters of male *Anguilla bicolor* in different hormone treatments.

Hormone	n	Growth pattern	b, slope of regression	Intercept (a)	R^2	TL range(cm)	BW range (cm)	Kn, Relative condition factor
Control	3	b<3	2.63	0.1180	0.9933	43.9-65.6	154.4-439.2	0.48±0.02
HCG	3	b<3	2.05	0.3060	0.0611	47.8-49.0	163.3-200.1	1.35±0.14*
PG	3	b<3	2.53	0.1575	0.6692	45.3-50.3	229.1-304.8	0.66±0.06
OVP	3	b<3	2.37	0.1998	0.9370	48.3-50.2	251.3-274.5	0.88±0.01

Negative allometric (b<3); Isometric (b=3); Positive allometric (b>3), n = 3. Statistically significant differences between groups (P<0.05) are indicated by *.

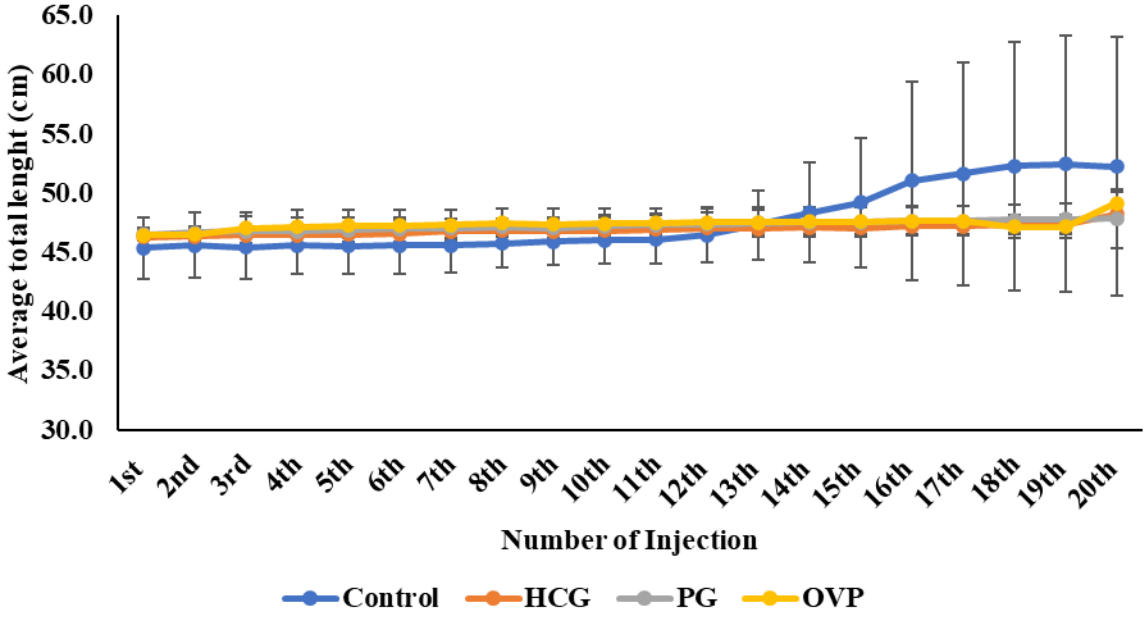

Figure 2. Effects of HCG, PG, and OVP hormone treatments compared to the control group on male *Anguilla bicolor*; total length changes (g) at 5-day intervals from the 1st to the 20th injection.

the untreated group.

Regarding the relationship between body weight and total length for male *A. bicolor*, the control The treatment showed a strong positive relationship between BW and TL, with the equation $W = 0.0073 \times TL^{2.643}$ ($R^2 = 0.9968$). In the HCG treatment, the relationship between BW and TL is less pronounced, with a power equation of $W = 0.0655TL^{2.0496}$ ($R^2 = 0.0611$) (Fig. 3). The low R^2 indicates that HCG treatment may lead to variable growth responses that are less dependent on length. The PG treatment shows a moderate relationship between BW and TL, with a power equation of $W=0.0142TL^{2.5309}$ ($R^2 = 0.7077$), indicating a reasonably consistent relationship between body weight and total length. The OVP treatment exhibits a strong positive relationship between BW and TL, with a power equation of $W=0.0245_{TL}{}^{2.3792}$ ($R^2 = 0.9404$), indicating that OVP treatment closely mimics natural growth patterns, with a strong correlation between length and weight, similar to the control group, mirroring natural growth patterns with slightly higher weight gains for length increases. The results demonstrate that different hormone treatments impact the length-weight relationship in male *A. bicolor* (Fig. 3). The control group and OVP-treated *A. bicolor* show strong, predictable growth patterns, while HCG-treated eels exhibit the weakest correlation between body weight and total length, suggesting that this treatment may disrupt typical growth processes. PG moderately affects growth, maintaining a relatively strong relationship between BW and TL, though with more variability than in the control or OVP groups.

Table 1 presents allometric growth pattern

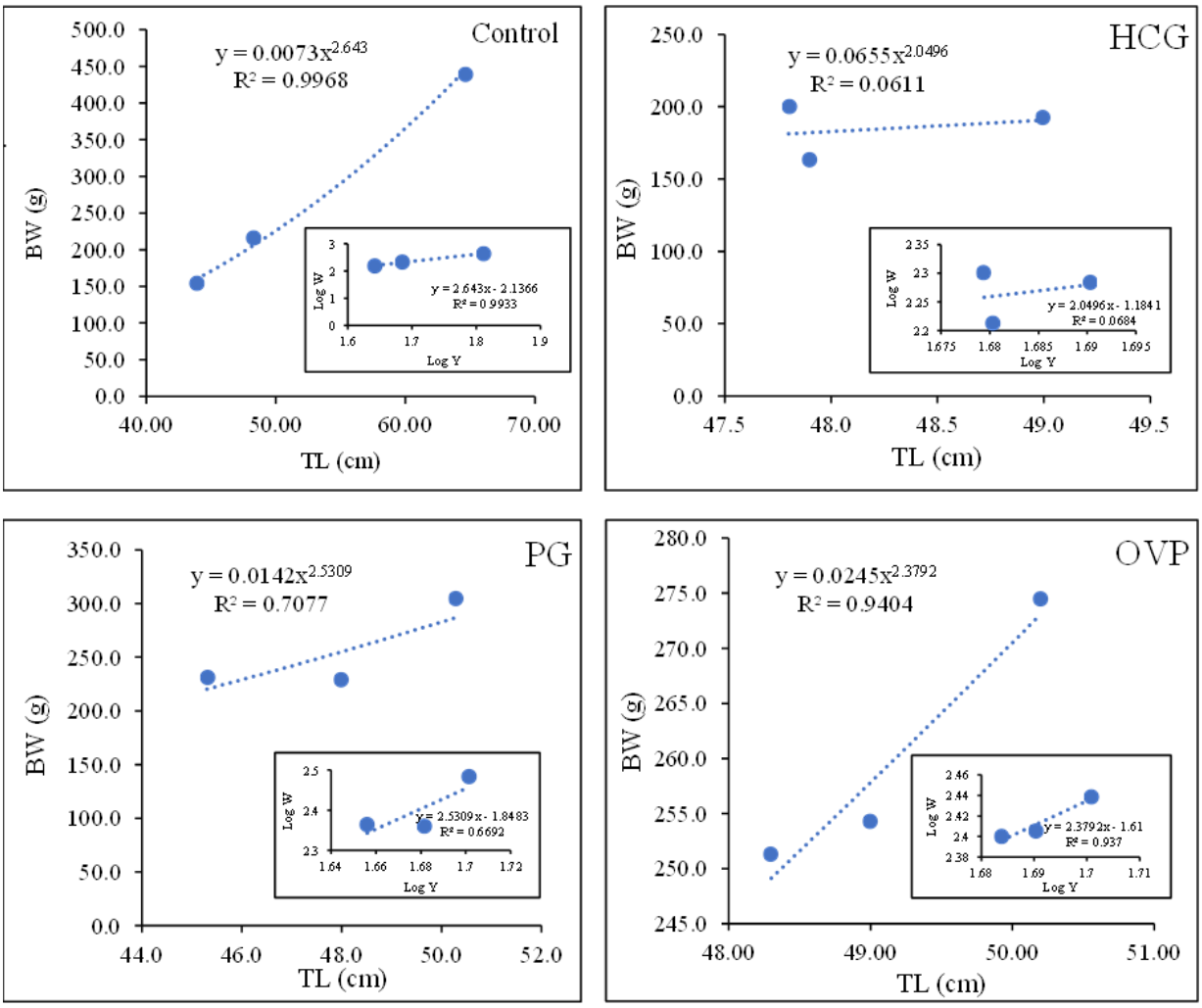

Figure 3. Relationship between BW (body weight) and TL (total length) of HCG, PG, and OVP hormone treatments compared to the control group at the end of the culture period; (n=3).

