Enhancing Water Quality in Intensive Asian Seabass (*Lates calcarifer*) Aquaculture Using Green Mussels (*Perna viridis*): A Biomass–Normalized Apparent Reduction

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ABSTRACT

Integrated multi–trophic aquaculture (IMTA) is a promising strategy to mitigate water quality deterioration in intensive marine aquaculture, although continuous water renewal may obscure the role of extractive species. This study evaluated green mussel (*Perna viridis*) integration with Asian seabass (*Lates calcarifer*) under simulated marine IMTA conditions. A 60–day experiment with four mussel biomass treatments was conducted at constant seabass density, with routine water exchange (50% every three days). Key water quality parameters, including dissolved oxygen (DO), temperature, power of hydrogen (pH), salinity, total ammonia nitrogen (TAN), nitrite–nitrogen (NO_2^-–N), nitrate–nitrogen (NO_3^-–N), alkalinity (ALK), total suspended solids (TSS), and turbidity, were monitored throughout the experiment. Asian seabass growth performance, survival, and biomass production were also assessed. Mussel–integrated treatments showed significantly lower concentrations of ALK, TAN, TSS, and turbidity, along with higher NO_3^-–N, compared with the control ($p<0.05$), whereas DO, temperature, pH, NO_2^-–N, and salinity did not differ significantly among treatments. Despite identical water–exchange regimes, the presence of mussels contributed to improved water quality stability. Fish growth, survival, and biomass production were enhanced in mussel–integrated systems, particularly at intermediate to high mussel biomass. These results demonstrate that green mussels can enhance water quality and production performance in intensive Asian seabass culture beyond the effects of routine water exchange alone, supporting the application of biomass–based IMTA as a practical approach for sustainable marine aquaculture.

Keywords: Asian seabass, Green mussel, IMTA, Water quality

INTRODUCTION

Asian seabass (*Lates calcarifer*), also known as barramundi, is a high–value aquaculture species globally. Its market value is projected to increase from USD 3.5 billion in 2020 to USD 7.9 billion by 2030, driven by rising demand and advancements in aquaculture technology (Razzak *et al*., 2019; Domingos *et al*., 2021; Mojjada *et al*., 2024). In Thailand and other Asian countries, intensive seabass farming is widely practiced to meet market demand. However, such systems often result in waste accumulation, particularly excess nitrogen, which degrades water quality (Muangrerk *et al*., 2024).

Asian seabass is a carnivorous species that requires high–protein feed (30–45%), but only

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20–40% of the nitrogen in the feed is assimilated into fish biomass. The remainder is excreted as total ammonia nitrogen (TAN), nitrite–nitrogen (NO_2^-–N), and nitrate–nitrogen (NO_3^-–N), which are toxic compounds that induce stress and mortality in fish and contribute to eutrophication, especially in open–sea cages (Tacon and Metian, 2008; Yang *et al*., 2023; Chen *et al*., 2024; Yan *et al*., 2024).

To address these challenges, the concept of integrated multi–trophic aquaculture (IMTA) has gained attention. IMTA incorporates species from different trophic levels within a single system to optimize resource utilization, reduce waste, and enhance ecosystem stability (Chopin *et al*., 2008; Hughes and Black, 2016). Green mussels (*Perna viridis*) are widely recognized as efficient biofilters in IMTA systems due to their ability to remove suspended solids and excess nutrients (van der Schatte Olivier *et al*., 2020).

Recent IMTA research has emphasized that the effectiveness of extractive species is strongly influenced by system openness and hydrodynamic conditions. In offshore and semi–open IMTA systems, culture units are continuously exposed to surrounding water masses, resulting in ongoing water renewal that can dilute or mask the apparent contribution of extractive organisms to nutrient removal (Buck *et al*., 2018). Under such conditions, direct quantification of absolute filtration or clearance rates may be methodologically challenging.

Green mussels are also economically important in Thailand, particularly in the Sriracha Bay area within the eastern economic corridor (EEC). They are highly adaptable and capable of filtering organic matter, heavy metals, and microplastics, making them useful both as bioindicators and bioremediation agents (Beyer *et al*., 2017; Phaksopa *et al*., 2023). Previous studies have evaluated the role of bivalves in IMTA systems. For instance, Kong *et al*. (2023) reported that bivalve integration improved dissolved oxygen (DO) levels and reduced ammonia and NO_2^-–N in multi–species aquaculture. Kumara *et al*. (2023) demonstrated that hard clams enhanced water quality and improved the survival rates of shrimp and fish. Similarly, Nurfadillah *et al*. quality in black tiger shrimp culture. Mechanistically, bivalves improve water quality primarily through suspension feeding, which removes particulate organic matter and suspended solids from the water column. This process reduces turbidity and organic nitrogen loading, potentially lowering subsequent ammonification and oxygen demand. In IMTA systems, such filtration can contribute to more stable DO conditions and reduced physiological stress in co–cultured species, thereby supporting improved survival and growth performance. The cited studies were conducted in multi–species or pond–based IMTA systems where bivalves were integrated as extractive components under semi–open conditions, enabling interception of particulate wastes and modulation of nutrient dynamics. These ecosystem-level benefits of IMTA configurations, including nutrient load moderation through extractive species such as bivalves, have been reviewed and synthesized in Rusco *et al*. (2024).

