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Effects of stocking density of broodstock on semi-artificial propagation and dietary protein levels on fry performance of swamp eel *Monopterus albus* in tank conditions

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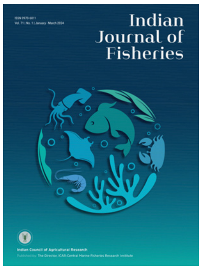

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Abstract

This study examined the effects of broodstock density on semi-artificial propagation efficiency and the influence of dietary protein levels on the rearing performance of swamp eel (*Monopterus albus*) fry under hatchery conditions. Two experiments were conducted using a completely randomised design with three replicates each. In Experiment 1, sexually mature eels (100–180 g) were stocked in $0.5\ m^3$ composite spawning tanks at densities of 3, 4, and 5 individuals per tank. Each tank contained three artificial nesting substrates, while aquatic vegetation (*Ludwigia adscendens*) was grown on the tank surface to enhance spawning conditions. Artificial rainfall was applied to stimulate reproductive behavior, and broodstock were fed a commercial pellet diet containing 45% protein. Eggs were incubated in round plastic basins (20 cm dia) with gentle aeration. In Experiment 2, fry (0.023 g) were reared for 45 days in 16.4 l plastic trays at a density of 100 individuals per tray and fed diets containing 45, 50, and 55% protein. Among the broodstock densities that resulted in successful spawning, no significant differences were detected between tanks stocked with 3 and 4 individuals, with respect to nest formation, number of eggs per nest, hatching rate, number of newly hatched larvae per nest, or larval body size ($p>0.05$). In contrast, no nests were formed at a stocking density of five individuals per tank. Dietary protein levels significantly affected fry growth performance, with 55% protein level producing the highest growth during the first 15 days of rearing ($p<0.05$). However, fry fed 50% and 55% protein diets exhibited comparable growth performance during the later rearing stages ($p>0.05$), and both achieved significantly higher growth than those fed the 45% protein diet ($p<0.05$). Overall, a broodstock stocking density of three individuals per spawning tank and a feeding regime using a 55% protein diet during the early rearing phase, followed by a 50% protein diet later, may be beneficial under the conditions of this study.

Introduction

The swamp eel (*Monopterus albus* Zuiew, 1793), also known as the rice field eel or Asian swamp eel, is a freshwater species that can be found all over South-east Asia, China, and parts of the United States (Froese and Pauly, 2008; Shafland *et al*., 2010). This eel species, part of the Synbranchidae family, is highly prized for its high protein content, bioactive compounds, and its remarkable ability to thrive in various freshwater habitats, such as rice paddies, ponds, ditches, and swamps (Siang *et al*., 2007; Zhang *et al*., 2019; Mao *et al*., 2024; Pintar *et al*., 2024; Zhang *et al*., 2025). The swamp eel is widely cultured in Vietnam, China, Thailand, Philippines, and other South-east Asian countries (Khanh and Ngan, 2010; Yang *et al*., 2018; Ame and Mayor, 2021). In Vietnam, the demand for swamp eel broodstock has recently increased alongside the development of farming systems, shifting from traditional earthen ponds and rice fields to intensive recirculating aquaculture and aquaponics systems, driven by strong domestic demand and growing export potential (Khanh and Ngan, 2010; Hien *et al*., 2019; Nhan *et al*., 2019; Nhan *et al*., 2020).

A distinctive biological characteristic of *M. albus* is its protogynous hermaphroditism, whereby individuals first mature as females, and some later transition to males after the initial spawning. This trait creates challenges for broodstock management in aquaculture (Cheng *et al*., 2003; He *et al*., 2010; Matsumoto *et al*., 2011; Zhou and Gui, 2016). Consequently, artificial propagation and commercial seed production remain challenging. Broodstock supply for swamp eel farming still depends largely on wild capture, resulting in seasonal shortages, fluctuations in fry availability and pricing, and increased pressure on natural populations (Ly *et al*., 2008; Guerrero, 2014; Phuong *et al*., 2017; Yang *et al*., 2018; Lv *et al*., 2020; Susatyo *et al*., 2020). For sustainable swamp eel aquaculture, it is important to have reliable ways to produce broodstock and the right feeding strategy for the early stages of eels.