parameters for male *A. bicolor* across all treatments, including the regression slope (b), intercept (a), R^2, total length (TL), body weight (BW), and the relative condition factor (Kn). All groups displayed negative allometric growth ($b < 3$), indicating a faster increase in length compared to weight. The control group exhibited a strong negative allometric pattern ($b = 2.63$) with a high correlation ($R^2 = 0.9933$), a TL range of 43.9-65.6 cm, a BW range of 154.4-439.2 g, and a low Kn of 0.48±0.02, indicating suboptimal fish condition. In the HCG-treated group, the growth in weight relative to length was weaker ($b = 2.05$) with a low correlation ($R^2 = 0.0611$), a narrow TL range (47.8-49.0 cm), a BW range of 163.3-200.1 g, and the highest Kn of 1.35±0.14, indicating good condition despite the weak length-weight relationship. The PG-treated group displayed moderate growth ($b = 2.53$) with a fair correlation ($R^2 = 0.6692$), a TL of 45.3-50.3 cm, a BW of 229.1-304.8 g, and a Kn of 0.66±0.06, indicating a relatively good condition compared to the control group but still below optimal levels. This indicates that while PG treatment may stimulate some growth, it does not necessarily translate into superior health outcomes (Wakiya et al., 2019). The OVP group showed balanced growth ($b = 2.37$), a high correlation ($R^2 = 0.9370$), a TL of 48.3-50.2 cm, a BW of 251.3-274.5 g, and a Kn of 0.88±0.01, indicating strong overall health and condition.

Table 2 presents the results of the relationship between gonad and liver weight and total length in male *A. bicolor* under various hormone treatments. For the gonad weight–total length relationship, data

Table 2. Linear regression analysis for gonad and liver weight relationship with different hormone treatment on male *Anguilla bicolor*.

Items	n	R^2	Intercept (a)	Slope (b)	S.E	t-stat	Weight range (g)	P-values
Gonad weight–Total length relationship								
Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HCG	3	0.1018	-5.3644	3.7234	0.0898	-0.2995	6.7-8.9	0.814
PG	3	0.9986	-3.0601	1.6263	0.0020	-30.6936	0.43-0.51	0.020*
OVP	3	0.9995	-80.0149	46.331	0.0088	-64.6971	0.01-0.06	0.2874
Liver weight–Total length relationship								
Control	3	0.8941	-1.4912	1.2258	0.0521	-2.0624	3.10-5.22	0.0108*
HCG	3	0.6423	-6.2728	3.9372	0.0255	-1.2350	2.10-2.40	0.4333
PG	3	0.9369	-2.6076	1.7974	0.0150	-3.3306	2.31-2.78	0.1857
OVP	3	0.9296	-3.3674	2.2745	0.0052	-4.5813	2.91-3.18	0.1368

N/A: Not available. Statistically significant differences between groups (P<0.05) are indicated by *.

for the control group are unavailable. The HCG-treated group showed a low correlation (R^2 = 0.1018) with a slope of 3.7234 and a high P-value (0.814), indicating no significant relationship. In the PG-treated group, a strong correlation (R^2 = 0.9986) with a slope of 1.6263 and a *P*-value of 0.020 is observed, indicating a significant positive relationship. The OVP-treated group exhibits a robust correlation (R^2 = 0.9995) with a high slope of 46.331, although the P-value is not statistically significant (P = 0.2874).

For the relationship between liver weight and total length, the control group shows a strong correlation (R^2 = 0.8941), with a slope of 1.2258 and a significant P-value (0.0108). The HCG treatment shows a moderate correlation (R^2 = 0.6423), with a slope of 3.9372 and a non-significant *P*-value (P = 0.4333). In the PG treatment, a strong correlation is observed (R^2 = 0.9369) with a slope of 1.7974 and a *P*-value of 0.1857, indicating non-significance. The OVP treatment shows a high correlation (R^2 = 0.9296) with a slope of 2.2745, but its *P*-value (0.1368) is not significant. Overall, the PG treatment is significant for gonad weight, and the control group is significant for liver weight relative to total length. Other treatments do not show significant relationships.

The gonadosomatic index revealed significant differences between treatments. The HCG-treated group exhibited the highest mean GSI (4.36±0.205), indicating a notable enhancement in gonadal development, with a significant difference (P<0.001). In contrast, the PG treatment resulted in a mean GSI of 0.195±0.032, and the OVP treatment had the lowest mean GSI at 0.0118±0.010. The control group showed no measurable GSI, indicating no gonadal development (Fig. 4).

The hepatosomatic index (HSI) showed variability across treatments but did not differ significantly from the GSI (P=0.081). The control group had an HSI mean of 1.70±0.445. The HCG treatment had a mean HSI of 1.24±0.182, suggesting a possible metabolic response to the treatment. The PG group recorded a mean HSI of 1.08±0.206, whereas the OVP treatment had a mean HSI of 1.17±0.093 (Fig. 5). These results suggest that although HCG treatment significantly enhances gonadal development, as indicated by the GSI, HSI across groups shows moderate variation, indicating differences in energy allocation related to reproduction.

Figure 6 shows the gonadal development of male *A. bicolor* under different hormonal treatments. The control group exhibited only significant gonadal enlargement, accompanied by notable fat accumulation, without any detectable gonad tissue. The HCG-treated group showed visibly elongated and compact gonadal tissue, indicating strong hormonal stimulation, whereas the PG treatment exhibited moderate gonadal growth. The OVP treatment showed minimal gonadal growth, as evidenced only by adipose gonadal tissue. These results suggest that hormone treatments, particularly HCG, effectively

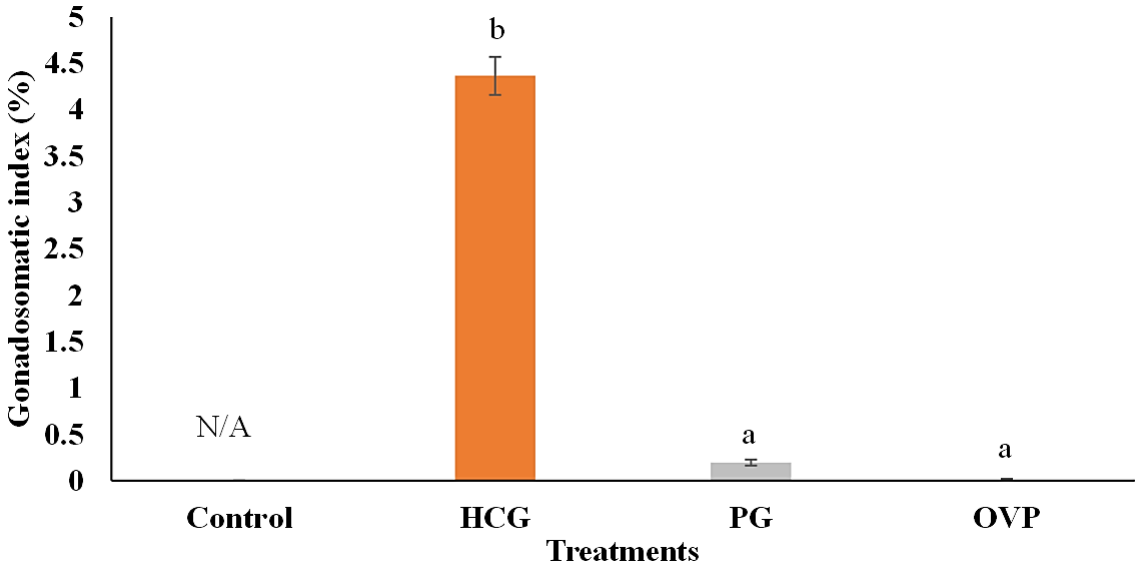

Figure 4. The mean ± SD of gonadosomatic index (GSI) in different treatments. Significant differences between groups are indicated by different letters (P<0.05).

