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Evaluation of Size-Based Performance of Blood Cockles *Anadara granosa* as a Biofiltration Agent in Whiteleg Shrimp *Litopenaeus vannamei* Aquaculture Systems

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ABSTRACT

Effluent from intensive whiteleg shrimp ponds can promote excessive phytoplankton growth in natural aquatic ecosystems. One potential mitigation strategy is the use of blood cockles, which can assimilate excess nutrients present in the effluent. Cockle size is an important consideration, as it may affect their nutrient uptake capacity. This study evaluated the performance of blood cockles from different size groups in improving the quality of effluent from intensive shrimp farming. The 90-day experiment was conducted at a semi-outdoor scale using shrimp pond effluent as the culture medium. Four treatments were applied: blood cockles with shell sizes of 25– 30, 30– 35 & 35–40mm, and a control without cockles. Each treatment was replicated three times. Results showed that cockles in all size groups reduced phytoplankton abundance as well as concentrations of inorganic and organic nutrients in the medium. The 30– 35mm size group achieved the highest efficiency and overall performance. Survival rates exceeded 90% across all treatments, and steady growth was observed throughout the culture period. In conclusion, blood cockles in the 30– 35mm size group provided the most efficient and optimal reduction of nutrient levels in shrimp pond effluent, while maintaining high survival and consistent growth over the 90-day period.

INTRODUCTION

Blood cockles (*Anadara granosa*) are marine bivalves widely distributed in Southeast Asia, particularly in Indonesia, Malaysia, and Thailand **(Khalil *et al*., 2017)**. They inhabit muddy-bottom sediments **(Rozirwan *et al*., 2023; Effendi *et al*., 2025)**. Deriving most of their nutrition from microalgae **(Hamli *et al*., 2019)**, they are classified as bottom filter feeders as well as detritus feeders **(Saffian *et al*., 2020)**. Blood cockles can be cultivated on mud substrates, relying entirely on natural food sources such as phytoplankton without the need for artificial feed **(Prasetiyono *et al*., 2022a)**. Their growth depends on nutrient availability in the sediment and the natural food present in the culture medium **(Nicholaus *et al*., 2019)**.

Whiteleg shrimp (*Litopenaeus vannamei*) farming generates nutrient-rich sludge and wastewater. Sludge originates from uneaten feed and detritus in shrimp ponds **(Junda *et al*., 2019)**, and pond effluent contains high levels of nitrate and phosphate **(Prasetiyono *et al*., 2022b)**. These nutrients are essential for microalgae growth **(Ramli *et al*., 2015)**. The elevated nutrient content is primarily due to organic matter from uneaten feed, fecal waste, and metabolic residues, which decompose into compounds that fuel algal proliferation **(Prasetiyono *et al*., 2024)**. These organic-rich sediments and wastewaters can also serve as food sources for detritus feeders, including blood cockles **(Sulistiyaningsih & Arbi, 2020)**.

Blood cockles have been used to improve the quality of the whiteleg shrimp pond effluent **(Prasetiyono *et al*., 2022a)**. Their ability to survive, grow, and absorb nutrients in such effluent is influenced by shell size, which affects resistance to shell damage and predation **(Mu *et al*., 2018; Belgrad *et al*., 2023)**, as well as tolerance to environmental stressors **(Dethier *et al*., 2019)**. Smaller cockles are more vulnerable to abiotic stress, such as high temperatures and low dissolved oxygen, due to higher metabolic demands for optimal conditions **(Dethier *et al*., 2019)**. Shell size also influences nutrient uptake, as filtration rates generally increase with body size **(Qiao *et al*., 2022)**.

Blood cockles assimilate nutrients from microalgae, detrital organic matter, and waste produced in intensive whiteleg shrimp systems **(Ihwan *et al*., 2025)**. However, nutrient absorption efficiency likely varies with organism size. This study investigated the relationship between blood cockle shell size and their capacity to improve whiteleg shrimp pond effluent quality, aiming to identify the most effective size class for maximizing biofiltration performance.

MATERIALS AND METHODS

1. Time and place

The study was conducted in a semi-field setting at the Lubuk Menur Jaya Belinyu Fish Farming Group, Bangka Regency, from October 2023 to January 2024. Analyses of test parameters were carried out at the Fish Nutrition and Feed Technology Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences (FPIK), IPB University; the Central Proteina Prima (CPP) Water Quality Laboratories in Belinyu and Pangkalpinang, Bangka; and the Fisheries Laboratory, Department of Fisheries and Marine Sciences, Faculty of Fisheries and Marine Sciences (FPPK), University of Bangka Belitung.

2. Blood cockle husbandry

Experimental animals were blood cockles (*Anadara granosa*) obtained from an aquaculture site in the coastal waters of Sukal Beach, West Bangka. The cockles were stocked in tarpaulin ponds (100 × 100 × 75cm) at a density of 50 individuals per container, based on **Syahira *et al*. (2021)**. Continuous aeration was applied throughout the 90-day maintenance period, with no artificial feed provided. Regular monitoring included survival rate, growth, and water quality.