However, the performance of bivalves in IMTA systems cannot be evaluated independently of system–scale nutrient dynamics. Nederlof *et al*. (2021) demonstrated that nutrient retention efficiency in IMTA systems is strongly modulated by water exchange and hydrodynamic connectivity, highlighting that comparative and biomass–normalized approaches may be more appropriate than absolute filtration measurements in open or semi–open systems. Furthermore, a comprehensive review by Batır *et al*. (2025) emphasized the functional role of bivalves as extractive components in IMTA, while also noting substantial variability in reported filtration efficiencies due to differences in species, biomass, experimental design, and environmental conditions.

However, many of these studies lack quantitative assessments of waste filtration efficiency per unit of mussel biomass, and few focus on high–density Asian seabass systems, which produce more waste. In addition, limited attention has been given to experimental designs that simulate realistic water–exchange regimes representative of practical marine IMTA operations. This study aims to evaluate the performance of green mussels in high–density seabass culture and to quantify biomass–dependent

parameters under standardized routine water exchange. The objective is not to determine organism–level filtration kinetics, but to provide a comparative system–level assessment of water quality stabilization across treatments. From a commercial IMTA perspective, identifying an optimal biomass of extractive species under routine water exchange provides production–relevant guidance for improving nitrogen stability and feed efficiency beyond dilution effects alone.

MATERIALS AND METHODS

Asian seabass fingerlings and green mussels

Four hundred Asian seabass fingerlings (approximately 20 g·Ind^{-1}) were obtained from Issarapong Farm, Samut Songkhram Province, Thailand, and acclimatized at the Sriracha Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Chonburi. The fish were reared in two 2 m^{-3} concrete tanks at a stocking density of 200 Ind^{-1}·tank^{-1} (100 Ind^{-1}·m^{-3} or 2 kg·m^{-3}), following the recommendations from Cotou *et al*. (2024) and Jongjaraunsuk *et al*. (2025), who suggested a maximum density of 2 kg·m^{-3} in polyculture systems. Salinity was gradually increased from 19–20 psu to 30–32 psu by adding filtered seawater (25 µm mesh) every two days while maintaining a full tank volume of 2.0 m^{-3}. This gradual adjustment was applied to minimize osmotic stress and avoid sudden physiological shock in Asian seabass. Abrupt exposure to the target salinity can disrupt osmoregulatory processes, whereas gradual adjustment allows sufficient time for physiological adaptation before the experimental period. Once the target salinity (30–32 psu) was reached, it was maintained throughout the experiment to ensure stable and comparable rearing conditions across treatments. Continuous aeration was provided using eight air stones per tank. The fish were acclimatized for two weeks before the experiment and fed twice daily (09:00 a.m. and 04:00 p.m.) with floating pellet feed (Profeed 902; Thai Union Feed Meal Ltd., Samut Sakhon Province, Thailand), containing at least 42% crude protein, 8% crude fat, 3% crude fiber, and no more than 12% moisture, at a feeding rate of 3% body weight per day.

Green mussels with a mean length of 3.8 cm were collected from the cultivation site in Sriracha Bay and acclimatized for five days in 1.0 m^{-3} concrete tanks similar to those used for fish acclimation. Continuous aeration was supplied using eight air stones. Because the mussels were already adapted to seawater conditions, no salinity adjustment was required. During the acclimation period, water quality was measured daily between 07:00 a.m. and 08:00 a.m. to ensure that all parameters remained within optimal ranges for both Asian seabass and green mussels. The target ranges were as follows: DO ≥ 5 mg·L^{-1}, Temperature 25–30 °C, pH 6.5–8.5, alkalinity (ALK) 100–150 mg·L^{-1} to stabilize pH and enhance buffering capacity, TAN < 0.5 mg·L^{-1} to minimize toxicity, and NO_2^-–N < 0.5 mg·L^{-1} to reduce stress and health risks. These criteria followed Boyd and Tucker (1998), APHA (2017), Anh *et al*. (2022), and Sukhsangchan *et al*. (2024). Salinity was expressed in practical salinity units (psu) to maintain consistency with standard marine monitoring practices, whereas mg·L^{-1} was used for dissolved water quality parameters such as DO, ALK, TAN, and NO_2^-–N.

Experimental design

After acclimation, the initial weights of the fish and mussels were measured using a digital CST–CDR scale (CST Instruments [Thailand] Ltd., Bangkok, Thailand). The experiment was conducted in circular fiberglass tanks (0.25 m^{-3}; 0.8 m diameter), each filled with 0.15 m^{-3} of water. The average initial fish weight was 34 g, and 20 fish were stocked per tank, giving a total biomass of 680 g or 0.68 kg per 0.15 m^{-3} (4.53 kg·m^{-3}). This stocking density was higher than the 2 kg·m^{-3} recommendation for polyculture systems with biofilters (Cotou *et al*., 2024; Jongjaraunsuk *et al*., 2025). The higher density was intentionally used to evaluate the waste treatment efficiency of green mussels under intensive culture conditions.

Water was exchanged every three days at a rate of 50% in all tanks to maintain suitable water quality for fish and mussels and to prevent excessive deterioration. The same water exchange regime was applied to all treatments to control for

the effect of water renewal and to allow direct comparisons among treatments.

The experiment consisted of four treatments, each with three replicates: T1 (control, no green mussels); T2 (25 green mussels·tank^{-1}; 95 g per 0.15 m^{-3}, equivalent to 0.63 kg·m^{-3}); T3 (50 green mussels·tank^{-1}; 190 g per 0.15 m^{-3}, equivalent to 1.27 kg·m^{-3}); and T4 (75 green mussels·tank^{-1}; 285 g per 0.15 m^{-3}, equivalent to 1.90 kg·m^{-3}).