In the wild, *M. albus* exhibits distinct reproductive behaviours, with males constructing burrows and foam nests and providing paternal care during the breeding season (Yin *et al*., 2005; Yin and Liu, 2010; Matsumoto *et al*., 2011). Spawning usually happens in shallow, vegetated areas during the warm, rainy season. These complex natural behaviors have led to difficulties in breeding swamp eels in captivity (Yin *et al*., 2004; Ishimatsu *et al*., 2018). Observations of natural gonadal development and spawning behaviour under captive and semi-controlled conditions have been documented (Guan *et al*., 1996; Do *et al*., 2008; Ly *et al*., 2008; Yang *et al*., 2018). Hormone-induced spawning and embryonic development were characterized by Guan (1996). The combination of luteinising hormone-releasing hormone analog (LHRHa) and human chorionic gonadotropin (HCG) was shown to effectively induce ovulation (Yeung *et al*., 1993; Phuong *et al*., 2017). Recent endeavours have concentrated on enhancing broodstock conditioning *via* optimised nutrition, photothermal regulation, and environmental simulation (Luo *et al*., 2020). The glycoprotein-rich hatching froth produced during spawning protects embryos and maintains oxygenation (Yin and Liu, 2010). Nevertheless, reproductive performance remains inconsistent due to variations in broodstock quality and spawning habitat conditions (Phuong *et al*., 2017; Liu *et al*., 2023).

On the other hand, diet and nutrient formulation play crucial roles in early fish development, with dietary protein serving as the main source of amino acids essential for growth, tissue repair, enzyme synthesis, and immune function (Wilson, 1986; Conceição, 1997). Protein demand is high during the fry and juvenile stages; however, excessive levels can increase nitrogen excretion and deteriorate water quality (Daiqing *et al*., 2000; Yue *et al*., 2019; Jiang *et al*., 2022). Amino acid balance and digestibility also strongly influence nutrient utilisation and intestinal health (Hien *et al*., 2019; Yang *et al*., 2024), while nutritionally optimised diets enhance enzyme activity, physiological performance, and protein retention efficiency (Jiang *et al*., 2022). Studies have shown that the best amount of protein in the diet for *M. albus* is between 35 and 55%, depending on the amount of fat, the stage of life, and the culture system (Daiqing *et al*., 2000; Ma *et al*., 2014; Lam *et al*., 2019; Tung *et al*., 2019; Yue *et al*., 2019; Nhan *et al*., 2020). To improve fry survival and growth, it is important to optimise protein nutrition during early nursing. This study aims to develop a tank-based semi-artificial spawning method and evaluate the effects of dietary protein levels on fry-rearing performance under hatchery conditions, contributing to sustainable aquaculture development in Vietnam.

Materials and methods

Experimental animals and conditions

The broodstock eels (*M. albus*) used for the semi-artificial propagation experiment (100–180 g in weight and 35–45 cm in length) (Fig. 1) were reared from the adult individuals to sexual maturity over a period of 12–14 months at the Freshwater Aquaculture Experimental Hatchery, Tra Vinh University, Vinh Long Province, in the south of Vietnam, while fry eels for the nursery experiment (mean weight and length of 0.023 g and 0.30 cm) were obtained from a local eel artificial propagation hatchery nearby. Freshwater for the experiments was sourced from a tributary river. It was pumped into a treatment tank and allowed to settle for three days. The water was then disinfected with potassium permanganate ($KMnO_4$) at 15 g m^{-3} and stored for 5–7 days before use. The 0.5 m^3 composite tanks maintained at a water depth of 40–50 cm were used as spawning units. Each spawning tank was placed with three nesting substrates for the broodstock eels (Fig. 2). Each nesting substrate was prepared using a 20 l plastic barrel. Each barrel had two drilled holes positioned 10 cm above the bottom, allowing passage for the broodstock, and was centrally and vertically installed with a PVC pipe (Ø114 mm) and filled with highly flexible clay soil obtained from rice fields. The top of each PVC pipe was covered with a ceramic tile,

