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Effect of Stocking Density on the Performance of Glass Eels (*Anguilla mamorata*) in an Indoor Culture System in Da Nang, Vietnam

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Abstract

The giant mottled eel (*Anguilla marmorata*) is a commercially valuable species that is commonly farmed in Vietnam. The survival rate of glass eels transitioning to the fingerling stage remains a challenge due to high mortality during the nursery phase. A 90-day experiment was conducted in triplicate to evaluate the effects of different stocking densities (200, 225, and 250 fish m^{-3}) in an indoor system at a farm in Da Nang. Additionally, the eels were fed imported formulated feed from Taiwan. Throughout the experiment, water quality parameters were monitored: the water temperature averaged 27.96 ± 1.55°C, pH was 7.85 ± 0.18, DO was 6.29 ± 0.27 mg L^{-1}, NH_3 was 0.22 ± 0.11 mg L^{-1}, and NO_2^- was 0.06 ± 0.07 mg L^{-1}. The survival rate was 61.36 ± 10.51% (ranging from 49.91% to 76.99%), and weight gain was 1.47 ± 0.05 g $fish^{-1}$. Average survival rates (SR%) were recorded at 64.46 ± 9.97%. Overall, these results highlight that, for the nursery rearing of *Anguilla marmorata* using commercial feed, the optimal stocking density is 200 fish m^{-3}.

Keywords

Indoor culture, glass eel, growth performance, giant mottled eel, parameters, survival rate and weight gain

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Introduction

The giant mottled eel (*Anguilla marmorata*) is a widely distributed species, ranging from the western to the central regions of the Pacific and Indian Oceans. Its geographical distribution extends from the eastern coast of Africa to the central South Pacific, covering areas from the southwestern Sea of Japan, Taiwan, and southeastern China to southern South Africa (Yuuki *et al.*, 2015). The species undergoes a complex life cycle, beginning as eggs that hatch into larvae, which later develop into transparent glass eels that migrate upstream into estuarine environments. Upon reaching maturity, the eels migrate back to the deep sea to spawn (Alessandro, 2019a). Some individuals spend the majority of their lives in freshwater before returning to the ocean to reproduce, whereas others reside in marine environments throughout their life cycle. All anguillid eel species are semelparous, meaning they die after spawning (Arai *et al.*, 2002). *A. marmorata* can grow up to 2 meters in length (Pike *et al.*, 2019) and exhibits a complex migratory life history, traveling distances ranging from hundreds to thousands of kilometers between oceanic spawning areas and inland freshwater habitats (Arai, 2014; 2016). This species inhabits a wide range of environments, including freshwater, brackish, and marine waters (Lin *et al.*, 2012). It spawns in the ocean and matures in rivers, estuaries, or coastal areas (Tsukamoto *et al.*, 2009).

The migratory patterns, reproductive strategies, life history traits, and habitat use of *A. marmorata* are known to vary among different populations, reflecting its ecological adaptability (Arai & Abdul, 2017; Arai & Chino, 2018). This species is capable of spawning year-round (Arai and Abdul, 2018). One of its known spawning grounds is located in a North Pacific island chain, within the same oceanic current system—the North Equatorial Current—as the spawning grounds of the Japanese eel (*A. japonica*) (Arai *et al.*, 2020).

A. marmorata is a high-value commercial fish species currently cultivated in Vietnam (Vo Thi Thanh Truc *et al.*, 2018). The primary area for commercial *A. marmorata* farming in Vietnam is the Mekong Delta, with Ca Mau Province having the largest eel farming area in the country. Vietnam hosts over 1,000 eel farming facilities, producing approximately 2,000-5,000 tons of commercial eels annually. Around 95% of these facilities focus on farming *A. marmorata*, while the remaining 5% are dedicated to farming *A. bicolor*. Currently, approximately 70% of the total eel production is consumed domestically, while the remaining 30% is exported to neighboring countries such as China, Japan, and Taiwan (Anh *et al.*, 2018; Tuan & Duat, 2021).

Anguillid eels are commercially important, yet some species, such as *Anguilla japonica*, are increasingly threatened. According to the International Union for Conservation of Nature (IUCN), *A. japonica* has been listed as an endangered species on the Red List. The natural population decline of this species is attributed to multiple factors, including widespread habitat modification, environmental pollution, and overexploitation of wild stocks (Sungchul & Ali, 2017). Similarly, the European eel (*A. anguilla*), a catadromous species inhabiting rivers of the Adriatic watershed in Croatia, is classified as critically endangered by the IUCN due to severe population declines caused by overfishing, illegal trafficking, and infections from nematodes, pathogenic bacteria, and viruses (Ivana *et al.*, 2022).