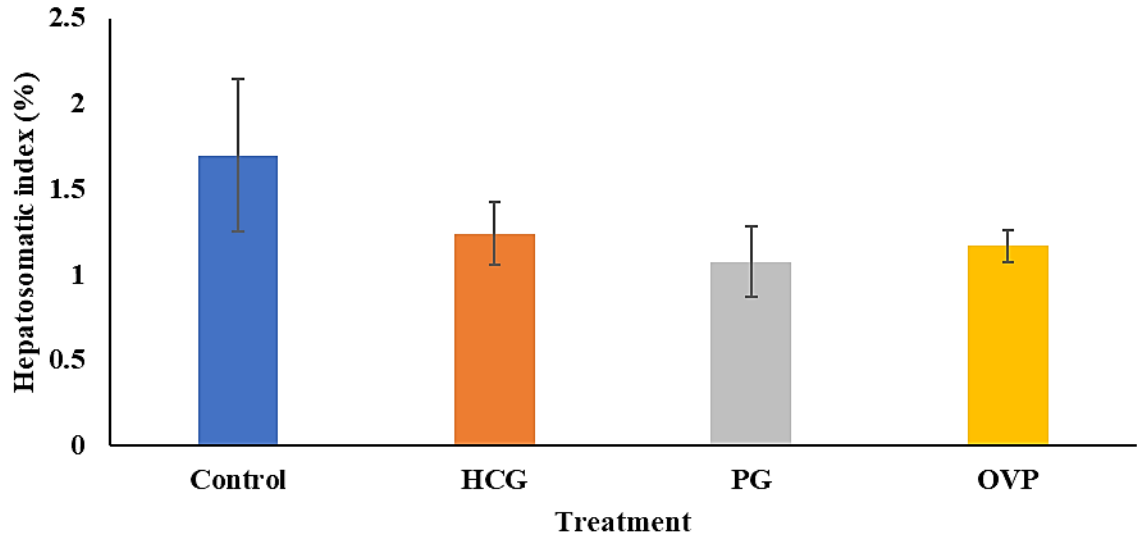

Figure 5. The mean ± SD of hepatosomatic index (HSI) in different treatments. Significant differences between groups are indicated by different letters (P<0.05).

promote gonadal development in A. bicolor, with varying degrees of efficacy across hormones.

The histological section in Figure 7 shows the stages of gonadal development under different treatments. HCG treatment resulted in abundant spermatozoa (SPZ), indicating gonadal maturation. PG treatment showed the presence of both spermatocytes (SC) and spermatozoa (SPZ), signifying a developing gonad. OVP treatment revealed only spermatogonia (SPG) and spermatocytes (SC), representing an immature gonad.

Hormone steroid level: Figure 8 presents the changes in 11-Ketotestosterone (11-KT) levels in male *A. bicolor* subjected to hormone treatments with HCG, PG, and OVP over a series of injections. Initially, all treatment groups had relatively low 11-KT levels (8.33±1.53, 9.33±3.06, 10.0±2.00, and 8.672±2.08 ng/mL, respectively) with no significant differences (P>0.05). By the 5th injection, slight increases are observed across most groups, with the control and HCG groups showing slightly elevated levels (10.00±1.00 and 11.50±2.61 ng/mL) with a significant

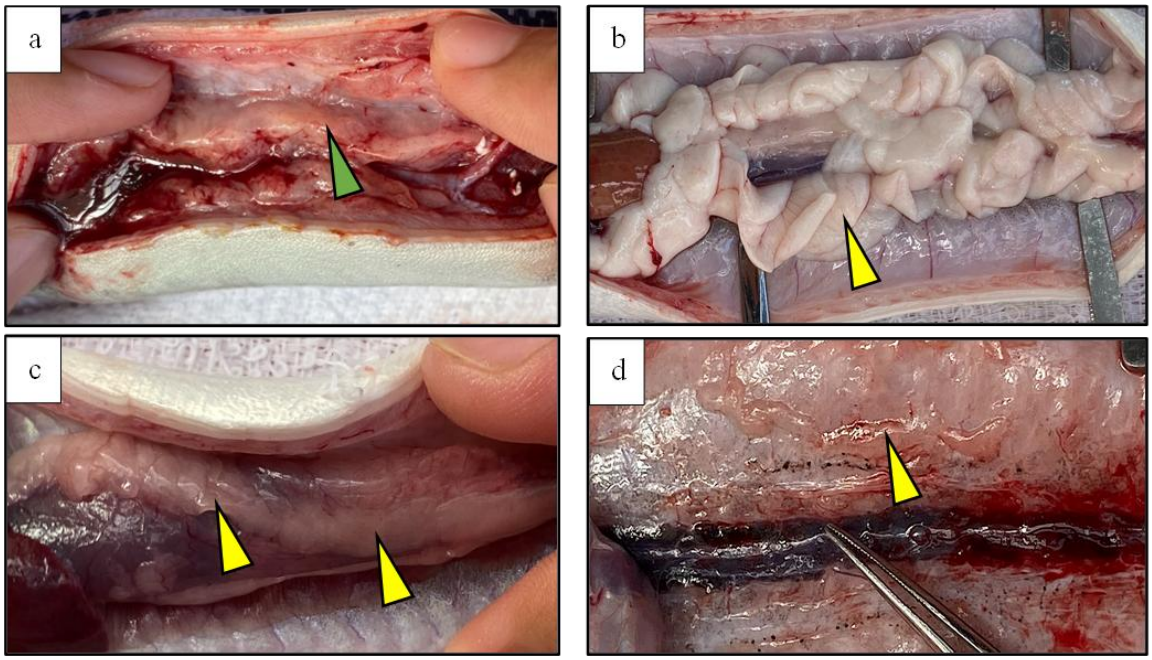

Figure 6. The gonad morphology development in different treatment groups. (a) Control, (b) HCG, (c) PG, and (d) OVP. The green arrow indicated fat only, while the yellow arrow indicated the gonad morphology (Scale = 2.0 cm).

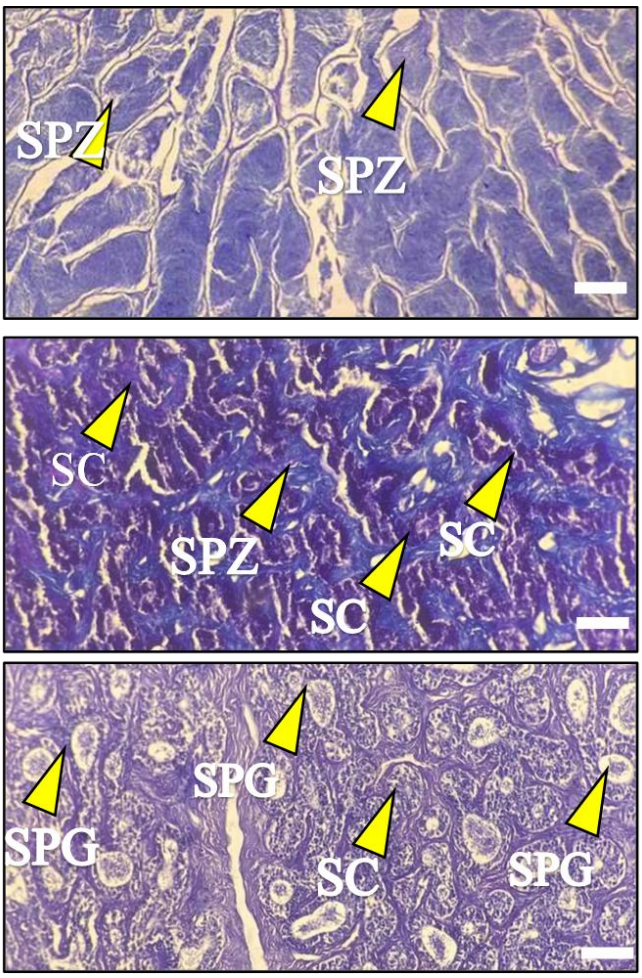

Figure 7. The histological section from different treatment groups. (a) HCG, (b) PG, and (c) OVP. The yellow arrow indicated the sperm cells: spermatozoa (SPZ), spermatocytes (SC), and spermatogonia (SPG) (Scale = 50 μm).