Shrimp pond wastewater and sludge were sourced from an intensive whiteleg shrimp (*Litopenaeus vannamei*) farm in the coastal waters of North Bangka (1°32′35.4″S, 105°48′51.4″E). Effluent was collected on day 60 of the shrimp culture cycle and treated by aeration for 14 days prior to use. The treated wastewater and sludge were then transferred into experimental containers, each with a 5cm sludge layer and a maintained water depth of 65cm.

An acclimatization period coincided with the wastewater aeration phase. Blood cockles were maintained in aerated media consisting of water and sediment from their native habitat. Water and sludge levels in acclimation containers were gradually increased over 10 days to match experimental conditions, followed by 4 days of stabilization. Cockles were then transferred to the pre-treated experimental containers, which were continuously aerated without water replacement or supplementation throughout the study.

3. Experimental design

A Completely Randomized Design (CRD) was used, with four treatments and three replicates per treatment:

- **A (Control):** No blood cockles
- **B:** Shell size 25– 30mm (26.91 ± 0.24mm)
- **C:** Shell size 30– 35mm (33.51 ± 0.25mm)
- **D:** Shell size 35– 40mm (37.52 ± 0.43mm)

Performance was evaluated based on reductions in phytoplankton abundance, nutrient concentrations, survival rate, and absolute growth in weight and shell length.

4. Phytoplankton abundance

Water and sediment samples were collected on days 0, 15, 35, 60, and 90. Water samples were preserved in Lugol’s iodine solution and examined microscopically at 40×–100× magnification. Phytoplankton were identified following **Stafford (1999)**, and abundance was calculated using the formula in **APHA (2017)**.

5. Blood cockles' survival and growth

Survival and growth were assessed on days 0, 15, 35, 60, and 90. Survival rate (%) was calculated as per **Maoxiao *et al*. (2019)**. Growth performance included absolute increases in shell length, width, and height, measured following **Rumondang *et al*. (2024)**. Changes in body weight were evaluated using the specific growth rate (SGR) formula described by **Prato *et al*. (2020)**.

6. Data analysis

Data were expressed as mean ± standard deviation (SD) and analyzed using one-way ANOVA at a 95% confidence level. Significant differences were further examined using Duncan's multiple range test. Analyses were performed using XLSTAT 2019 software, with significance set at $P < 0.05$.

RESULTS

1. Phytoplankton abundance

The results of the study on intensive shrimp farming effluent cultivated with blood cockles showed differences in phytoplankton abundance among the four treatments: Treatment A (control, without blood cockles), Treatment B (blood cockles 25– 30mm), Treatment C (30– 35mm), and Treatment D (35– 40mm), as illustrated in Fig. (1).

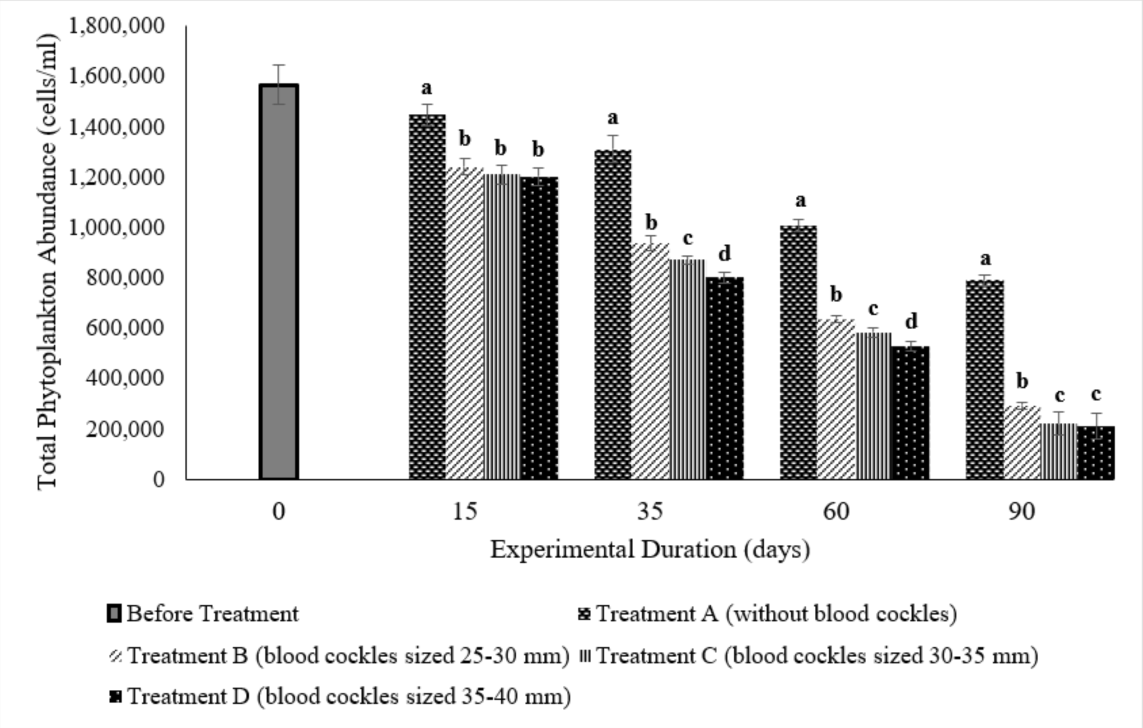

Fig. 1. Phytoplankton abundance in the culture media of blood cockles with varying shell sizes