The 60–day experiment was designed to evaluate the effects of different mussel biomass densities on fish growth, health, and water quality, and to determine the most suitable stocking density for production efficiency.

Feeding during the experiment

The same feed used during the acclimation period was provided throughout the experiment. Profeed 902 (Thai Union Feed Meal Ltd., Samut Sakhon Province, Thailand) contained at least 42% protein, 8% fat, 3% fiber, and no more than 12% moisture. Fish were fed twice daily at 09:00 a.m. and 04:00 p.m. at a feeding rate of 3% of body weight. To minimize cannibalism, the fish were size–matched at the beginning of the experiment and were provided with sufficient feed to support optimal growth.

Water quality measurements

During the experimental period, water quality was monitored more intensively than during the acclimation phase. The parameters measured included DO, temperature, salinity, pH, TAN, NO_2^-–N, NO_3^-–N, ALK, total suspended solids (TSS), and turbidity. DO, temperature, and pH were measured using a Rinko Profiler Multi–Parameter CTD Model ASTD 102 (JFE Advantech Co., Ltd., Tokyo, Japan). Salinity was measured using a Ponsel Mesure–ODEON multi–parameter handheld device (Aqualabo Services SA, Champigny–sur–Marne, France). Turbidity was assessed using a Lovibond TB211–IR Turbidity Meter Kit (Tintometer GmbH, Dortmund, Germany). Water samples for TAN, NO_2^-–N, NO_3^-–N, ALK, and TSS were collected and analyzed according to the standard methods of APHA (2017).

Fish growth performance

All fish in each treatment were weighed at 10–day intervals using the procedure described in the experimental design section. Growth performance was evaluated using standard indicators, including weight gain (WG), average daily gain (ADG), specific growth rate in weight (SGRW), survival rate, production, and feed conversion ratio (FCR). WG (g·Ind^{-1}) was calculated as the difference between final weight (FW) and initial weights (IW), whereas ADG (g·Ind^{-1}·day^{-1}) was calculated as weight gain divided by the culture period. SGRW (%·Ind^{-1}·day^{-1}) was calculated using the natural logarithm of body weight, as shown in equation (1) – (6):

$$\text{WG (g·Ind}^{-1}\text{)} = \text{FW - IW} \quad (1)$$

$$\text{ADG (g·Ind}^{-1}\text{·day}^{-1}\text{)} = [(\text{FW}) - (\text{IW})]/\ \text{Cultured days} \quad (2)$$

$$\text{SGRW (\%·Ind}^{-1}\text{·day}^{-1}\text{)} = [[(\ln \text{FW}) - (\ln \text{IW})]/\text{Cultured days}] \times 100 \quad (3)$$

$$\text{Survival (\%)} = [(\text{Final number/Initial number})] \times 100 \quad (4)$$

$$\text{Production (kg·tank}^{-1}\text{)} = \text{Final total biomass - Initial total biomass} \quad (5)$$

$$\text{FCR} = \text{Total feed provided/ Total weight gain} \quad (6)$$

In Equation (3), ln denoted the natural logarithm (base *e*), which accounts for the exponential growth pattern of fish and provides a more accurate estimate of specific growth rate.

Green mussel performance

Green mussel performance was evaluated using growth and production indicators based on the same equations used for fish growth performance. WG, ADG, and SGRW were calculated using Equations (1), (2), and (3), respectively. Survival and production also were calculated using Equations (4) and (5). Production was expressed as the net increase in total mussel biomass per tank and reported as $kg \cdot tank^{-1}$.

Data analysis

All data were analyzed using one–way analysis of variance (ANOVA) to assess treatment effects, using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Duncan's new multiple range test was used to identify significant differences among treatment means at $p<0.05$. Results are presented as mean±standard deviation.

Water quality parameters that showed significant differences ($p<0.05$), with lower mean values in mussel–integrated treatments than in the fish–only control under the same water–exchange conditions, were further evaluated using a biomass–normalized comparative approach. To allow comparison among treatments with different mussel biomasses, a biomass–normalized apparent reduction (BNAR) was calculated per kilogram of green mussel biomass. This calculation was adapted from the filtration rate concept described by Argente *et al*. (2024), but was modified to fit the experimental conditions of the present study.

Unlike classical filtration or clearance rate measurements, which require flow–through system and residence–time control, the present experiment used routine water exchange applied equally to all treatments. Under these conditions, absolute filtration rates could not be quantified without interference from water renewal. Therefore, the original filtration–rate concept was modified and used here as a comparative, biomass–normalized indicator rather than as a mechanistic measure of filtration. The BNAR was calculated as follows:

$$BNAR = \frac{\text{(Value before water exchange - Value after water exchange)}}{\text{Average biomass}} \quad (7)$$

The average biomass was adjusted according to mussel survival during each sampling interval. The resulting BNAR values represent the relative reduction in water quality parameters associated with mussel biomass under the same water–exchange regimes, rather than absolute filtration or clearance rates. These values were then analyzed statistically among mussel–integrated treatments using the same procedures described above.

Ethical statement

This animal study protocol was approved by the Ethics Committee of the Kasetsart University Institutional Animal Care and Use Committee (ACKU67–FIS–025), Bangkok, Thailand, on 10 October 2024.

RESULTS

Water quality

Among the ten water quality parameters measured, five parameters (DO, temperature, pH, NO_2^-–N, and salinity) did not differ significantly among treatments ($p>0.05$). This indicates that similar physicochemical conditions were maintained across treatments throughout the experimental period, providing a consistent background for evaluating treatment effects on the remaining parameters.