difference (P=0.024). By the 10th injection, levels in the HCG groups continue to increase, whereas PG, OVP, and control remain relatively consistent with their initial levels (8.83±1.04, 8.07±2.10, and 8.87±1.67, respectively), with only minor fluctuations. From the 15th to the 20th injection, there is a significant increase in 11-KT levels (P=0.028), particularly in the HCG group, which peaks at approximately 20.13±1.10 ng/mL by the 20th injection compared with other treatments. OVP and PG also show moderate increases, and the control group exhibits a moderate increase, reaching approximately 9.10±0.69 and 11.70±1.87 ng/mL by the 20th injection, respectively, and remaining lower than the other groups.

In the E2 analysis (Fig. 9), initial injections show low concentrations across all groups, with slight increases in the HCG and PG group (3.67±0.58, 3.83±0.76 ng/ml, respectively) and OVP reaching up to 4.33±1.15 ng/ml, while one group maintains lower levels (2.33±0.58 ng/ml). By the 5th injection, OVP shows a significant difference (P<0.05) in E2 (6.67±1.15 ng/ml) compared with other treatments,

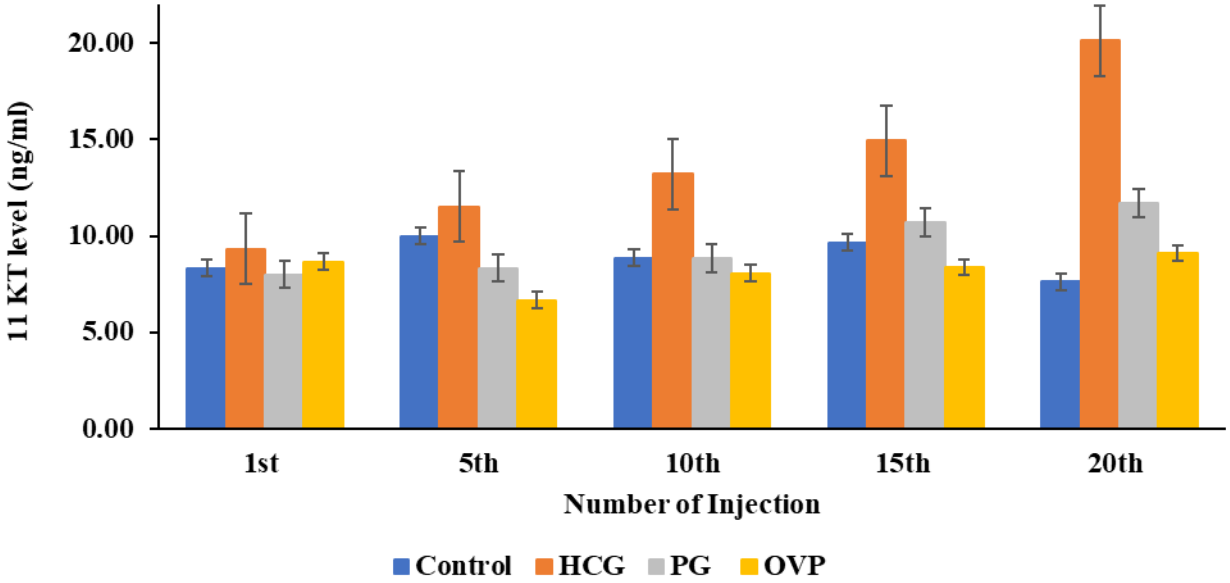

Figure 8. Steroid hormone 11 KT levels (ng/ml) of each treatment. Statistically significant differences between groups are indicated by different letters (P<0.05).

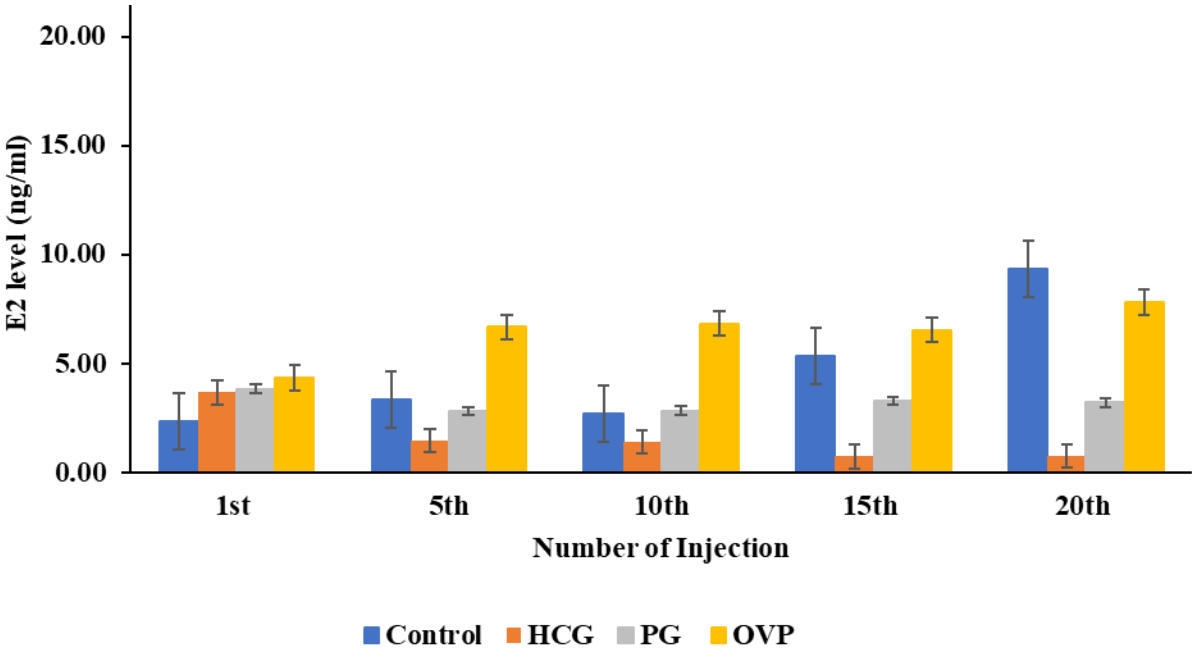

Figure 9. Steroid hormone E2 levels (ng/ml) of each treatment. Statistically significant differences between groups are indicated by different letters (P<0.05).

whereas control and PG stabilize at moderate levels (3.33±1.53 and 2.80±0.71 ng/ml), and HCG shows the lowest levels (1.47±0.47 ng/ml). This trend continues with subsequent injections: OVP consistently shows elevated E2 levels that are significantly different (P<0.05), followed by PG and Control, whereas HCG remains at minimal E2 levels. By the 20th injection, OVP and control show further increases in E2 (7.80±2.00 and 9.33±1.53 ng/ml, respectively), while PG exhibits a steady increase (3.20±0.90 ng/ml), and HCG remains low (0.77±0.31 ng/ml).

Fatty acid profiling: Table 3 displays the fatty acid composition (%) of total saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in *A. bicolor* under different hormone treatments (Control, HCG,

Table 3. Fatty acid composition (%) of total saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) of different hormone treatments.