Based on Fig. (1), the application of blood cockles of different sizes had a measurable effect on phytoplankton abundance. Before treatment (day 0), phytoplankton abundance in the water was 1,567,167 ± 79,114 cells/ml. On day 15, abundance in treatments B (25– 30mm), C (30– 35mm), and D (35– 40mm) did not differ significantly from one another but was significantly lower than in treatment A (control, without blood cockles). By days 35 and 60, significant differences were observed among all treatments. On day 90, phytoplankton abundance in treatments C and D remained statistically similar but was significantly lower than in treatments B and A.

On day 90, phytoplankton abundance was 789,667 ± 21,368 cells/ml in treatment A, 292,500 ± 15,207 cells/ml in treatment B, 220,333 ± 46,702 cells/ml in treatment C, and 210,000 ± 51,061 cells/ml in treatment D. Overall, phytoplankton abundance decreased across all treatments, including the control. However, the greater reduction observed in treatments with blood cockles (B, C, and D) compared to the control indicates the effectiveness of blood cockles in reducing phytoplankton levels. The percentage decrease in phytoplankton abundance for each treatment relative to the control is presented in Fig. (2).

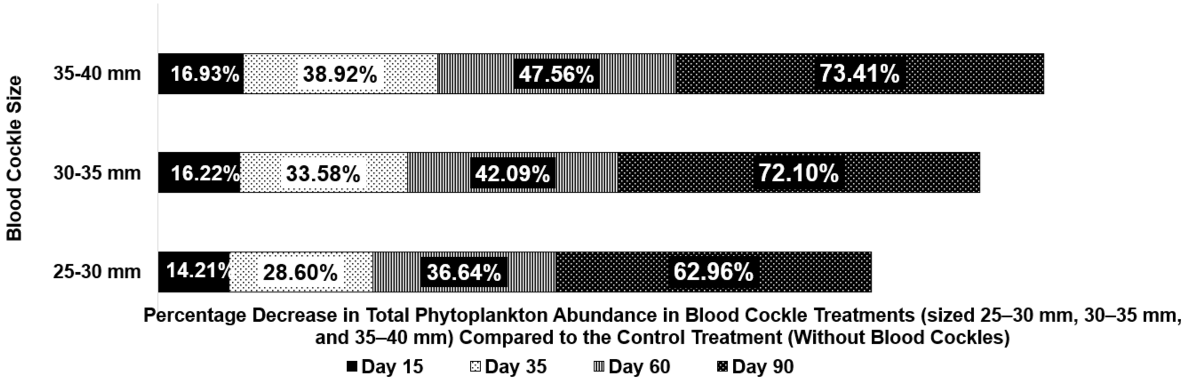

Fig. 2. Percentage decrease in total phytoplankton abundance in culture media with blood cockles of various sizes compared to the control treatment (Without blood cockles) in each culture period

The percentage decrease in total phytoplankton abundance in the blood cockle treatments (25–30, 30–35, and 35– 40mm) compared to the control (without blood cockles) increased with cultivation time (Fig. 2). The greatest reduction occurred on day 90, with treatments C (30– 35mm) and D (35– 40mm) showing the highest decreases at 72.10 and 73.41%, respectively. These values were significantly greater than treatment B (25– 30mm), which showed a 62.96% reduction.

Phytoplankton in the experimental whiteleg shrimp pond effluent belonged to six taxonomic groups: Chlorophyta, Cyanophyta, Cryptophyta, Bacillariophyta, Euglenophyta, and Pyrrophyta. Chlorophyta, characterized by chlorophyll *a* and *b* pigments, impart a green color and are common primary producers in both freshwater and marine environments. Cyanophyta (blue-green algae, BGA) are photosynthetic but have prokaryotic cell structures; some species are associated with harmful algal blooms (HABs). Cryptophyta possess plastids containing chlorophyll *a* and *c*, along with pigments such as phycoerythrin and phycocyanin. Bacillariophyta (diatoms) have silica-based, box-shaped cell walls. Euglenophyta contain photosynthetic plastids and move via flagella, while Pyrrophyta (dinoflagellates) possess two flagella and, in some species, produce harmful toxins.

Among these groups, Chlorophyta were the most abundant, while Cyanophyta were the least abundant. The abundance of each phytoplankton group by treatment and the corresponding percentage reductions are shown in Table (1).