In contrast, significant differences ($p<0.05$) were observed for ALK, TAN, NO_3^-–N, TSS, and Turbidity (Table 1, Figure 1). The distribution patterns presented in Figure 1 further support these findings, showing narrower variability and reduced upper ranges for TAN, TSS, and turbidity in mussel–integrated treatments compared to the control. ALK was significantly lower in Treatment 4 (75

green mussels·tank^{-1}) than in Treatment 1 (no green mussels), Treatment 2 (25 green mussels·tank^{-1}), and Treatment 3 (50 green mussels·tank^{-1}) ($p<0.05$). TAN levels were lowest in Treatments 3 and 4, followed by Treatment 2, whereas the highest value was recorded in Treatment 1, which contained no mussels ($p<0.05$). This pattern suggests improved nitrogen conditions in the mussel–integrated treatments, particularly at intermediate to high mussel biomass. For NO_3^-–N, Treatment 1 had the lowest mean value and differed significantly from Treatments 2, 3, and 4 ($p<0.05$). TSS was significantly lower in Treatments 2, 3, and 4 than in Treatment 1 ($p<0.05$). Turbidity was lowest in Treatment 4 and differed significantly from the other treatments ($p<0.05$). Overall, these results Treatment 4 and differed significantly from the other treatments ($p<0.05$). Overall, these results indicate that mussel integration affected key water quality parameters related to nitrogen dynamics and suspended solids, whereas the other physicochemical parameters remained stable under identical water–exchange conditions.

Growth performance of Asian seabass

Growth performance of Asian seabass differed significantly among treatments ($p<0.05$). Fish cultured in mussel–integrated treatments exhibited higher average FW, WG, ADG, SGRW, production, as well as lower FCR, compared with the control. In particular, Treatments 3 and 4, which incorporated intermediate to high green mussel biomass, showed significantly higher ADG and

Table 1. Mean±SD and ranges (in brackets) of water quality parameters in rearing tanks under different experimental treatments after 60 days (N = 60).

Parameters/ Treatments	T1 (Control)	T2 (A. seabass +25 green mussels)	T3 (A. seabass +50 green mussels)	T4 (A. seabass +75 green mussels)	F	p-value
DO (mg·L^{-1})	6.89±0.19	6.91±0.19	6.90±0.19	6.91±0.17	0.17	0.92
	(6.43–7.41)	(6.43–7.44)	(6.30–7.39)	(6.56–7.39)		
Temperature (°C)	24.59±2.14	24.41±2.05	24.38±1.99	24.36±2.01	0.15	0.93
	(19.80–28.66)	(19.70–28.72)	(19.76–28.66)	(19.81–28.61)		
pH	7.92±0.08	7.95±0.08	7.93±0.09	7.93±0.09	0.59	0.62
	(7.70–8.16)	(7.77–8.16)	(7.69–8.15)	(7.72–8.16)		
ALK (mg·L^{-1})	96.78±7.55^{b}	97.61±9.93^{b}	97.15±9.59^{b}	88.04±8.79^{a}	15.52	0.00
	(85.00–119.00)	(68.00–123.67)	(68.00–121.00)	(68–112.33)		
TAN (mg·L^{-1})	0.30±0.32^{c}	0.22±0.23^{b}	0.16±0.17^{a,b}	0.12±0.13^{a}	7.40	0.00
	(0.01–1.08)	(0.01–1.03)	(0.01–0.61)	(0.00–0.45)		
NO_2^-–N (mg·L^{-1})	2.52±0.41	2.59±0.32	2.58±0.29	2.58±0.29	0.62	0.60
	(0.62–3.00)	(1.04–3.01)	(1.07–3.12)	(0.94–3.02)		
NO_3^-–N (mg·L^{-1})	1.30±0.56^{a}	1.59±0.61^{b}	1.70±0.61^{b}	1.77±0.57^{b}	7.43	0.00
	(0.00–2.46)	(0.00–2.58)	(0.05–2.72)	(0.68–2.69)		
Salinity (psu)	32.56±0.50	32.61±0.50	32.71±0.48	32.65±0.39	1.38	0.25
	(31.50–33.80)	(31.53–33.80)	(31.40–33.80)	(31.43–33.33)		
TSS (mg·L^{-1})	0.08±0.02^{b}	0.07±0.02^{a}	0.07±0.02^{a}	0.07±0.02^{a}	8.04	0.00
	(0.05–0.13)	(0.05–0.12)	(0.05–0.13)	(0.04–0.13)		
Turbidity (NTU)	2.58±1.52^{c}	2.36±1.29^{b,c}	2.00±1.10^{b}	1.51±1.03^{a}	8.45	0.00
	(0.88–7.27)	(0.68–5.91)	(0.53–6.37)	(0.48–7.51)		

Note: Mean±SD in each row with different lowercase superscripts are significantly different ($p<0.05$). DO = dissolved oxygen; Temperature = water temperature; pH = power of hydrogen; ALK = alkalinity; TAN = total ammonia nitrogen; NO_2^-–N = nitrite nitrogen; NO_3^-–N = nitrate nitrogen; TSS = total suspended solids.