Fig. 1. Broodstock eels (*M. albus*) used for semi-artificial propagation

Fig. 2. Nesting substrates positioned in the spawning tank

while aquatic vegetation (*Ludwigia adscendens*) was grown on the water surface of the barrels to provide a natural nesting habitat (Fig. 3). Before being added to the barrels, the clay soil was thoroughly cleaned of debris and disinfected using lime powder (CaO) at a rate of 4–5 kg per 40 m³. It was then soaked in freshwater and sun-dried for seven days prior to use. Round plastic basins, featuring a bottom diameter of 20 cm and a water depth of 20 cm, served as egg incubation devices and included gentle aeration. For the fry-rearing experiments, 16.4 l plastic trays (31 × 22 × 24 cm) were filled with water to a depth of 4 cm. Each tray included a nylon mesh that covered approximately one-fifth of the bottom area, serving as an artificial substrate, and was lightly aerated throughout the experimental period (Fig. 4). Additionally, Fooshee commercial pellet diets (produced by Xiamen Biotime Biotechnology Co., Ltd., Vietnam) containing 45, 50, or 55% protein, with a particle size of 1.2 mm, were fed to the fry to assess their rearing performance.

Fig. 3. Ceramic tiles placed over the PVC pipes, with *L. adscendens* grown on the surface of the barrels

Fig. 4. Fry-rearing units comprising plastic trays (31 × 22 × 24 cm) equipped with artificial substrate and slight aeration

Experimental protocols and data collection

This study was carried out from November 2024 to April 2025 at Tra Vinh University's Aquaculture and Agriculture Wet Lab in Vietnam. It comprised two tests utilising a completely randomised design with three replications to assess the effectiveness of semi-artificial propagation in tanks and the impact of varied protein levels in commercial pellet feed on swamp eel fry rearing performance.

Experiment 1 evaluated the effectiveness of semi-artificial propagation techniques in tank systems. Thirty-six healthy, uniform-sized swamp eel broodstock were randomly assigned to nine spawning tanks at stocking densities of 3, 4, and 5 individuals per tank, with each density replicated three times. Prior to stocking, broodstock were bathed in a 5% sodium chloride (NaCl) solution for prophylactic disinfection. After a two-day acclimation period under experimental conditions, the trial was initiated. Broodstock were fed a commercial pellet diet containing 45% protein twice daily (07:30 and 16:30) at a feeding rate of approximately 5–7% of body weight per day. Artificial rainfall was applied throughout the culture period to stimulate spawning, and half of the tank water was replaced every three days. Egg occurrence in the spawning nests was monitored every five days through careful lifting of the ceramic tiles covering the PVC pipes, followed by a visual inspection. Upon detecting eggs, their developmental stages were evaluated, with orange-yellow eggs (Fig. 5) identified as the most suitable for collection and incubation. The eggs were carefully scooped using a fine-mesh net, rinsed with clean freshwater, and transferred to aerated incubation basins (Fig. 7) at a density of 300 eggs l⁻¹. The eggs hatched after 4 to 7 days of incubation. The parameters monitored in this experiment included:

Fig. 5. Orange-yellow eel eggs of *M. albus*

The number of nests formed in each spawning tank was recorded after 30 days of broodstock culture. The number of eggs per nest was counted after all eggs in a nest were collected.

Hatching rate (HR %) = (No. of newly hatched larvae/No. of stocked eggs at the beginning of incubation) × 100

The number of newly hatched larvae per nest was determined by counting them after all incubated eggs had completely hatched. In addition, the body size of newly hatched larvae was assessed by randomly sampling five newly hatched larvae from each incubation basin. Larval length was measured to the nearest millimeter using a divided ruler, and weight was recorded using a 4-digit analytical balance.