Table 1. Abundance of phytoplankton groups in pond effluent treated using blood cockles of different sizes

DOC	Abundance of Phytoplankton (cells/ml)	Treatments			
		A (without blood cockles)	B (blood cockles sized 25- 30mm)	C (blood cockles sized 30- 35mm)	D (blood cockles sized 35- 40mm)
0	Chlorophyta	1,248,667 ± 59,591			
	Cyanophyta	18,667 ± 4,072			
	Cryptophyta	174,333 ± 8,221			
	Bacillariophyta	58,333 ± 20,817			
	Euglenophyta	32,500 ± 11,456			
	Pyrrophyta	34,833 ± 9,751			
15	Chlorophyta	1,149,333 ± 46,023^{a}	1,015,900 ± 51,930^{b}	992,500 ± 22,913^{b}	986,233 ± 35,220^{b}
	Cyanophyta	17,500 ± 866^{a}	10,667 ± 1,528^{b}	5,750 ± 1,299^{b}	5,167 ± 1,258^{b}
	Cryptophyta	170,667 ± 7,522^{a}	130,000 ± 13,919^{b}	129,167 ± 16,266^{b}	127,500 ± 16,394^{b}
	Bacillariophyta	53,333 ± 3,819^{a}	36,667 ± 8,780^{b}	35,333 ± 6,825^{b}	33,500 ± 9,042^{b}
	Euglenophyta	28,667 ± 10,563^{a}	24,167 ± 5,204^{a}	23,833 ± 3,215^{a}	25,000 ± 5,000^{a}
	Pyrrophyta	26,667 ± 2,887^{a}	23,333 ± 11,273^{a}	25,000 ± 8,660^{a}	24,000 ± 7,858^{a}
35	Chlorophyta	1,105,000 ± 50,329^{a}	818,333 ± 25,166^{b}	759,667 ± 14,785^{c}	693,333 ± 20,207^{d}
	Cyanophyta	12,500 ± 1,000^{a}	2,667 ± 764^{b}	1,500 ± 1,500^{b}	1,167 ± 1,041^{b}
	Cryptophyta	140,000 ± 10,000^{a}	89,167 ± 6,292^{b}	86,667 ± 7,638^{b}	85,000 ± 6,614^{b}
	Bacillariophyta	25,000 ± 10,000^{a}	10,000 ± 2,500^{b}	8,333 ± 2,887^{b}	7,833 ± 4,509^{b}
	Euglenophyta	13,333 ± 3,253^{a}	6,583 ± 1,588^{b}	6,167 ± 1,258^{b}	5,833 ± 1,443^{b}
	Pyrrophyta	15,000 ± 2,500^{a}	9,167 ± 1,443^{b}	8,333 ± 1,443^{b}	7,500 ± 2,500^{b}
60	Chlorophyta	851,000 ± 28,579^{a}	552,833 ± 5,204^{b}	522,833 ± 4,752^{c}	471,667 ± 11,815^{d}
	Cyanophyta	8,667 ± 1,258	500 ± 866	-	-
	Cryptophyta	117,500 ± 6,614^{a}	79,500 ± 7,365^{b}	56,333 ± 22,329^{b}	53,500 ± 26,301^{b}
	Bacillariophyta	16,167 ± 9,570^{a}	2,000 ± 3,464^{b}	1,667 ± 1,443^{b}	1,500 ± 1,323^{b}
	Euglenophyta	4,167 ± 3,819	-	-	-
	Pyrrophyta	8,333 ± 3,819^{a}	2,500 ± 2,500^{b}	1,667 ± 1,443^{b}	833 ± 1,443^{b}
90	Chlorophyta	670,833 ± 27,538^{a}	235,000 ± 28,395^{b}	173,167 ± 31,426^{c}	169,667 ± 34,356^{c}
	Cyanophyta	4,667 ± 577	-	-	-
	Cryptophyta	102,500 ± 6,614^{a}	56,667 ± 23,761^{b}	46,667 ± 19,858^{b}	40,333 ± 23,539^{b}
	Bacillariophyta	6,667 ± 1,443	-	-	-
	Euglenophyta	833 ± 1,443	-	-	-
	Pyrrophyta	4,167 ± 5,204	833 ± 1,443	500 ± 866	-

Based on Table (1), the abundance of most phytoplankton groups decreased from the initial measurement (day 0) to the end of the culture period (day 90) across all treatments.

On day 15, the abundance of Chlorophyta, Cyanophyta, Cryptophyta, and Bacillariophyta in treatments B (25– 30mm), C (30– 35mm), and D (35– 40mm) did not differ significantly from each other but was significantly lower than in treatment A (control, without blood cockles). In contrast, Euglenophyta and Pyrrophyta showed no significant differences across all treatments on this date.

On day 35, Chlorophyta abundance differed significantly among treatments, with treatment D showing the lowest abundance and treatment A the highest. For the other phytoplankton groups, treatments B, C, and D did not differ significantly from each other but were significantly lower than treatment A.