Anguilla sp. is one of a number of high-priced export commodities, and while glass eels are still abundant, if there is a ban on exporting glass eels, it could be stunting to the industry, as many investors are interested, but the number of elver available for growing up to the size of consumption does not currently meet demand (Purnam *et al.*, 2018). For the giant mottled eel (*Anguilla marmorata*), the IUCN currently classifies the species as of Least Concern (LC) (Pike *et al.*, 2020). Despite this, anguillid eels remain ecologically and economically significant for global fisheries. Their declining populations not only threaten biodiversity but also jeopardize the livelihoods of communities dependent on these species. Therefore, urgent efforts are required to understand and mitigate these threats through effective conservation strategies. The capture and cultivation of this species play an increasingly important role, with most juvenile eels at the glass eel stage either imported from neighboring countries such as Indonesia and the

Philippines or naturally sourced within Vietnam. However, the survival rate and growth rate of glass eels transitioning to the elver stage remains a challenge due to high mortality during the nursery phase. The nursery stage of the glass eel is a critical phase in eel aquaculture. Nitrite (NO_2) and ammonia (NH_3) are two key water quality parameters that require close monitoring during this period. Maintaining stable environmental conditions during the nursery phase of *Anguilla marmorata* is essential (Nguyen Minh Ty, 2023). In addition, bloodworms, which are commonly used as a feed source for glass eels, pose a potential risk of pathogen transmission. Therefore, the exclusive use of commercial feed in this study represents a practical solution to address the aforementioned concerns. The aquaculture of anguillid eels continues to face significant challenges, mainly due to their complex life cycle and the ongoing reliance on wild-caught juveniles for seed stock. One of the most critical and demanding phases is the capture of glass eels during their upstream migration, followed by the transition to artificial feeding. During the early stages of nursery rearing, high mortality rates are frequently observed, primarily as a result of difficulties in feed adaptation (Fredson *et al.*, 2024). The objective of this study was to assess survival rates and weight gain of glass eels reared in indoor culture systems during the nursery period.

Materials and Methods

Experimental design

The experiment utilized nine circular rearing tanks, each with an average volume of 6.39 m^3 (3.0 m diameter x 1.5 m height). An aeration and water circulation system were installed in the nursery tanks to promote vertical mixing between the surface and bottom water layers (**Figure 1**). Glass eels are imported annually during the period from November to February of the following year, primarily from Indonesia or other source countries and were transported to three indoor tanks in Da Nang for two days for acclimation. They were randomly selected, counted, and stocked into the tanks. The fish stocking densities were set at 200, 225, and 250 individuals per cubic meter for each treatment group of glass eels. The experimental design included triplicates for each treatment. The average initial weight of a glass eel was 0.16 g per individual. The three experimental treatments were conducted over the same period, using the same sources of glass eel, feed, and supplied water. This study utilized imported glass eels and exclusively relied on commercial feed. This approach represents a future trend, as the availability of wild-caught eel seed in Vietnam is becoming increasingly scarce (Tuan *et al.*, 2022).

The feed was thoroughly mixed with clean water to form a paste before being administered to the fish. Feeding was conducted twice daily, at 7:00 and 16:00. The daily feeding amount was calculated using the formula for determining feed quantity during the nursery phase of eel culture, as described by Cuvin *et al.* (2019). Throughout the experimental period, the fish were fed at a daily rate of 4-5% of their body weight.

Water quality

Temperature, pH, and dissolved oxygen were monitored twice daily at 08:00 and 17:00 (Purnama *et al.*, 2018) with a digital oxygen-meter (YSI 55, Yellow Springs, OH, USA) and a pH meter (Hanna HI98128 pH meter), respectively. The NH_3/NH_{4+} and NO_{2-} values were measured weekly at 17:00 using Sera test kits. Throughout the experiment, water quality parameters were monitored, namely water temperature, pH, dissolved oxygen (DO), ammonia (NH_3), and nitrite (NO_2^-).