Fatty acid	Control	HCG	PG	OVP
N	**3**	**3**	**3**	**3**
C6:0	0.03±0.12	0.07±0.12	0.10±0.01	0.14±0.322
C10:0	0.67±0.10	1.52±0.43	0.07±0.28	0.15±0.265
C12:0	36.4±0.23	16.04±0.14	0.05±0.03	0.04±0.109
C14:0	2.71±0.12	2.34±0.23	6.13±0.17	4.77±0.112
C15:0	0.16±0.05	0.20±0.12	0.55±0.43	1.63±0.156
C16:0	17.42±1.12	0.23±0.21	4.59±0.59	5.18±0.102
C17:0	0.10±0.82	0.02±0.421	28.53±0.11	27.17±0.124
C18:0	0.70±0.03	0.10±0.875	1.82±0.06	1.53±0.231
C20:0	0.45±0.11	0.05±0.642	0.12±0.09	0.14±0.542
C21:0	0.11±0.07	N.D	0.16±0.08	0.07±0.05
C22:0	0.11±0.12	0.10±0.645	0.14±0.21	0.59±0.109
Σ SAFA	**58.87±11.07^{c}**	**20.67±4.57^{a}**	**42.28±1.078^{b}**	**41.43±0.733^{b}**
C14:1	0.08±0.02	1.50±0.085	0.09±0.134	0.69±0.145
C15:1	0.05±0.11	0.04±0.245	0.05±0.234	0.28±0.054
C16:1	3.27±0.47	2.69±1.034	2.75±2.131	4.80±0.311
C17:1	0.36±0.12	1.78±0.16	6.88±0.132	13.16±0.834
C18:1n9t	18.98±0.41	4.43±0.751	0.05±0.211	1.80±0.423
C18:1n9c	0.42±0.132	1.50±0.95	8.50±0.70	5.00±0.088
C20:1n9	0.14±0.04	11.89±0.384	0.15±0.297	0.79±0.016
Σ MUFA	**23.29±0.704^{b}**	**23.83±1.89^{b}**	**18.48±0.778^{a}**	**26.51±0.813^{b}**
C18:2	2.23±0.34	0.08±0.17	0.15±0.341	0.05±0.602
C18:2n6c	3.80±0.231	0.07±0.723	0.17±0.218	0.16±0.712
C18:3n6	3.81±0.11	0.10±0.34	3.30 ±0.18	0.44±0.104
C20:2	2.22±0.34	0.07±0.11	0.54±0.08	3.38±0.012
C20:3n6	1.93±0.251	0.45±0.48	0.56±0.241	0.16±0.054
C20:3n3	3.11±0.431	0.77±0.678	0.85±0.231	2.30±0.421
C20:4n6 (ARA)	0.05±0.421	10.03±0.03	9.98±0.412	10.71±0.998
C20:5n3 (EPA)	0.50±0.012	9.42±0.453	5.41±0.631	0.25±0.341
C22:2	0.15±0.121	0.11 ±0.451	0.13±0.856	0.30 ±0.341
C22:6n3 (DHA)	0.04±0.56	34.40±0.856	18.16±0.111	14.32±0.526
Σ PUFA	**17.84±0.229^{a}**	**55.50±0.249^{d}**	**39.24±0.117^{c}**	**32.06±0.374^{b}**

N.D: Not detected

PG, and OVP). In terms of SAFA, the control group had the highest content (58.87±11.07 %), with a significant difference (P<0.01), followed by the PG (42.28±1.078 %), OVP (41.43±0.733 %), and HCG (20.67±4.57 %) groups. For MUFA, the OVP group showed the highest composition (26.51±0.813%) with a significant *P*<0.01, while the control, HCG, and PG groups had lower values (23.29±0.704, 23.83±1.89, and 18.48±0.778 %, respectively). In terms of PUFA, the HCG treatment had the highest composition (55.50±0.249%), which was significantly different (*P*<0.01) from the other groups, followed by PG (39.24±0.117%), OVP (32.06±0.374%), and the control (17.84±0.229%).

Discussions

Allometric growth pattern: In the present study, the control group exhibited a steady increase in weight, whereas fish treated with hormones (HCG, PG, and OVP) showed more variable weight changes over time, although these differences were not statistically significant. Specifically, the HCG-treated fish maintained initial weight stability but experienced a gradual decline. This trend aligns with findings by Zahri et al. (2015), who reported that HCG primarily promotes gametogenesis, potentially at the expense of somatic growth. Similarly, *A. bicolor* injected with PG showed a sharp weight decline by the 4th injection, followed by fluctuations and a slight upward trend, reaching around 246.2±53.4 g at the 20th injection.

This pattern may reflect the metabolic costs associated with PG-induced spermatogenesis (Blakeslee et al., 2018).

The control group exhibited a consistent increase in total length, peaking at approximately 52.3±10.9 cm by the 20th injection. In contrast, fish treated with hormones such as HCG, PG, and OVP exhibited minimal fluctuations in total length, maintaining a relatively stable range of 47.9±2.5 cm to 49.2±1.0 cm, with no statistically significant differences (P>0.05). These findings suggest that while hormonal treatments may initially influence growth, their long-term impact on somatic growth appears limited (Nuraini et al., 2017). For instance, research on the African catfish (*Clarias gariepinus*) has demonstrated that Ovaprim effectively induces breeding and enhances reproductive parameters, such as fertilization and hatching rates (Ameer et al., 2021).

However, this study did not examine long-term somatic growth post-injection, leaving a gap in understanding the potential trade-offs between reproduction and growth in hormone-treated fish. Additionally, a study on the siban fish (*Cyclocheilichthys apogon*) found that higher doses of OVP (7 mL kg^{-1} body weight) significantly improved spawning performance, including an increased relative number of ovulated eggs and fertilization rates (Nuraini et al., 2017). While this indicates OVP's efficacy in improving reproductive outcomes, the study did not assess subsequent weight changes, making it difficult to directly compare the somatic growth effects observed.

The length-weight relationship is a fundamental metric in evaluating fish growth and overall health. In this study, the control group of *A. bicolor* demonstrated a strong positive relationship between body weight (BW) and total length (TL). This near-perfect correlation suggests that, in the absence of hormonal manipulation, body weight growth scales predictably with length, indicating a healthy, allometrically consistent growth trajectory. Such patterns of strong, predictable negative allometric growth have also been observed in wild eels and other freshwater fish species, supporting the idea that natural somatic development prioritizes proportional scaling (Putri and Syamsudin, 2021).

In contrast, the HCG-treated group showed a much weaker correlation (R^2 = 0.0611) with a slope (b = 2.05), indicating a disrupted or irregular length-weight relationship. Although the condition factor (Kn = 1.35±0.14) was the highest among all groups, suggesting that individuals were apparently healthy or well-nourished, the lack of correlations among size metrics indicates that HCG may drive internal physiological or reproductive processes rather than proportional somatic growth. This observation aligns with findings by Palstra et al. (2023), who noted that HCG tends to accelerate gonadal development at the expense of muscle or length gains, potentially explaining the disconnect between TL and BW. A similar trend was noted in a study by Lukas et al. (2019), which reported that while HCG improved gonad index and reproductive readiness in rainbow trout (*Oncorhynchus mykiss*), it did not yield substantial gains in length-weight metrics.

PG-treated eels showed a moderate relationship between BW and TL (R^2 = 0.7077, b = 2.53), with a condition factor of Kn = 0.66±0.06, slightly better than the control but still below optimal. This suggests variability in response, likely due to individual sensitivity to PG and timing of spermatogenic effects. Similar findings were reported by Wakiya et al. (2019), who observed that PG administration in salmonids produced inconsistent length-weight relationships, largely influenced by hormone dose, application timing, and fish maturity stage, supporting the moderate yet variable growth trend observed in this study. Interestingly, the OVP treatment produced one of the strongest correlations between BW and TL (R^2 = 0.9404), with a slope of b = 2.37 and Kn = 0.88±0.01. These findings suggest that OVP treatment closely mirrors natural growth trajectories and may enhance energy allocation to somatic growth. Similarly, Nazir et al. (2023) reported that OVP enhances growth efficiency and maturation in catfish (*Clarias batrachus*) by supporting both reproductive and muscular development, a result that aligns with the higher BW seen for corresponding TL in the

present study.