Experiment 2 evaluated the effects of dietary protein levels on the rearing performance of swamp eel fry. A total of 900 fry were randomly allocated to nine rearing trays, with each tray stocked with 100 individuals. The fry were fed diets containing 45, 50, or 55% protein twice daily at 7:30 and 16:30, at a daily ration of approximately 5–7% of body weight. Throughout the experiment, the water in each tray was completely replaced twice daily, two hours after each feeding, and the trays and substrates were cleaned simultaneously.

Every 15 days during the rearing period, five eels were randomly selected from each tray (15 individuals per each dietary protein level) to evaluate growth performance. Individual body weights were determined using an electronic balance with a precision of 0.01 grams. A scaled ruler was used to measure lengths (in millimeters). At the end of the experiment, the survival rate and feed conversion ratio (FCR) were recorded and calculated. Important performance indicators were calculated using the follwoing formulae:

$$\text{Daily weight gain (DWG, g day}^{-1}\text{)} = \frac{\text{(Final weight - Initial weight)}}{\text{No. of rearing days}}$$

$$\text{Daily length gain (DLG, cm day}^{-1}\text{)} = \frac{\text{(Final length - Initial length)}}{\text{No. of rearing days}}$$

$$\text{Specific growth rate in weight (SGRw, \% day}^{-1}\text{)} = \frac{[\text{(ln (final weight) - ln (initial weight)}]}{\text{No. of rearing days}} \times 100$$

$$\text{Specific growth rate in length (SGRw, \% day}^{-1}\text{)} = \frac{[\text{(ln (final length) - ln (initial length)}]}{\text{No. of rearing days}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Final fish number}}{\text{Initial fish number}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry feed fed (g)}}{\text{Total wet weight gain (g)}}$$

In both experiments, key water quality parameters were regularly monitored. Temperature (29.19–29.87°C) and pH (8.32–8.37) were measured using a HANNA meter (Model HI98103, Romania), while total ammonical nitrogen (TAN) and nitrite ($N{-}NO_2^-$) concentrations were determined using Sera test kits (Germany). All measured parameters were within the optimal ranges for *M. albus* culture (Nhan *et al*., 2019; Lukistyowati *et al*., 2022; Yuan *et al*., 2024).

Data analysis

SPSS v20.0 for Windows was used to analyse the data. Differences between treatments were analysed using one-way ANOVA and Tukey's *post hoc* test at a significance level of $p<0.05$. Levene's test was used to assess variance homogeneity, and percentage data was arcsine-transformed before analysis.

Results

Broodstock density influenced the nesting success of *M. albus* under semi-artificial propagation conditions in tanks. Tanks stocked with three numbers of broodfishes produced more nests than those containing four, although this difference was not significant ($p>0.05$; Table 1). No nests were observed at the highest density (five broodstocks per tank). Furthermore, no significant differences were observed in the number of eggs per nest, hatching rate, number of newly hatched larvae per nest, or larval size (mean weight and length) among the tested broodstock densities ($p>0.05$, Table 1).

In the first 15 days, both mean weight (MW_{15}) and mean length (ML_{15}) of the fry increased significantly as dietary protein rose from 45 to 55% ($p<0.05$, Tables 2 and 3). From day 30 onwards, although MW continued to increase significantly with higher protein levels ($p<0.05$, Table 2), other growth parameters in terms of weight, including MW_{30}, WG, DWG, and SGRw, at a 55% dietary protein level were significantly higher than at 45% ($p<0.05$, Table 2) but did not differ from the 50% protein level ($p>0.05$, Table 2). A similar pattern was observed for growth parameters in terms of length (ML_{30}, ML_{45}, LG, DLG, and SGR_L), which were significantly greater at the 55% dietary protein level than those at 45% ($p<0.05$, Table 3) but were comparable to the 50% level ($p>0.05$, Table 3).

Survival rates in the fry were very high, ranging from 94.67% to 97.33%. The feed conversion ratio (FCR) varied between 2.00 and 2.33. Neither of these parameters showed significant difference among the tested dietary protein levels ($p>0.05$, Table 4).