This protocol was an environment-friendly aquaculture system considered a sustainable approach for eel rearing. The system was based on the balance of macro and microminerals in water using organic buffer substances to control the pH and other environmental parameter factors. The role of these factors was to increase cultural feasibility by reducing harmful substances in the water and increasing the growth and survival rates. In aquaculture, management and environmental factors that induce stress in fish probably increase mortality significantly (Mark *et al.*, 2019). The present study was

Figure 1. Glass eel (*Anguilla marmorata*) rearing tank system in Da Nang

conducted to evaluate the results of ecological parameters and stocking density on the performance of glass eels in this system during the nursery stage. This study assessed the survival rate of glass eels reared in indoor culture systems during the nursery period with formulated feed. The commercial feed utilized in this study was imported from Taiwan. The formulated feed composition consisted of more than 47% crude protein, more than 3% crude fat, less than 1.2% crude fiber, less than 16.5% ash, and less than 11% moisture.

Growth rate

Fish were harvested after 90 days and daily weight gain (DWG), specific growth rate (SGR), and survival rate (SR) were calculated using the following equations: DWG = g/fish/day; SGR (%/day) = ln final weight − ln initial weight/ days× 100; and SR (%) = 100 × (final fish count/initial fish count).

Statistical analysis

Data were processed using Microsoft Excel 2019 and SPSS version V28.01. The collected data were analyzed for mean and standard deviation, and subjected to one-way analysis of variance (One-way ANOVA). Tukey's HSD test was employed as a post hoc test to determine significant differences between group means (P <0.05).

Results and Discussion

Water quality parameters

During the study, the parameters of water quality were maintained within the tolerance range for most freshwater species used in aquaculture.

Water quality parameters in our eel rearing systems

The recorded water quality parameters in the eel rearing system treatments are presented in (**Table 1**).

Temperature

The average temperature was 28.14 ± 1.65°C, with a range of 24.5 to 31°C. These temperatures fall within the optimal range for *Anguilla marmorata*, which has been reported as 18-33°C (Mingzhong *et al.*, 2013), 17.7-30°C (Luong Quang Tuong *et al.*, 2017), and 25-30°C (Tain-Sheng, 2016).

The optimal temperature for growth has been reported as 27.6°C (Mingzhong *et al.*, 2013), 29.2°C (Tain-Sheng, 2016), 27-28°C (Utama *et al.*, 2018), 28°C (Curvin *et al.*, 2019), and 29-30°C (Rowena *et al.*, 2020). Therefore, the temperatures in the rearing system was highly suitable for the growth of *A. marmorata*.

pH

The optimal pH range for *A. marmorata* has been reported as 7.0-8.0 (Chenxi *et al.*, 2018), 7.4-7.81 (Utama *et al.*, 2018), 6.8-8.2 (Luong Quang Tuong *et al.*, 2017), and 7.1-8.4 (Rowena *et al.*, 2020). The observed average pH was 7.85 ± 0.18 in Da Nang with a range of 7.5-8.2). These pH levels were within the optimal range for *A. marmorata* growth.

Dissolved Oxygen (DO)

The required DO levels for *A. marmorata* were reported as 4-6 mg L^{-1} (Luong Quang Tuong *et al.*, 2017), 6.5-7.5 mg L^{-1} (Chenxi *et al.*, 2018), and 6-7 mg L^{-1} (Rowena *et al.*, 2020). The average DO level was 6.29 ± 0.27 mg L^{-1} in our study with a range of 5.9-6.6 mg L^{-1}). These DO levels were suitable for the culture of *A. marmorata*.

Ammonia (NH3)

The observed NH_3 levels showed no statistical differences among the treatments ($P > 0.05$). The maximum NH_3 concentration was 0.5 mg L^{-1} and the minimum was 0 mg L^{-1}. These values were suitable for *A. marmorata*, as NH_3 levels should be <5 mg L^{-1} (Chenxi *et al.*, 2018) or 0.05-0.07 mg L^{-1} (Rowena *et al.*, 2020).

Nitrite (NO_2-)

NO_2- is a critical parameter for eels, including *A. marmorata*. The acceptable range is 0.25-1.0 mg L^{-1} (Chenxi *et al.*, 2018) or <0.01 mg L^{-1} (Rowena *et al.*, 2020). The NO_2- levels observed in this study were highly suitable for *A. marmorata* culture.

The results of the environmental parameter monitoring in the rearing tanks indicated that all the measured values across the treatments remained within the optimal ranges for the growth and development of *Anguilla marmorata* glass eels (Chenxi *et al.*, 2018; Cuvin *et al.*, 2019; Rowena *et al.*, 2020; Nguyen Minh Ty, 2023).