All treatments exhibited negative allometric growth ($b < 3$), with the control group showing the steepest decline in proportional weight gain relative to length ($b = 2.63$). This is consistent with patterns observed in eels under natural environmental conditions, in which energy is often directed toward survival and length-based foraging rather than bulk accumulation (Putri and Syamsudin, 2021). The high condition factor in HCG-treated fish, despite a poor length-weight correlation, further supports the notion that hormonal treatments, particularly those targeting reproductive maturation, may enhance physiological condition metrics (e.g., liver and gonad indices) without corresponding somatic growth (Lukas et al., 2019).

The analysis of the relationships between gonad and liver weights and total length in male *A. bicolor* under different hormone treatments shows varied patterns: the PG-treated group exhibits a strong positive correlation ($R^2 = 0.9986$), and the control group also shows a significant relationship with liver weight. These findings can be further contextualized by recent research on other species. For example, a study on the slimy sculpin (*Cottus cognatus*) found that gonadal and liver sizes fluctuated in synchrony with the reproductive cycle, indicating significantly energy allocation to reproductive organ development, particularly during breeding periods (Brasfield et al., 2013). This observation supports the hypothesis that hormonal treatments, such as PG, can significantly influence gonadal growth.

Similarly, in a study of goldfish (Carassius auratus), metabolic changes were observed across reproductive phases, indicating that liver metabolism adjusts to support gonadal development during energetically demanding reproductive periods (Ladisa et al., 2021). This supports your observation that liver weight correlates with total length in the control group, suggesting that the liver is an essential organ for energy storage and distribution, particularly under natural growth conditions. It also highlights that hormonal treatments, such as PG and OVP, may affect liver weight. However, the P-values for the correlations between liver weight and PG and OVP were not significant, suggesting that these hormones may not directly affect liver growth, unlike gonadal development.

Moreover, research on tilapia (*Oreochromis niloticus*) has shown that gonadal development directly influences growth by regulating growth hormones and insulin-like growth factors (IGF-I) (Bhatta et al., 2012). This is particularly relevant to the significant relationship between gonad weight and total length observed in the PG-treated group. The direct hormonal interaction between the gonads and growth factors may explain why PG-treated A. bicolor showed stronger correlations in gonad weight than in liver weight, supporting the idea that hormonal treatments, such as PG, primarily affect reproductive organ development.

The significantly higher mean GSI observed in the HCG-treated group (4.36 ± 0.205) aligns with studies demonstrating that HCG effectively stimulates gonadal development in fish. For instance, research on greater amberjack (*Seriola dumerili*) reported that HCG treatment led to notable enhancements in spermatogenesis, emphasizing its role in promoting gonadal maturation (Ventriglia et al., 2024). In contrast, the low GSI observed in the PG-treated group (0.195 ± 0.032) and the minimal GSI in the OVP-treated group (0.0118 ± 0.010) suggest that these hormones may have a less pronounced effect on gonadal development in *A. bicolor*. This finding is supported by studies in which different hormonal treatments produced varying degrees of gonadal maturation, indicating species-specific responses to hormonal induction. (Rozenfeld, 2019).

The observed HSI values across treatments in the study suggest that although HCG treatment significantly enhances gonadal development, it does not proportionally increase liver weight, as indicated by the lower HSI. This could indicate a diversion of energy resources towards reproductive development, a phenomenon also observed in other species (Kumar et al., 2021). For instance, research on emperor snakehead (*Channa marulioides*) found that follicle-stimulating hormone (FSH) induction influenced both

GSI and HSI, with correlations to estradiol-17β levels, suggesting a link between hormonal treatments, gonadal development, and liver condition (Kumar et al., 2021).

Furthermore, studies have reported a positive correlation between HSI and GSI across various fish species, indicating that higher energy reserves, as indicated by HSI, are often associated with enhanced reproductive success (Lal et al., 2023). However, the lack of significant differences in HSI among the treatment groups suggests that, although hormonal treatments effectively stimulate gonadal development, they may not substantially alter liver weight, possibly because the liver's role in energy metabolism supports reproductive processes (Lal et al., 2023).

These findings highlight the differential effects of various hormonal treatments on gonadal development in male *A. bicolor*. The control group exhibited significant gonadal enlargement accompanied by notable fat accumulation; however, gonadal tissue was not detected. In contrast, the HCG-treated group showed visibly elongated, compact gonadal tissue, indicating effective hormonal stimulation. The PG treatment resulted in moderate gonadal growth, whereas the OVP treatment showed minimal gonadal development, as evidenced solely by adipose tissue. These observations suggest that hormone treatments, particularly HCG, effectively promote gonadal development in *A. bicolor*, with varying degrees of efficacy among the different hormones.

Recent studies have further elucidated the role of hormonal treatments in fish reproduction. For instance, research on snakehead (*Channa striata*) demonstrated that combinations of pregnant mare serum gonadotropin (PMSG) and anti-dopamine treatments significantly enhanced gonadal maturation compared to controls (Ath-thar et al., 2021). Similarly, a study on the longtooth grouper (*Epinephelus bruneus*) emphasized the importance of exogenous hormones, such as HCG, for inducing maturation in broodstock, findings that parallel those observed in *A. bicolor* (Oh et al., 2013). These studies collectively highlight the critical role of hormonal manipulation in improving reproductive outcomes in aquaculture.

HCG treatment resulted in abundant spermatozoa (SPZ), indicating gonadal maturation. This finding aligns with studies that hormonal treatments significantly enhanced spermatogenesis. For example, research on the chameleon goby (*Tridentiger trigonocephalus*) has demonstrated that hormonal manipulation can accelerate the transition from primordial germ cells to mature gametes, highlighting the role of hormones in gonadal development (Cho et al., 2014). PG treatment showed the presence of both spermatocytes (SC) and spermatozoa (SPZ), signifying a developing gonad. This observation supports earlier research indicating that various growth factors and hormones can promote different stages of spermatogenesis. OVP treatment revealed only spermatogonia (SPG) and spermatocytes (SC), representing an immature gonad. This finding is consistent with previous reports showing that certain treatments can inhibit or delay gonadal maturation (Cho et al., 2014). These findings collectively underscore the efficacy of hormonal treatments, particularly HCG, in promoting gonadal development and maturation in *A. bicolor*, aligning with observations in other fish species subjected to similar hormonal manipulations.

Hormone steroid level: At the start of the experiment, 11-KT levels were low in all groups, with no significant differences. This baseline finding aligns with previous studies, including those by Sudo et al. (2012), which reported that male silver eels maintain low androgen levels prior to hormonal stimulation. As the injections progressed, small increases in 11-KT levels were observed across most groups, with the HCG-treated group showing the largest increase, while the control group showed only minor increases. This finding is consistent with the study by Wang et al. (2020), which demonstrated that prolonged exposure to 11-KT notably accelerated oocyte development, correlating with increased androgen receptor expression in ovarian tissues. The rising 11-KT levels observed in the HCG-treated group indicate successful stimulation of the hypothalamic-pituitary-gonadal axis, resulting in increased androgen production.

By the later stages of the experiment, the HCG-treated group showed a marked rise in 11-KT levels, reaching a peak, whereas the PG, OVP, and control groups exhibited relatively stable or minor fluctuations. This trend reinforces the critical role of HCG in spermatogenesis, as previously indicated by Sudo et al. (2022). The HCG treatment appears to have the most substantial effect on 11-KT production, likely facilitating both spermatogenesis and metabolic processes in male *A. bicolor*, as observed in other studies on fish reproduction (Diver et al., 2010). Recent studies have supported these findings, demonstrating that 11-KT not only promotes oocyte development but also plays a crucial role in lipid metabolism during gonadal maturation. For example, Wang et al. (2020) observed that 11-KT treatment increased lipoprotein lipase activity in ovarian tissues, thereby aiding lipid uptake, which is essential for oocyte growth. This suggests that the enhanced 11-KT levels in *A. bicolor* may similarly support spermatogenesis and metabolic functions, highlighting the hormone's multifaceted role in reproductive maturation.