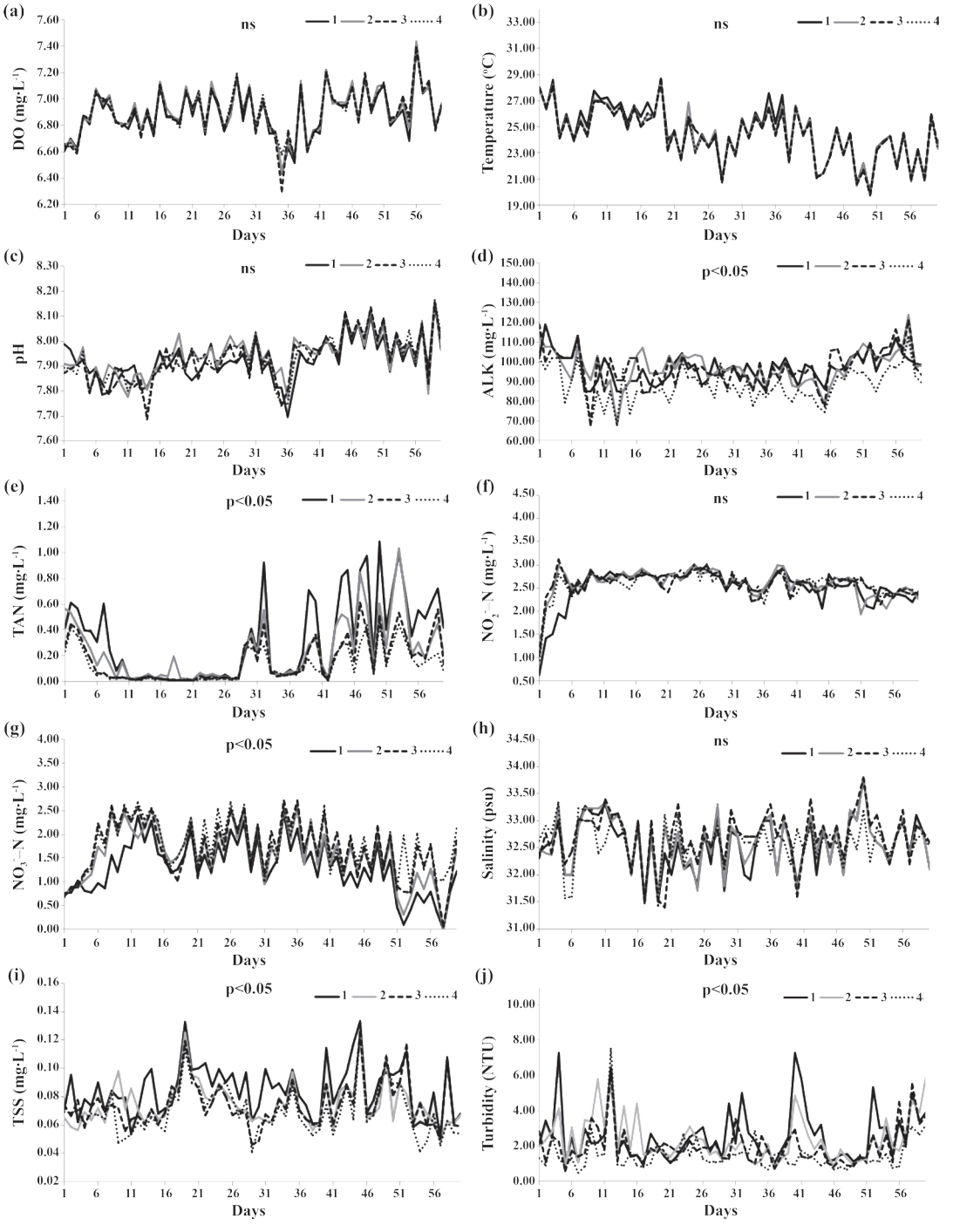


Figure 1. Distribution of water quality parameters during the experiment: (a) dissolved oxygen (DO); (b) water temperature (Temperature); (c) power of hydrogen (pH); (d) alkalinity (ALK); (e) total ammonia nitrogen (TAN); (f) nitrite–nitrogen (NO_2^-–N); (g) nitrate–nitrogen (NO_3^-–N); (h) salinity; (i) total suspended solids (TSS); and (j) turbidity. Treatments: 1 = control; 2 = seabass + 25 green mussels; 3 = seabass + 50 green mussels; 4 = seabass + 75 green mussels; "ns" indicates no significant difference ($p>0.05$); $p<0.05$ indicates a significant difference among treatments.

production than Treatment 2 and the control. The improved growth performance observed in Treatments 3 and 4 coincided with lower concentrations of TAN relative to the other treatments. This pattern suggests that the improved nitrogen conditions associated with mussel integration contributed to improved fish growth and biomass accumulation. Survival rates remained high and did not differ significantly among treatments, indicating that differences in production were primarily driven by growth performance rather than mortality (Table 2).

Biomass and growth of green mussel

The growth performance of green mussels under different stocking densities in the co–culture system showed no statistically significant differences ($p>0.05$) in terms of FW, WG, ADG, or SGRW (Table 3). However, significant differences ($p<0.05$) were observed in survival rate and total production. Treatments 3 and 4 recorded higher survival rates compared to Treatment 2. Total production was highest in Treatment 4, followed by Treatments 3 and 2, respectively.

Table 2. Growth performance of Asian seabass reared under different green mussels density treatments for 60 days.