On day 60, Chlorophyta abundance again differed significantly among treatments, with treatment D recording the lowest abundance. Cryptophyta, Bacillariophyta, and Pyrrophyta abundances in treatments B, C, and D did not differ significantly from each other but were significantly lower than in treatment A. Cyanophyta were absent in treatments C and D, while Euglenophyta were absent in all cockle treatments (B, C, and D).

On day 90, Chlorophyta abundance in treatments C and D did not differ significantly but was significantly lower than in treatments B and A. Treatments B and A also differed significantly from each other. By this stage, Chlorophyta, Cyanophyta, and Bacillariophyta were absent in treatments B, C, and D, while Pyrrophyta were absent in treatment D.

Survival and growth of blood cockles

The survival rate is a key indicator of the adaptability and resilience of blood cockles during cultivation. It reflects not only their capacity to withstand environmental conditions over the 90-day period but also their sustained ability to utilize available natural food sources, thereby contributing to nutrient reduction in the effluent water. In this study, survival was calculated as the percentage of individuals alive at the end of the cultivation period relative to the initial stock. Differences in survival across size groups are shown in Fig. (3).

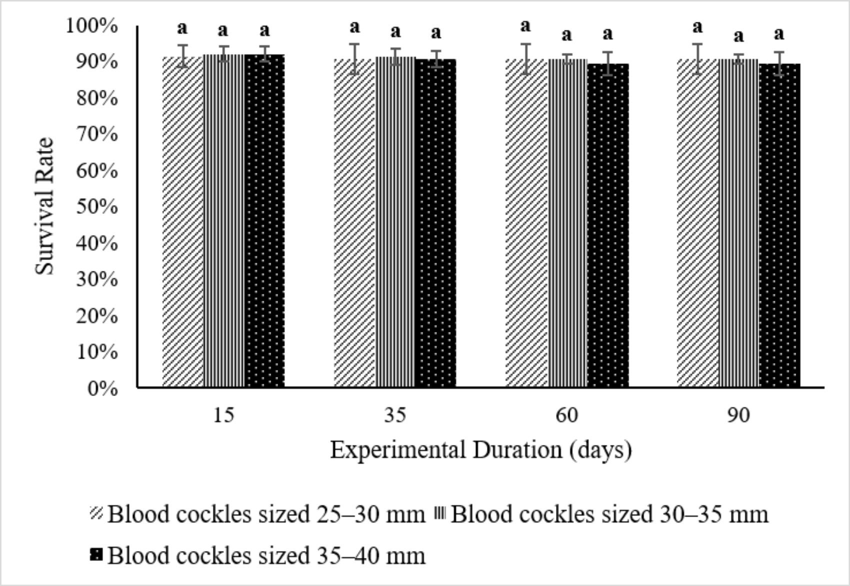

Fig. 3. Survival of blood cockles cultivated at different sizes in sludge and wastewater from whiteleg shrimp ponds (25– 30, 30– 35, and 35– 40mm).

Based on Fig. (3), blood cockles of different size classes (25–30, 30–35, and 35–40mm) cultured in sludge and wastewater from intensive whiteleg shrimp ponds showed no significant differences in survival rates on days 15, 35, 60, and 90.

On day 15, survival rates were 91.33 ± 3.06%, 92.00 ± 2.00%, and 92.00 ± 2.00% for the 25–30, 30–35, and 35– 40mm groups, respectively. On day 35, all groups maintained survival rates above 90%, with values of 90.67 ± 4.16%, 91.33 ± 2.31%, and 90.67 ± 2.31%, respectively. On day 60, survival rates were 90.67 ± 4.16%, 90.67 ± 1.15%, and 89.33 ± 3.06%, respectively, and these values remained unchanged on day 90.

Growth

Growth in shell length and body weight served as indicators of nutrient assimilation over the experimental period. Increases in shell length for each size group are shown in Fig. (4).