The results (**Table 2**) indicated that the survival rate of the nursery-reared eels after three months ranged from a minimum of 61.97% to a maximum of 66.88%. The survival rates observed in this study were higher than those reported for *Anguilla marmorata* in Indonesia (51.33%; Purnama *et al.*, 2018) and the 60% survival rate recorded under nursery culture conditions in Vietnam (Tuan & Duat, 2021). However, these values were substantially lower than the survival rates of 65-75% reported by Nguyen Thuc Tuan *et al.* (2021) under improved nursery conditions. It should be noted that differences in feed sources were present between these studies, which may have influenced the outcomes; thus, these comparisons should be interpreted with caution.

However, our findings were significantly lower than the survival rate of 86.63 ± 7.42% for *Anguilla bicolor* in a recirculating aquaculture system in Indonesia (Tatang *et al.*, 2022). They were also lower than the 78.9-87.8% survival rate reported by Luong Cong Trung & Tran Tho Dan (2022) in their study on the effect of stocking density on the growth and survival of *A.*

Table 1. Water quality parameters of the *Anguilla marmorata* rearing system

Density (fish m^{-3})	200	225	250	Average
pH	7.83^{a} ± 0.11	7.85^{a} ± 0.13	7.86^{a} ± 0.10	7.85 ± 0.18
DO (mg L^{-1})	6.59^{a} ± 0.01	6.09^{b} ± 0.06	6.19^{ab} ± 0.03	6.29 ± 0.27
Temperature (°C)	26.31^{a} ± 0.02	29.39^{a} ± 0.01	28.16^{a} ± 0.2	27.96 ± 1.55
NO_{2-} (mg L^{-1})	0.01^{a} ± 0.02	0.01^{a} ± 0.03	0.12^{b} ± 0.13	0.05 ± 0.07
NH_3/NH_{4+} (mg L^{-1})	0.21^{ab} ± 0.01	0.12^{b} ± 0.12	0.33^{a} ± 0.09	0.22 ± 0.11

Note: Values in the same row with different superscript letters are significantly different (P <0.05).

Table 2. Survival rate of glass eels (*Anguilla marmorata*) after three months of nursery culture in the study area (Da Nang)

Density (fish m^{-3})	200	225	250
Survival rate (%) Trial 1st	56.97	49.91	56.23
Survival rate (%) Trial 2nd	66.86	65.37	62.32
Survival rate (%) Trial 3rd	76.80	78.34	67.35
Average Survival Rate (%)	66.88^{a} ± 9.92	64.54^{a} ± 14.23	61.97^{a} ± 5.57

Note: Values in the same row with different superscript letters are significantly different (P <0.05).

marmorata at the fingerling stage. Furthermore, our results were considerably lower compared to the 91-100% survival rate of European eels in Japan (Abdalla *et al.*, 2007) and the exceptionally high survival rate of 98.37% for *A. marmorata* reported by Fredson *et al.* (2024).

At the end of the culture period, the weight of the eels averaged 1.6g ± 0.074g per individual, with an absolute weight gain - average daily weight gain (ADWG) - of 0.017g per day (**Figure 2)**. This result exceeded the ADWG of 0.001g per day observed in the *Anguilla bicolor* nursery culture using feather meal supplementation reported by Muhammad *et al.* (2018) in Japan, and the ADWG of 0.004 g per day for American eels (*A. rostrata*) in the United States reported by Carrie *et al.* (2018).

However, our results were lower compared to those of Nguyen *et al.* (2015), who reported an ADWG of 0.032-0.033g per day in a first-grade eel nursery culture (initial stocking sizes of 0.15-5g per individual) in a recirculating aquaculture system supplied with pure oxygen. The higher growth rates in their study could be attributed to the significantly larger initial sizes of the eels (up to 5g per individual) compared to the initial average weight of 0.167g per individual in our study. The growth performance observed in this study was lower compared to previous studies, primarily due to the exclusive use of commercial feed during the nursery rearing phase. Although the average daily gain (ADG) was relatively low, this study represents a preliminary step toward the development of a fully controlled feeding strategy for future nursery cultures of *Anguilla marmorata* glass eels.