Parameter	T1 (Control)	T2 (A. seabass +25 green mussels)	T3 (A. seabass +50 green mussels)	T4 (A. seabass +75 green mussels)	F	p-value
IW ($g \cdot Ind^{-1}$)	34.83±2.97	34.68±2.59	34.98±4.19	34.02±3.51	0.32	0.81
FW ($g \cdot Ind^{-1}$)	60.45±6.49^{a}	61.78±6.54^{a}	68.68±9.24^{b}	67.37±8.29^{b}	5.52	0.00
WG ($g \cdot Ind^{-1}$)	25.62±2.00^{a}	27.10±1.07^{b}	33.70±2.53^{c}	33.35±0.51^{c}	17.80	0.00
ADG ($g \cdot Ind^{-1} \cdot day^{-1}$)	0.42±0.03^{a}	0.45±0.02^{a}	0.57±0.03^{b}	0.55±0.01^{b}	23.67	0.00
SGRW ($\% \cdot Ind^{-1} \cdot day^{-1}$)	0.85±0.02^{a}	0.96±0.02^{b}	1.13±0.05^{c}	1.09±0.01^{c}	58.68	0.00
Survival (%)	100	100	100	100		
Production (kg)	1.15±0.04^{a}	1.17±0.02^{b}	1.31±0.07^{c}	1.31±0.06^{c}	7.76	0.01
FCR	1.41±0.08^{b}	1.32±0.01^{b}	1.15±0.09^{a}	1.11±0.08^{a}	5.08	0.00

Note: Mean±SD in each row with different lowercase superscripts are significantly different ($p<0.05$). IW = initial weight; FW = final weight; WG = weight gain; ADG = average daily gain; SGRW = specific growth rate based on body weight; FCR = feed conversion ratio.

Table 3. Biomass and growth performance of green mussels under different treatments after 60 days.

Parameter	T1 (Control)	T2 (A. seabass +25 green mussels)	T3 (A. seabass +50 green mussels)	T4 (A. seabass +75 green mussels)	F	p-value
IW ($g \cdot Ind^{-1}$)	–	3.83±0.24	3.86±0.26	3.85±0.31	0.21	0.89
FW ($g \cdot Ind^{-1}$)	–	5.05±0.82	4.93±0.67	5.21±0.88	0.09	0.91
WG ($g \cdot Ind^{-1}$)	–	1.33±0.27	1.10±0.22	1.35±0.10	1.27	0.35
ADG ($g \cdot Ind^{-1} \cdot day^{-1}$)	–	0.02±0.005	0.02±0.004	0.02±0.002	1.34	0.33
SGRW ($\% \cdot Ind^{-1} \cdot day^{-1}$)	–	0.45±0.36	0.32±0.04	0.50±0.13	0.48	0.64
Survival (%)	–	42.00±2.00^{a}	61.00±3.61^{b}	63.89±3.47^{b}	42.70	0.00
Production (kg)	–	0.07±0.02^{a}	0.11±0.02^{b}	0.24±0.01^{c}	53.46	0.00

Note: Mean±SD in each row with different lowercase superscripts are significantly different ($p<0.05$). IW = initial weight; FW = final weight; WG = weight gain; ADG = average daily gain; SGRW = specific growth rate based on body weight.

A biomass–normalized apparent reduction (BNAR) interpretation

BNAR values for TAN, TSS, and turbidity are shown in Figure 2. These parameters, which were significantly lower in the mussel–integrated treatments than in the control (Table 1), showed positive biomass–normalized apparent reduction values throughout the 60–day period.

For TAN, BNAR values increased over time across all mussel treatments. In Treatment 2,