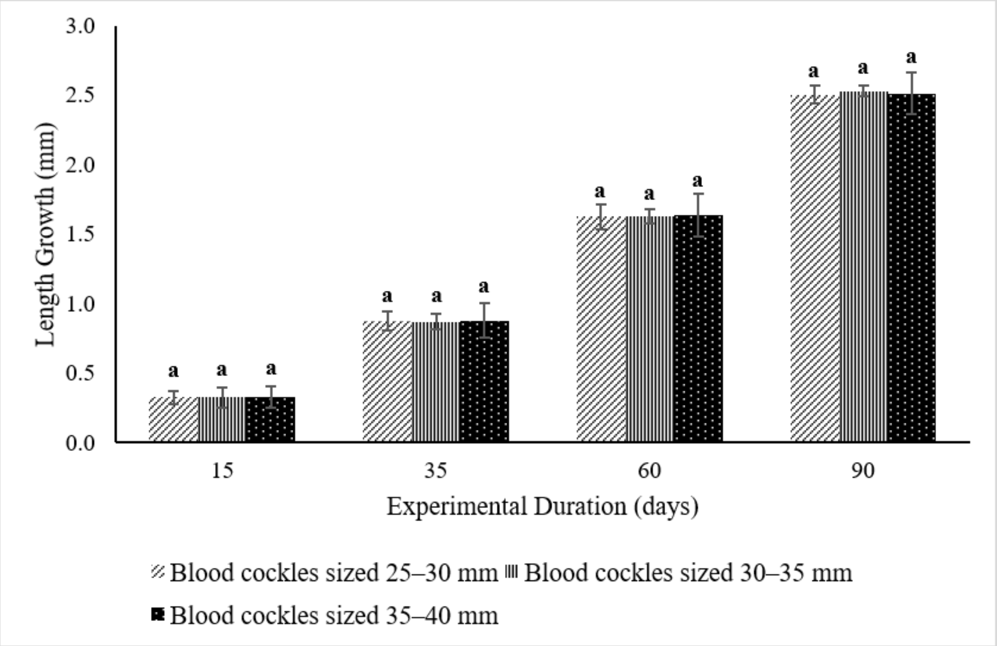

Fig. 4. Length growth of blood cockles of different sizes cultivated in sludge and pond wastewater: 25–30, 30–35, and 35– 40mm

Shell length growth

Based on Fig. (4), body length increased progressively across all culture periods. Blood cockles of different initial shell sizes, when cultured in shrimp pond effluent, showed no significant differences in length growth among size groups at any sampling period (days 15, 35, 60, and 90).

On day 15, length growth was 0.327 ± 0.048 mm, 0.325 ± 0.075 mm, and 0.328 ± 0.074 mm for the 25–30 mm, 30–35 mm, and 35–40 mm groups, respectively. By day 35, increases reached 0.874 ± 0.068 mm, 0.871 ± 0.053 mm, and 0.879 ± 0.125 mm, respectively. On day 60, growth was 1.624 ± 0.092 mm, 1.626 ± 0.051 mm, and 1.637 ± 0.153 mm, respectively. At the end of the culture period (day 90), total increases in shell length were 2.504 ± 0.060 mm, 2.528 ± 0.040 mm, and 2.510 ± 0.150 mm, respectively.

Weight growth

Weight growth was assessed as the total weight (shell and internal organs) of the blood cockles during the culture period. The absolute weight growth for each treatment group is shown in Fig. (5).

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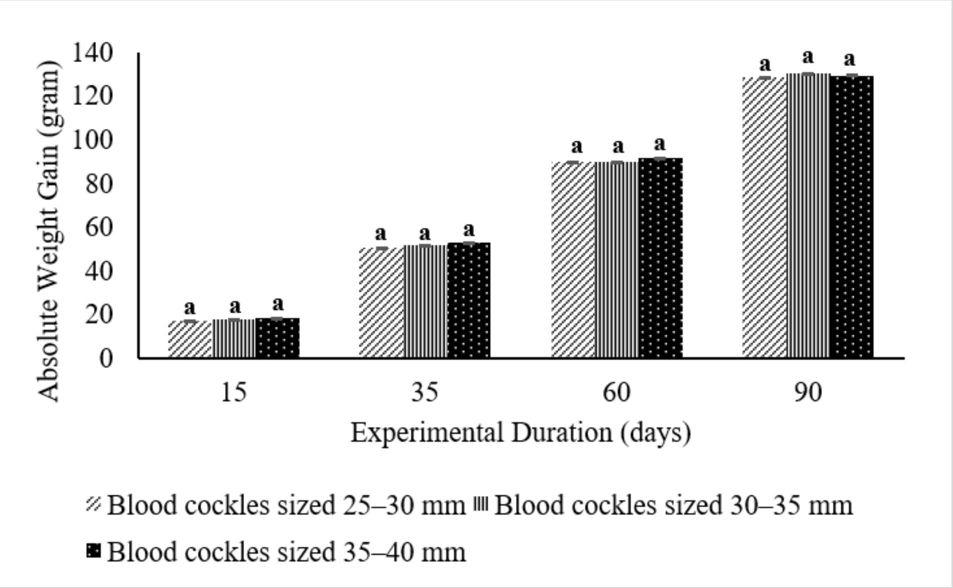

Fig. 5. Absolute weight growth of blood cockles of different sizes cultivated in pond water and sludge: 25–30, 30–35, and 35– 40mm

Fig. (5) shows that the absolute weight of blood cockles increased steadily with the length of the cultivation period. No significant differences in absolute weight growth were observed among the 25–30, 30–35, and 35– 40mm size groups at any sampling period (days 15, 35, 60, and 90).