In this study, the water temperature averaged 27.96 ± 1.55°C (ranging from 24.5 to 30.5°C), pH was 7.85 ± 0.18 (ranging from 7.5 to 8.2), DO was 6.29 ± 0.27 mg L^{-1} (ranging from 5.9 to 6.6 mg L^{-1}), NH_3 was 0.22 ± 0.11 mg L^{-1} (ranging from 0 to 0.5 mg L^{-1}), and NO_{2-} was 0.06 ± 0.07 mg L^{-1} (ranging from 0 to 0.2 mg L^{-1}). The survival rates (SR%) observed were 64.87 ± 9.97%. The results indicated that stocking density did not have a statistically significant effect on the final weight, weight gain, daily weight gain (DWG), specific growth rate (SGR), or survival rate (SR). However, differences were observed in the survival rate percentages, with stocking densities of 200 fish m^{-3} and 250 fish m^{-3} achieving SR values of 66.88 ± 9.92% and 61.97 ± 5.57%, respectively, reflecting a survival rate difference of 4.9% between these two treatments. These findings highlight the impact of stocking density on the survival of *Anguilla marmorata* glass eels cultured in indoor culture systems in Da Nang, Vietnam.

Meristic variation

Table 3 shows the meristic parameters ED, DE, BD, HL, HL/TL, and BD/TL between males

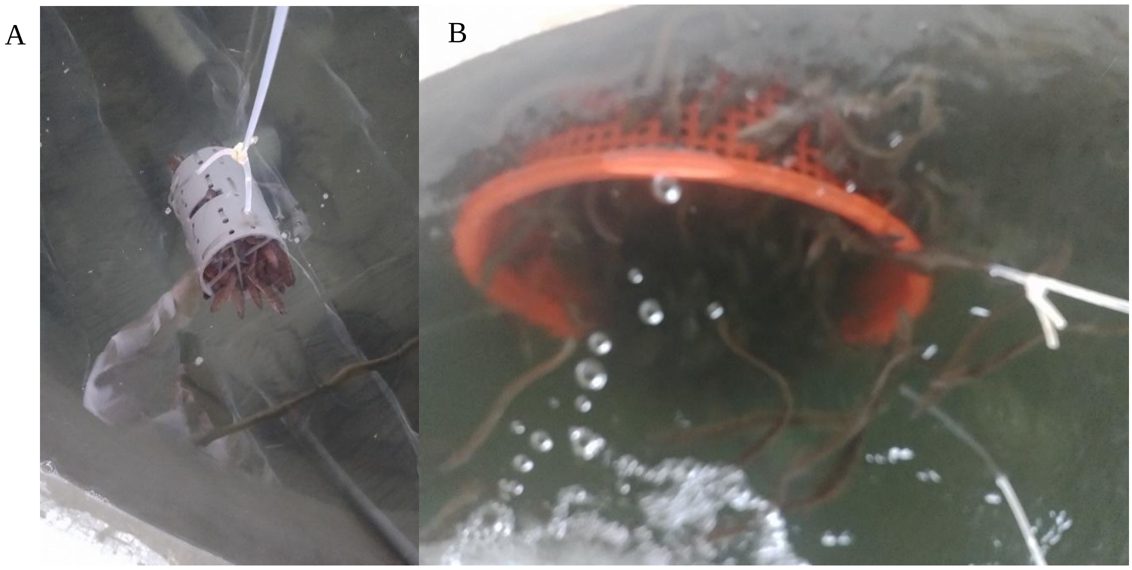

Figure 2. Eel (*Anguilla marmorata*) rearing tank system after three months

Table 3. Growth rate of *Anguilla marmorata* glass eels after three months of nursery culture in the study area

Parameters	Density (fish m^{-3})		
	200	225	250
Average final weight (g fish^{-1}) (first trial)	1.65	1.71	1.79
Average final weight (g fish^{-1}) (second trial)	1.61	1.62	1.72
Average final weight (g fish^{-1}) (third trial)	1.60	1.64	1.55
Total average final weight (g fish^{-1})	1.62 ± 0.02	1.66 ± 0.05	1.69 ± 0.13
Weight gain (g fish^{-1})	1.56 ± 0.07	1.49 ± 0.06	1.44 ± 0.05
Daily weight gain (g fish^{-1})	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Specific growth rate (% day^{-1})	2.58 ± 0.02	2.61 ± 0.03	2.63 ± 0.84

Note: Values in the same row with different superscript letters are significantly different (P <0.05).

and females of the mudsleeper B. koilomatodon. The results revealed that some of the factors depended on the gender. For example, BD, HL, and DE/HL had significant differences between females and males at the 5.0% level (one-way ANOVA, P <0.05). The reason for these differences was that the body size of males was more massive than females. There were no statistical differences in the other parameters (ANOVA, P >0.05).

Conclusions

The results of this study demonstrate the potential feasibility of using commercial feed in the nursery culture of *Anguilla marmorata* within indoor rearing systems. Regular grading is essential for successful eel cultivation. Further studies are needed to evaluate the production performance of glass eels at higher stocking densities.

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