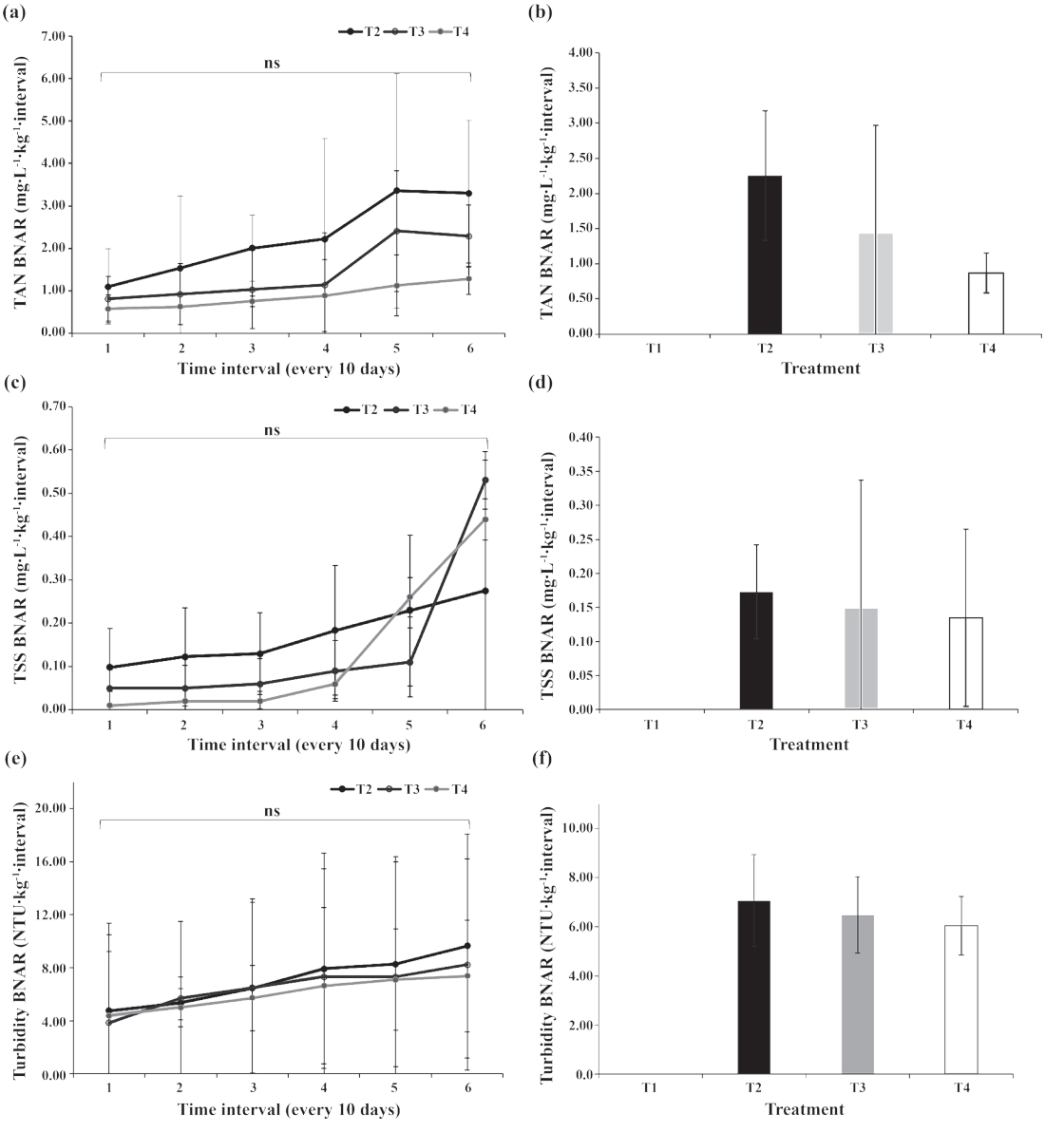


Figure 2. Biomass–normalized apparent reduction (BNAR) values of green mussels for water quality parameters, including total ammonia nitrogen (TAN), total suspended solids (TSS), and turbidity, under different stocking densities over 60 days. Panels (a), (c), and (e) show BNAR trends at 10–day intervals, whereas panels (b), (d), and (f) show the average BNAR values for each parameter over the full 60–day period. "ns" indicates no significant difference ($p>0.05$).

BNAR values for TAN ranged from 1.09±0.89 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval on day 10 to 3.30±1.72 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval on day 60. Treatment 3 showed BNAR values between 0.81±0.53 and 2.29±0.73 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval, while Treatment 4 ranged from 0.58±0.33 to 1.28±0.37 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval across the same intervals. The average TAN BNAR values for the full trial were 2.25±0.92, 1.43±1.53, and 0.87±0.28 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval in Treatments 2, 3, and 4, respectively. No statistically significant differences were found among treatments ($p>0.05$).

TSS BNAR values also followed an increasing trend throughout the experiment. In Treatment 2, BNAR values ranged from 0.10±0.09 to 0.27±0.30 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval. Treatment 3 recorded BNAR values from 0.05±0.04 to 0.53± 0.07 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval, and Treatment 4 ranged from 0.01±0.03 to 0.44±0.05 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval. The mean TSS BNAR values over 60 days were 0.17±0.07 (T2), 0.15±0.19 (T3), and 0.14±0.13 $mg \cdot L^{-1} \cdot kg^{-1} \cdot$interval (T4), with no statistically significant differences among treatments ($p>0.05$).

For turbidity, BNAR values similarly increased over the experimental period. Treatment 2 recorded BNAR values from 4.76±5.73 to 9.64±8.45 $NTU \cdot kg^{-1} \cdot$interval. Treatment 3 ranged from 3.85± 7.53 to 8.23±7.96 $NTU \cdot kg^{-1} \cdot$interval, and Treatment 4 from 4.40±4.82 to 7.38±4.21 $NTU \cdot kg^{-1} \cdot$interval. The average turbidity BNAR values were 7.07± 1.87, 6.48±1.55, and 6.04±1.19 $NTU \cdot kg^{-1} \cdot$interval in Treatments 2, 3, and 4, respectively, with no statistically significant differences ($p>0.05$).

DISCUSSION

The results of this study demonstrate that integrating green mussels into an Asian seabass aquaculture system significantly improved several key water quality parameters known to influence fish performance. Mean TAN concentrations declined with increasing mussel biomass, while TSS and turbidity were also significantly lower in mussel–integrated treatments, supporting the role of green mussels as effective extractive components in co–culture systems (Kong *et al*., 2023; Kumara *et al*., 2023). These findings are consistent with previous work showing that nitrogen loading in Asian seabass culture systems is closely linked to feed utilization and waste production, thereby influencing water quality dynamics (Plaipetch *et al*., 2014).

Based on the experimental data, statistically significant reductions were observed only for ALK, TAN, NO_3^-–N, TSS, and turbidity, whereas DO, temperature, pH, NO_2^-–N, and salinity did not differ significantly among treatments. Therefore, these parameters should be interpreted independently rather than in direct contrast. ALK was significantly lower in Treatment 4, which had the highest mussel biomass, compared with the other treatments. This reduction is likely attributable to the utilization of carbonate and bicarbonate ions for shell formation by green mussels (Boyd and Tucker, 1998). Although mussels contribute to the apparent reduction of TSS and nitrogenous waste in the system, they also excrete ammonia (NH_4^+–N), thereby increasing the nitrogen pool available for nitrification. This process likely explains the higher NO_3^-–N concentrations observed in mussel–integrated treatments (Bruesewitz *et al*., 2008; Rong *et al*., 2021).