On day 15, absolute weights were 16.733 ± 0.663 g, 17.630 ± 0.682 g, and 18.257 ± 1.285 g for the 25–30, 30–35, and 35– 40mm groups, respectively. By day 35, weights increased to 50.550 ± 0.875 g, 51.570 ± 1.326 g, and 52.753 ± 2.523 g, respectively. On day 60, the respective weights were 89.753 ± 8.854 g, 89.743 ± 0.864 g, and 91.597 ± 4.879 g. The highest values were recorded on day 90, reaching 128.397 ± 4.377 g, 130.417 ± 2.670 g, and 129.370 ± 5.084 g, respectively.

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DISCUSSION

Intensive farming of whiteleg shrimp (*Litopenaeus vannamei*) often generates significant waste, primarily consisting of organic residues from feed and shrimp excretions. These waste materials accumulate in shrimp ponds, creating nutrient-rich conditions that promote excessive phytoplankton growth. As primary producers, phytoplankton readily utilize the available nutrients, leading to blooms. **Gao *et al.* (2024)** observed that elevated phytoplankton abundance is strongly correlated with high levels of organic matter in the water. While nutrient availability plays a central role in supporting phytoplankton proliferation, other factors such as water quality, environmental conditions, and the presence of plankton-feeding organisms also contribute. **Saeedi *et al.***

(2022) reported that benthic communities feeding on phytoplankton can significantly influence phytoplankton abundance in aquatic ecosystems. Blood cockles are filter feeders that primarily consume phytoplankton and other organic matter suspended in the water column **(Jaowatana *et al.*, 2024)**.

As shown in Fig. (1), phytoplankton abundance declined across all treatments from day 0 to day 90. This reduction is likely linked to the decreasing concentrations of key nutrients—namely ammonium, nitrate, and orthophosphate—in the culture medium. Phytoplankton rely on these nutrients, particularly nitrogen and phosphorus, for growth and reproduction. In closed or semi-closed systems, the absence of a continuous nutrient supply can limit phytoplankton proliferation over time **(Ramos *et al.*, 2017)**. The feeding activity of blood cockles may further contribute to the reduction in phytoplankton abundance, reinforcing their potential role in bioremediation.

The cultivation of blood cockles in treatments B (25– 30mm), C (30– 35mm), and D (35– 40mm) resulted in a more pronounced reduction in phytoplankton abundance compared to treatment A, which lacked blood cockles. This decrease can be attributed to the filtration activity of blood cockles which consume phytoplankton and organic detritus as part of their diet. Larger or more numerous individuals are likely contributed to greater phytoplankton uptake, leading to a faster decline in phytoplankton populations than in control conditions without cockles **(Yurimoto *et al.*, 2021)**. The presence of blood cockles has been shown to reduce phytoplankton concentrations in aquatic systems, with chlorophyll-a levels serving as a reliable indicator of this effect **(Saif *et al.*, 2020)**.

On day 15 of the experiment, no significant differences in total phytoplankton abundance were observed among treatments B (25– 30mm), C (30– 35mm), and D (35– 40mm), all of which contained blood cockles of varying sizes. This uniformity is likely due to suboptimal phytoplankton filtration during the early stages of cultivation, as the blood cockles were still undergoing adaptation to the new environmental conditions. During this acclimatization phase, organisms tend to prioritize homeostasis, which can manifest in altered feeding behavior, reduced activity, and physiological adjustments in response to environmental stressors **(Schubert *et al.*, 2017)**. Even larger individuals may exhibit limited feeding efficiency during this period. As noted by **Nemova (2023)**, aquatic organisms often show a temporary decline in feed intake when adjusting to new surroundings, which may explain the initially low impact of blood cockles on phytoplankton abundance.

By days 35 and 60 of the cultivation period, differences in phytoplankton abundance began to emerge among the treatments with blood cockles of varying sizes, suggesting that the cockles had successfully adapted to the culture environment. During this phase, treatment D (35– 40mm) exhibited a significantly lower phytoplankton abundance compared to the other treatments. This trend reflects the greater filtration capacity of larger blood cockles, as nutrient absorption generally increases with body size. The biomass grows aligned with growing nutritional demands, resulting in higher feeding rates. Moreover, the development of feeding structures and improvements in absorption efficiency over time further enhance the capacity of larger individuals to reduce phytoplankton abundance in the culture medium **(Rosa *et al.*, 2018; Qiao *et al.*, 2022)**.

Although there was a size difference between treatment C (30– 35mm) and treatment D (35– 40mm), no significant difference in phytoplankton abundance was observed between the two groups by day 90. This outcome is likely due to a decline in the filtration rate of blood cockles in treatment D, as a result of the markedly reduced phytoplankton concentrations in the culture medium. Filtration activity in bivalves is influenced by several factors, including phytoplankton density, particle quality and size, and the size of the bivalve itself **(Khalil *et al.*, 2021)**. While larger individuals generally exhibit higher filtration rates under high phytoplankton concentrations **(Marion *et al.*, 2022)**, their activity may decrease when food availability becomes limited. As a physiological energy-conservation strategy, shellfish tend to reduce shell valve movement and filtration effort during periods of low microalgal density in the water column **(Larsen *et al.*, 2018)**.