Discussion

Efficiency of semi-artificial propagation at different broodstock densities

The present study demonstrated that broodstock density strongly influenced nest formation in *M. albus* under semi-artificial propagation conditions. Although broodstock densities of three and four individuals per 0.5 m³ spawning tank produced comparable numbers of nests, increasing the density to five individuals

Table 1. Reproductive performance of *M. albus* under semi-artificial propagation conditions in tanks

Parameters	Density (Ind. tank^{-1})		
	3	4	5
Nest number per spawning tank (nest)	1.00 ± 0.00^a	0.67 ± 0.58^a	-
Egg number per nest (egg)	164.33 ± 129.02^a	161.50 ± 159.09^a	-
Hatching rate (%)	96.94 ± 3.01^a	90.89 ± 1.56^a	-
Newly hatched larval number per nest (Ind nest^{-1})	157.67 ± 124.12^a	149.00 ± 148.49^a	-
Mean weight of newly hatched larvae (g ind^{-1})	0.017 ± 0.001^a	0.017 ± 0.001^a	-
Mean length of newly hatched larvae (cm ind^{-1})	2.72 ± 0.04^a	2.72 ± 0.04^a	-

Values are presented as mean±SD. Values with similar superscripts in the same row show no significant difference ($p>0.05$)

Table 2. Initial mean weight (IMW), mean weight (MW), weight gain (WG), daily weight gain (DWG), and specific growth rate in weight (SGRw) of *M. albus* fry over a 45-day rearing period under various dietary protein levels

Parameters	Dietary protein level (%)		
	45	50	55
IMW (g ind^{-1})	0.023 ± 0.001	0.023 ± 0.001	0.023 ± 0.001
MW$_{15}$ (g ind^{-1})	0.047 ± 0.005^{a}	0.062 ± 0.010^{b}	0.104 ± 0.012^{c}
MW$_{30}$ (g ind^{-1})	0.338 ± 0.047^{a}	0.438 ± 0.055^{b}	0.449 ± 0.033^{b}
MW$_{45}$ (g ind^{-1})	0.885 ± 0.126^{a}	1.331 ± 0.185^{b}	1.429 ± 0.155^{c}
WG (g ind^{-1})	0.862 ± 0.126^{a}	1.308 ± 0.185^{b}	1.406 ± 0.155^{b}
DWG g (day^{-1})	0.019 ± 0.003^{a}	0.029 ± 0.004^{b}	0.031 ± 0.003^{b}
SGRw (% day^{-1})	8.089 ± 0.318^{a}	8.998 ± 0.319^{b}	9.164 ± 0.237^{b}

Values are presented as mean±SD. Values with different superscripts in the same row show a significant difference ($p<0.05$)

Table 3. Initial mean length (IML), mean length (ML), length gain (LG), daily length gain (DLG), and specific growth rate in length (SGR$_L$) of *M/ albus* fry over a 45-day rearing period under various dietary protein levels

Parameters	Dietary protein level (%)		
	45	50	55
IML (cm ind^{-1})	3.00 ± 0.02	3.00 ± 0.02	3.00 ± 0.02
ML$_{15}$ (cm ind^{-1})	4.11 ± 0.02^{a}	4.63 ± 0.04^{b}	5.42 ± 0.03^{c}
ML$_{30}$ (cm ind^{-1})	7.92 ± 0.05^{a}	8.01 ± 0.04^{ab}	8.33 ± 0.03^{b}
ML$_{45}$ (cm ind^{-1})	9.90 ± 0.08^{a}	11.21 ± 0.09^{b}	11.72 ± 0.06^{b}
LG (cm ind^{-1})	6.90 ± 0.08^{a}	8.21 ± 0.08^{b}	8.72 ± 0.06^{b}
DLG (cm day^{-1})	0.15 ± 0.00^{a}	0.18 ± 0.02^{b}	0.19 ± 0.00^{b}
SGR$_L$ (% day^{-1})	2.64 ± 0.19^{a}	2.92 ± 0.18^{b}	3.03 ± 0.11^{b}

Values are presented as mean±SD. Values with different superscripts in the same row show a significant difference ($p<0.05$)