The role of green mussels in reducing TAN, TSS, and turbidity was evident, and BNAR became more pronounced with increasing mussel biomass. These results indicate that higher mussel biomass was associated with greater relative improvements in nitrogen–related water quality parameters under identical water–exchange conditions. Such patterns are consistent with previous studies demonstrating the capacity of bivalves to enhance water quality in co–culture systems involving shrimp and fish (Kong *et al*., 2023; Kumara *et al*., 2023; Nurfadillah *et al*., 2023). Although the calculation approach was conceptually adapted from conventional filtration–based metrics, the term "filtration rate" was deliberately avoided in this study. Classical filtration or clearance rates are typically derived under closed or semi–closed conditions, whereas the present experiment incorporated routine water exchange designed to simulate semi–open IMTA management. Under

such conditions, observed changes in water quality reflect the combined influence of biological uptake and water renewal, making it inappropriate to interpret the calculated values as mechanistic filtration or clearance rates.

Accordingly, BNAR was employed as a comparative indicator to describe biomass-dependent trends in apparent water quality improvement rather than absolute removal efficiency. Importantly, BNAR is not intended to replace conventional filtration or clearance rate measurements, but rather to provide a context–appropriate system–level comparison under routine water–exchange conditions. This reformulation allows robust comparison among treatments under standardized water–exchange regimes without implying direct physiological filtration performance. Similar biomass–related trends were discussed by Argente (2024), who highlighted the influence of mussel size and biomass on apparent removal performance under controlled conditions. Differences among studies are nevertheless expected because of variation in species characteristics, environmental conditions, and experimental design.

Co–culturing green mussels also had a positive effect on the growth performance of Asian seabass. Significant improvements in FW, WG, ADG, and SGRW were observed in Treatments 3 and 4, which also had lower FCR values. These results indicate enhanced feed efficiency under mussel–integrated conditions. The improved growth performance is likely linked to improved nitrogen-related water quality, particularly the reduction in TAN concentrations, which is known to directly influence fish metabolism and stress responses. This is consistent with previous finding showing that increasing TAN levels elevate oxygen consumption rates in Asian seabass, reflecting increased metabolic demand and physiological stress (Is–haak *et al*., 2022), thereby supporting the observed growth improvements under lower TAN conditions in the present study. Based on acute toxicity tests conducted on juvenile Asian seabass, the 96–h LC_{50} values for TAN and non–ionized ammonia (NH_3–N) were reported as 3.31 mg·L^{-1} and 1.30 mg·L^{-1}, respectively, under laboratory conditions (Økelsrud and Pearson, 2007). Given that the TAN concentrations observed in the present study were substantially lower than these thresholds, the interpretations pertain to sublethal differences in water quality rather than acute toxicity effects. Nevertheless, the stocking density used in this study (4.5 kg·m^{-3}) exceeded the IMTA–recommended threshold of ≤2 kg·m^{-3} (Cotou *et al*., 2024; Jongjaraunsuk *et al*., 2025), which may pose risks to long–term water quality if not managed effectively. Moreover, the 60–day duration of the experiment may not be sufficient to assess potential long–term impacts on fish health, such as immunological stress or the accumulation of metabolic waste. Future research should consider longer experimental durations and include physiological and biochemical indicators, such as cortisol levels and liver enzyme activity, to evaluate chronic stress responses (Nurfadillah *et al*., 2023). Farm–scale trials in open marine cages are also recommended to validate these findings under commercial conditions.

Green mussels survived and exhibited growth across all treatments. Treatment 4 recorded the highest survival rate and biomass production. Survival rates between 60% and 64% were maintained in Treatments 3 and 4, despite the absence of direct feeding. These rates are acceptable for biofilter species under co–culture conditions. However, the SGRW of 0.32–0.50%·Ind^{-1}·day^{-1} observed in this study was lower than the 1.45% ·Ind^{-1}·day^{-1} reported under natural conditions (Chaitanawisuti and Menasveta, 1987). This discrepancy is likely due to the limited availability of natural food sources such as phytoplankton and suspended organic matter, as well as the relatively stagnant conditions in tank–based systems compared to dynamic marine environments.

In summary, the integration of green mussels into intensive Asian seabass culture systems contributed to improved water quality stability and enhanced fish growth performance beyond the effects of routine water exchange alone. The use of 50–75 mussels per 0.25 m^3 tank resulted in measurable improvements in nitrogen–related parameters and biomass production. These findings support the application of green mussels as a functional component of IMTA systems aimed

at improving sustainability in intensive marine aquaculture. Further studies under farm–scale and open–water conditions are necessary to confirm the scalability and practical applicability of these results.

CONCLUSIONS

This study demonstrated that integrating green mussels into intensive Asian seabass aquaculture systems can significantly improve water quality, enhance fish growth performance, and support sustainable aquaculture practices. Green mussels effectively reduced TAN, TSS, and turbidity, indicating their potential role as natural biofilters. Fish co–cultured with mussels, particularly at moderate to high mussel densities, showed improved growth and feed efficiency. Although mussel growth was lower than that reported under natural conditions, their survival and contribution to waste reduction were notable despite the absence of supplemental feeding. These findings indicate that green mussels can serve as a functional component in polyculture systems, offering both ecological and production benefits. Further research should confirm these outcomes under commercial farming conditions, such as open–sea cage systems, and incorporate long–term health monitoring to ensure the sustainability and scalability of integrated multi–trophic aquaculture systems.

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