In the analysis of estradiol (E2) levels, initial values were low across all groups, with slight increases observed in HCG and PG, whereas OVP showed a slightly higher value. The control group maintained the lowest levels. By the midpoint of the injections, OVP showed a significant increase in E2 compared with the other groups, while the control and PG groups stabilized at moderate levels. Notably, the HCG group consistently recorded the lowest E2 levels. This trend persisted, with OVP showing the highest E2 levels at the end of the study, while HCG remained at minimal values. These findings align with research by Zhang et al. (2024), which highlighted the regulatory role of hormonal treatments in controlling estradiol levels and reproductive outcomes in various species. The elevated E2 levels observed in the OVP group suggest that this treatment may play a critical role in regulating estradiol in male *A. bicolor*, contributing to gonadal development. This supports the growing body of literature emphasizing the importance of specific hormonal treatments in influencing reproductive hormone dynamics across species, underscoring the need for targeted strategies in aquaculture to optimize reproductive success.

Fatty acid profiling: The control group showed the highest SAFA concentration, which was significantly higher than that of the other treatments. In comparison, the PG, OVP, and HCG groups exhibited lower SAFA levels. This indicates that the control treatment significantly enhances SAFA levels, which are essential for energy storage and cellular function (Widodo et al., 2021). For MUFA, the OVP treatment resulted in the highest concentration, significantly surpassing those of the control, HCG, and PG groups. This higher concentration of MUFAs in the OVP-treated group is associated with beneficial effects on cardiovascular health (Widodo and Musnina, 2021). These findings underscore the importance of optimizing MUFA levels to improve overall fish health in aquaculture systems.

In terms of PUFA, the HCG treatment exhibited the highest concentration, with a significant difference from the other groups. This suggests that hormonal treatments, particularly HCG, may increase PUFA levels, which play critical roles in reproductive processes such as gonadal development, gametogenesis, and hormone regulation (Jamaluddin et al., 2019). This supports findings from Wijayanti and Setiyorini (2018), which showed that PUFA-enriched diets significantly improved gonadal development in *A. bicolor*. The elevated PUFA levels observed under HCG treatment may be associated with improved reproductive outcomes. Moreover, studies have shown that docosahexaenoic acid (DHA), a crucial component of membrane phospholipids, facilitates sperm mobility and quantity (Butts et al., 2015). The current findings support this, with HCG-treated *A. bicolor* exhibiting the highest DHA levels and producing the greatest number of spermatozoa among the treatments. This aligns with previous research linking DHA to sperm membrane structure and function, which are essential for successful reproduction (Baeza et al., 2014).

Overall, the results suggest that hormonal treatments significantly influence the fatty acid

composition in *A. bicolor*. Specifically, PG and OVP treatments increase SAFA, OVP boosts MUFA, and HCG enhances PUFA, with each type of fatty acid playing an important role in various biological processes. These findings highlight the potential to optimize hormonal treatments to improve reproductive outcomes in aquaculture and suggest that future research should focus on developing dietary strategies to further enhance these beneficial effects.

Conclusion

The proper hormone for male *A. bicolor* is HCG, which has been shown to significantly boost gonadal development while reducing body weight, reflecting a trade-off favoring gametogenesis. The HCG treatments also altered hormone and fatty acid levels, with implications for energy storage and reproduction. These findings highlight the potential of tailored hormone regimens in aquaculture to optimize growth and reproductive success.

Acknowledgement

The authors thank the staff and students of the Fish Hatchery at the Borneo Marine Research Institute, Universiti Malaysia Sabah, for their unwavering support and technical assistance throughout the study. This study was funded by Universiti Malaysia Sabah (Grant code: GUG-0651). This project was also supported by the Higher Institution Centres of Excellence (HICoE), [JPT(BKPI)1000/016/018/35 (2), HIC2402].

Ethics approval*:* All experiments were conducted in accordance with the guidelines of the Code of Practice for the Care and Use of Animals for Scientific Purposes at Universiti Malaysia Sabah, under approval code AEC 0009/2024.

References

Abdel-Aziz M.F., Zied R.M., Hassan H.U., Sayed A.D., Ahmad H., Mushtaq S., Yaqoob H., Habib A., Arai T. (2022). Effects of replacement of dietary fish oil with plant oil on growth performance and fatty acid composition of spinefoot rabbitfish, *Siganus rivulatus*. Brazilian Journal of Biology, 84: e262969.

Abdulkadir S., Tsuchiya M. (2008). One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. Journal of Experimental Marine Biology and Ecology, 354(1): 1-8.

Ameer M.W., Jabeen F., Asad M., Kaukab G., Bashir A., Rasheed M., Akram M. (2021). Comparative efficacy of Ovaprim and hMG (menotropin) to induce breeding in African catfish (*Clarias gariepinus*). Fish Physiology and Biochemistry, 47(5): 1559-1564.

Arai T. (2022). Sustainable management of tropical anguillid eels in Southeast Asia. In: Natural resources conservation and advances for sustainability. Elsevier. pp: 461-480.

Ashraf S., Khizar F., Mushtaq I., Masud H., Shah S.A.H., Sarwar M.S. (2023). Journal of Aquatic Research and Sustainability. Journal of Aquatic Research and Sustainability, 1(1): 3-7.

Ath-Thar M.H., Gustiano R., Sundari S., Kurniawan K., Prakoso V.A., Kusmini I.I. (2021). Pregnant mare's serum gonad hormones and antidopamine treatments on the maturation of snakehead (Channa striata). Aquaculture, Aquarium, Conservation and Legislation, 14(1): 173-180.

Babatunde M.M., Abdullah N., Osho I.B., Ashiru O.M. (2017). Nutritional requirements of fish for optimum reproduction. Aquaculture Nutrition, 24(5): 1200-1211.

Bhatta S., Iwai T., Miura C., Higuchi M., Shimizu-Yamaguchi S., Fukada H., Miura T. (2012). Gonads directly regulate growth in teleosts. Proceedings of the National Academy of Sciences, 109(28): 11408-11412.

Blakeslee C.J., Galbraith H.S., Deems R.M. (2018). The effects of rearing temperature on American glass eels. Agricultural Sciences, 9(08): 1070.

Brasfield S.M., GR Tetreault, ME McMaster, J Bennett, KR Munkittrick (2013). Seasonal patterns of gonad size, liver size, and in vitro gonadal steroidogenic capacity in slimy sculpin (*Cottus cognatus*). Water Quality Research Journal of Canada, 48(3): 243-254.

Brown-Peterson N.J., Wyanski D.M., Saborido-Rey F., Macewicz B.J., Lowerre-Barbieri S.K. (2011). A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries, 3(1): 52-70.

Butts I.A.E, Baeza R., Støttrup J.G., Krüger-Johnsen M., Jacobsen C., Pérez L., Tomkiewicz J. (2015). Impact of dietary fatty acids on muscle composition, liver lipids, milt composition and sperm performance in European eel. Comparative Biochemistry and Physiology Part A:

Molecular and Integrative Physiology, 183: 87-96.

Cho H.C., Hwang I.J., Baek H.J. (2014). Histological analysis of early gonadal development and sex differentiation in chameleon goby, *Tridentiger trigonocephalus*. Development and Reproduction, 18(1): 51.

Damsteegt E.L., Thomson-Laing G., Wylie M.J., Lokman P.M. (2020). Effects of estradiol and 11-ketotestosterone pre-treatment on artificial induction of maturation in silver female shortfinned eels (*Anguilla australis*). Plos one, 15(2): e0229391.

FAO. (2022). The state of world fisheries and aquaculture. Towards blue transformation. FAO. Rome, Italy.

Froese R. (2006). Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology, 22(4): 241-53.

Geffroy B., Bardonnet A. (2016). Sex differentiation and sex determination in eels: consequences for management. Fish and Fisheries, 17(2): 375-398.