Table 4. Survival rates and feed conversion ratio (FCR) of *M. albus* fry after a 45-day rearing period under various dietary protein levels

Parameters	Dietary protein level (%)		
	45	50	55
Survival rate (%)	97.00 ± 1.73^{a}	97.33 ± 1.53^{a}	94.67 ± 0.58^{a}
FCR	2.33 ± 0.58^{a}	2.00 ± 0.00^{a}	2.00 ± 0.00^{a}

Values are presented as mean±SD. Values with similar superscripts in the same row show no significant difference ($p>0.05$)

per tank completely prevented nest formation. This pattern is consistent with previous descriptions of the reproductive ecology of *M. albus*, in which males perform complex courtship displays, construct burrows or bubble nests, and provide paternal care (Yin and Liu, 2010; Matsumoto *et al.*, 2011). These behaviours depend on the availability of adequate space and the species' territorial characteristics, as shown by several studies (Guan *et al.*, 1996; Do *et al.*, 2008; Yin and Liu, 2010; Matsumoto *et al.*, 2011; Phuong *et al.*, 2017). The absence of nests at the highest density tested suggests that overcrowding likely intensifies territorial competition or disrupts mating behaviours characteristic of this protogynous hermaphrodite (Cheng *et al.*, 2003; He *et al.*, 2010). At elevated densities, overlapping territories, increased competition, and heightened social stress can lead to pronounced conspecific aggression, ultimately suppressing nest-building activity (Ly *et al.*, 2008; Matsumoto *et al.*, 2011; Bessa *et al.*, 2022). Similar density-dependent inhibition of spawning has been documented in other species, including African catfish (*Clarias gariepinus*) (Addo *et al.*, 2022), pikeperch (*Sander lucioperca*) (Blecha *et al.*, 2016), European catfish (*Silurus glanis*) (Krasteva, 2022), and vimba bream (*Vimba vimba*) (Kujawa *et al.*, 2022).

Furthermore, no significant differences were observed between broodstock densities of three and four individuals per tank in egg number per nest, hatching rate, number of newly hatched larvae per nest, or the body size of newly hatched larvae. The high hatching rates observed in this study (90.89–96.94%) were comparable to, or higher than, the 60–87% reported in earlier propagation studies of *M. albus* (Guan *et al.*, 1996; Do *et al.*, 2008). The improved results may be attributed to the better semi-artificial propagation protocol. The combination of clay-filled barrels, PVC nesting chambers, aquatic plants, and artificial rain created a stable microhabitat that looked like natural spawning sites (Ishimatsu *et al.*, 2018; Krasteva, 2022). Clay substrates enhanced egg adhesion and protection, while *L. adscendens* plants created an environment reminiscent of flooded rice fields, thereby improving oxygenation, reducing broodstock stress (Guan *et al.*, 1996; Yin *et al.*, 2004; Yin and Liu, 2010; Ishimatsu *et al.*, 2018). Other teleost species that rely on nest-building for reproduction have demonstrated similar trends. For example, controlled breeding trials of *Clarias gariepinus* (Addo *et al.*, 2022) and *Silurus glanis* (Krasteva, 2022) showed that providing suitable nesting surfaces improved incubation outcomes. Furthermore, after establishing appropriate behavioural and environmental conditions, both *Vimba vimba* and *S. glanis* demonstrated stable reproductive performance (Krasteva, 2022; Kujawa *et al.*, 2022). The findings indicate that reproductive performance did not differ significantly between broodstock densities of three and four individuals per spawning tank. Therefore, a broodstock density of three individuals per spawning tank may be considered a practical option, as it provides comparable reproductive outcomes while requiring fewer broodstock and potentially lowering management demands.

Fry rearing performance under different dietary protein levels

Dietary protein is a crucial component influencing growth, metabolism, and tissue synthesis in fish (Wilson, 1986; Conceição, 1997). The current study indicated that feed protein levels and rearing time significantly influenced the growth performance of *M. albus* fry. Specifically, during the first 15 days, both MW$_{15}$ and ML$_{15}$ showed significant increases as dietary protein levels rose in a range of 45–55%. However, from day 30 onward, most growth parameters (MW$_{30}$, WG, DWG, SGRw, ML$_{30}$, ML$_{45}$, LG, DLG, and SGR$_L$) at the 50 and 55% protein levels were significantly higher than those at the 45% level, but did not exhibit significant differences between 50% and 55%. These results indicate that a dietary protein level of 55% yielded the highest growth performance among the tested levels during the first 15 days of rearing. In contrast, a protein level of 50% was sufficient to maintain high growth performance during the later rearing stages. The findings align with established principles of fish nutrition, which indicate that carnivorous or fast-growing species and small juveniles typically require higher protein levels to sustain rapid hypertrophy and high rates of protein deposition (Wilson, 1986; Conceição, 1997; Radhakrishnan *et al.*, 2020). During the early rearing phase, elevated dietary protein likely provides essential amino acids and sufficient nitrogen for tissue accretion and organ development, explaining the marked growth advantage observed with 55% protein in the first 15 days of rearing. By day 30, the absence of significant differences between 50 and 55% protein treatments indicates a declining requirement following the intensive early growth phase. This trend likely indicates that as the fry grow, their digestive efficiency improves, allowing for better nutrient assimilation from slightly lower-protein diets. General fish growth models have well established that energy allocation shifts from rapid somatic growth toward maintenance and organ maturation, while relative protein deposition declines as body size increases (Wilson, 1986; Luo *et al*., 2020). Elevating dietary protein to 54% improved growth and digestive enzyme activity in juvenile *M. albus*, beyond which additional increases yielded no further advantages. Ma *et al*. (2014) similarly found that high-protein diets enhance protease activity and increase amino acid absorption, which facilitates rapid myofibrillar protein synthesis (Radhakrishnan *et al*., 2020). However, providing excessive protein beyond the metabolic capacity can lead to an increase in nitrogenous waste and a reduction in overall efficiency (Ma *et al*., 2014; Nhan *et al*., 2020). The lack of further improvement at 55% protein, therefore, suggests that the threshold for efficient protein deposition may have been reached, with excess nitrogen excreted rather than utilised for growth. The superior performance at 50–55% protein in the present study likely reflects the high amino acid demand of eel fry during active tissue growth and organogenesis (Surjobala *et al*., 2019; Jiang *et al*., 2025). However, the absence of further improvement beyond 50% indicates a plateau effect, where excess protein contributes little to growth but increases nitrogen excretion, potentially impairing water quality (Yue *et al*., 2019). Thus, 50% dietary protein appears to be sufficient for fry-stage *M. albus*, balancing growth and feed utilisation while minimising environmental waste.

Survival rates (94.67–97.33%) and FCR values did not differ significantly among protein levels, indicating that the tested protein range did not negatively affect health or feed utilisation. This aligns with studies on early-stage carnivorous freshwater fish, which indicate that survival is predominantly influenced by environmental stability, water exchange frequency, and feeding frequency, rather than by moderate variations in dietary protein (Radhakrishnan *et al*., 2020). In general, dietary protein levels of 50–55% supported higher growth while maintaining high survival rates. Similar patterns have been reported in other eel species and carnivorous fishes, where increased dietary protein enhances growth but has little effect on survival once nutritional requirements are met (Daiqing *et al*., 2000; Lam *et al*., 2019; Zhang *et al*., 2019).

In conclusion, this study found that broodstock densities of three and four individuals per 0.5 m³ spawning tank produced comparable reproductive parameters, including nesting success, egg number per nest, hatching rate, number of newly hatched larvae per nest, and larval body size. Moreover, fry growth performance was significantly influenced by dietary protein levels, with the 55% protein diet promoting the greatest growth during the first 15 days of rearing, while the 50 and 55% protein diets supported comparable growth performance from day 30 onward. Overall, a broodstock stocking density of three individuals per spawning tank and a feeding regime using a 55% protein diet during the early rearing phase, followed by a 50% protein diet later, may be beneficial under the conditions of this study.

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