Jamaluddin, Muhsinah N.A., Widodo A. (2019). Comparative study on fatty acid profile of eel fish (*Anguilla bicolor*) in elver eel and silver eel phase from Palu River and Lake Poso. Research Journal of Pharmacy and Technology, 12(12): 5794.

Kacar Y., Polat N., Aydin M. (2016). Nutrient dynamics in gonad development of marine fishes. Journal of Fish Biology, 89(4): 907-916.

Kumar R., Mohanty U.L., Pillain B.R. (2021). Effect of hormonal stimulation on captive broodstock maturation, induced breeding and spawning performance of striped snakehead, *Channa striata* (Bloch, 1793). Animal Reproduction Science, 224: 106650.

Ladisa C., Ma Y., Habibi H.R. (2021). Seasonally related metabolic changes and energy allocation associated with growth and reproductive phases in the liver of male goldfish (*Carassius auratus*). Journal of Proteomics, 241: 104237.

Lal J., Biswas P., Singh S.K., Debbarma R., Deb S., Yadav N.K., Patel A.B. (2023). Effects of dietary aromatase inhibitors on masculinization of rosy barb (*Pethia conchonius*): Evidence from growth, coloration and gonado-physiological changes. Plos one, 18(11): e0287934.

Le Cren E.D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). The Journal of Animal Ecology, 201-19.

Lukas A.Y., Santoso P., Tobuku R. (2019). Physiological study of Indonesian shortfin eel *Anguilla bicolor* on different temperature medium using a recirculation system. Aquaculture, Aquarium, Conservation and Legislation, 12(6): 2227-2235.

Mahboob S., Sheri A.N. (2002). Relationships among gonad weight, liver weight and body weight of major, common and some Chinese carps under composite culture system with special reference to pond fertilization. Asian-Australasian Journal of Animal Sciences, 15(5): 740-744.

Martínez-Montaño E., González-Álvarez K., Lazo L.P., Audelo-Naranjo J.M., Vélez-Medel A. (2016). Morphological development and allometric growth of yellowtail kingfish *Seriola lalandi* V. larvae under culture conditions. Aquaculture Research, 47(4): 1277-1287.

Nazir S., Khan N., Azmat H., Naveed S., Ramzan M.M., Davies S.J. (2023). Efficacy of various concentrations of synthetic hormones on the induced breeding of *Channa marulius* (Sole). Journal of the World Aquaculture Society, 54(1): 143-155.

Nuraini N., Tanjung A., Warningsih T., Muchlisin Z.A. (2017) Induced spawning of siban fish *Cyclocheilichthys apogon* using Ovaprim. F1000Research, 6: 1855.

Oh S.R., HC Kang, CH Lee, SW Hur, YD Lee (2013) Sex reversal and masculinization according to growth in longtooth grouper *Epinephelus bruneus*. Development and Reproduction, 17(2): 79.

Palstra AP, I Van de Ven , P Jéhannet, L Kruijt, H Schipper, W Swinkels, LT Heinsbroek (2023) Human chorionic gonadotropin enhancement of early maturation and consequences for reproductive success of feminized european eel (Anguilla anguilla). *Fishes*, *8*(6), 281.

Pankhurst NW (2016) Reproduction and development. In: Fish physiology (Vol. 35). Academic Press. pp: 295-331.

Peña R., McGregor-Bravo C.U., Contreras-Olguín M. (2023). Allometric growth and larval development in Pacific red snapper *Lutjanus peru* under culture conditions. Journal of Fish Biology, 102(2): 413-425.

Politis S.N., Butts I.A., Tomkiewicz J. (2014). Light impacts embryonic and early larval development of the European eel, *Anguilla anguilla*. Journal of Experimental Marine Biology and Ecology, 461: 407-15.

Putri M.R.A., Syamsudin T.S. (2021). Population structure

of tropical eel (*Anguilla bicolor bicolor*) in Cikaso River, West Java. In: E3S Web of Conferences. EDP Sciences, 322: 05008

Ricker W.E. (1957) Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada, 191: 1-382.

Rousseau K., Lafont A.G., Pasquier J., Maugars G., Jolly C., Sébert M.E., Aroua S., Pasqualini C., Dufour S. (2013). Advances in eel reproductive physiology and endocrinology. Eel Physiology. Science Publishers, 1-43.

Rozenfeld C. (2019). Development of innovative methods for induction of European eel (Anguilla anguilla) spermatogenesis. Doctoral dissertation, Universitat Politècnica de València. 201 p.

Shanmughan A., Dahanukar N., Harrison A., Pinder A.C., Ranjeet K., Raghavan R. (2022). Demographics and exploitation of two Near Threatened freshwater eels, *Anguilla bengalensis* and *Anguilla bicolor*, in small-scale subsistence fisheries and implications for conservation. Aquatic Conservation: Marine and Freshwater Ecosystems, 32(2): 269-281.

Shiraishi H, Crook V. (2015). Eel market dynamics: an analysis of *Anguilla* production, trade and consumption in East Asia. Traffic. Tokyo, Japan.

Simat V., Bogdanovic T., Soldo B. (2020) Gametogenesis and energy demands in fish reproduction. Marine Ecology, 41(3): 178-192.

Sudo R., Kawakami Y., Nomura K., Tanaka H., Kazeto Y. (2022). Production of recombinant Japanese eel (*Anguilla japonica*) growth hormones and their effects on early-stage larvae. General and Comparative Endocrinology, 317: 113977.

Sudo R., Tosaka R., Ijiri S., Adachi S., Aoyama J., Tsukamoto K. (2012). 11-ketotestosterone synchronously induces oocyte development and silvering-related changes in the Japanese eel, *Anguilla japonica*. Zoological Science, 29(4): 254-259.

Ventriglia G., Fakriadis I., Papadaki M., Zupa R., Pousis C., Mandalakis M., Mylonas C.C. (2024) Effects of different hormonal treatments on spermatogenesis advancement in hatchery-produced greater amberjack *Seriola dumerili* (Risso 1810). General and Comparative Endocrinology, 348: 114447.

Wang W., Zhu H., Tian Z., Sun A., Dong Y., Dong T., Hu H. (2020) Effects of 11-ketotestosterone on development of the previtellogenic ovary in the sterlet, *Acipenser ruthenus*. Frontiers in Endocrinology, 11: 115.

Wakiya R., Itakura H., Kaifu K. (2019) Age, growth, and sex ratios of the giant mottled eel, *Anguilla marmorata*, in freshwater habitats near its northern geographic limit: a comparison to tropical regions. Zoological Studies, 58.

Widodo A., Ummiah R., Anam S. (2021) Analysis of fatty acid profile on Rono Lindu (*Oryzias sarasinorum*) and Rono Poso (*Adrianichthys oophorus*) fishes endemic of Central Sulawesi. Journal of Physics: Conference Series, 1763(1): 012081.

Widodo A., Musnina W.O.S. (2021) Comparative study of amino acids profile of eel fish (*anguilla bicolor*) in the yellow eel and silver eel phase from Lake Poso. International Journal of Pharmaceutical Research, 13(1): 09752366.

Wijayanti I., Setiyorini E.S.S. (2018). Nutritional content of wild and cultured eel (*Anguilla bicolor*) from Southern Coast of Central Java. *Ilmu Kelautan:* Indonesian Journal of Marine Sciences, 23(1).

Williamson M.J., Jacoby D.M., Bašić T., Walker A., Piper A.T. (2024). Social network analysis as a tool to inform anguillid eel conservation and management. ICES Journal of Marine Science. 81(2):402-10.

Zahri A., Sudrajat A.O., Junior M.Z. (2015). The gonad growth of *Anguilla bicolor bicolor* induced by hormone combination of HCG, MT, E2, and antidopamine. Jurnal Akuakultur Indonesia, 14(1): 69-78.

Zhang Y., Chen J., Gao Z., Wang H., Liang D., Guo Q., Liu Q. (2024). Identification of heterosis and combining ability in the hybrids of male sterile and restorer sorghum [*Sorghum bicolor* (L.) Moench] lines. Plos one, 19(1): e0296416.