Prior to the experimental period, the Chlorophyta group exhibited the highest abundance among all phytoplankton groups (Table 1). This pattern is likely linked to the application of probiotics in the whiteleg shrimp (*Litopenaeus vannamei*) production ponds, which contribute to elevated Chlorophyta levels in pond effluent. **Prasetiyono *et al.* (2024)** observed a high prevalence of Chlorophyta—particularly *Nannochloropsis* sp. and *Chlorella* sp.—in the effluent of intensive shrimp ponds on the northern coast of Bangka Island where probiotics were applied. Similarly, **Cao *et al.* (2014)** reported that probiotic use can enhance the dominance of Chlorophyta in shrimp pond ecosystems. This effect is attributed to the presence of nutrients and growth-promoting compounds in probiotic formulations that specifically favor Chlorophyta proliferation.

Phytoplankton abundance declined across all treatments over the maintenance periods on days 15, 35, 60, and 90, although the rate of decline varied among treatments. Treatment A (without blood cockles) consistently showed the highest phytoplankton abundance at each time point, indicating the influence of blood cockles on phytoplankton reduction. Among all phytoplankton groups, the Chlorophyta group showed the strongest alignment with total phytoplankton abundance trends (Table 1 & Fig. 1). This is attributable to its dominance throughout the study period, making it the primary contributor to total phytoplankton abundance. As **Kang *et al.* (2021)** noted, when environmental conditions favor a particular phytoplankton group, that group tends to dominate the community and significantly shape overall abundance, with such changes being closely tied to water quality and nutrient availability. Organic nutrients play a crucial role in shaping the structure and dynamics of phytoplankton communities across aquatic ecosystems **(Moschonas *et al.,* 2017)**. A decline in nutrient availability typically leads to a corresponding decrease in phytoplankton populations.

Beyond nutrient limitations, the presence of blood cockles in treatments B (25–30mm), C (30– 35mm), and D (35– 40mm) further influenced phytoplankton abundance due to their role as filter feeders. By consuming phytoplankton as a primary food source, blood cockles accelerate the reduction of phytoplankton biomass in the culture medium **(Mo *et al.,* 2023)**. Consequently, phytoplankton abundance in these treatments was significantly lower compared to treatment A, which lacked blood cockles. Blood cockles efficiently filter phytoplankton and small organic particles from their environment to support their growth and energy needs **(Tan *et al.,* 2024)**. **Yurimoto *et al.* (2021)** demonstrated that blood cockles absorb phytoplankton particles and digest them using cellulolytic enzymes within their digestive tract. These bivalves feed by filtering water through their bodies **(Gosling, 2015)**. Larger blood cockles possess wider gill surfaces and exhibit higher water pumping rates, which enable them to filter greater volumes of water and consume more phytoplankton and organic particles **(Bayne, 2017)**. This increased filtration capacity in larger individuals enhances their nutrient uptake potential, directly supporting higher growth rates and meeting elevated energy demands **(Karlson *et al.,* 2021)**.

Under favorable environmental conditions, blood cockles exhibit high survival rates. As shown in Fig. (3), survival remained consistently high across all treatments throughout the maintenance periods, with no significant differences observed between size groups. This success is largely attributed to the cultivation medium, which comprised aerated whiteleg shrimp (*Litopenaeus vannamei*) pond effluent that had undergone 14 days of intensive aeration. Aeration increases dissolved oxygen (DO) levels, preventing anoxic conditions—one of the leading causes of mortality in aquatic organisms **(Zhu *et al.,* 2020)**. By introducing atmospheric oxygen through bubbles, aeration not only elevates DO but also facilitates the removal of dissolved gases such as CO_2, H_2S, volatile organic compounds (VOCs), and metals **(Aytac *et al.,* 2024)**. Additionally, aeration promotes the aerobic decomposition of organic matter by microorganisms, aids in the oxidation of toxic ammonia and nitrite, and inhibits the formation of harmful sulfur compounds and methane through enhanced oxidation processes **(Fu *et al.,* 2023)**.

The high survival rates observed during the maintenance period can also be attributed to a 14-day acclimatization process conducted prior to the experiment. During acclimatization, blood cockles were gradually introduced to a maintenance medium consisting of water and mud from their natural habitat, supplemented incrementally with sludge and shrimp pond wastewater. This gradual adaptation allows aquatic organisms to adjust their physiological and behavioral responses to new environmental conditions, thereby enhancing their ability to survive and grow **(El-Dakar *et al.,* 2021)**.

Acclimatization encompasses physiological, anatomical, and morphological adjustments that improve an organism's performance and survival under changing environments **(Makaras *et al.,* 2021)**. Effective acclimatization increases the likelihood of successful survival when organisms are introduced to novel settings **(Teletchea, 2019)**. Consistently, survival rates in this study ranged from 89.33 to 90.67% across all treatments and maintenance periods (Fig. 3), comparable to the 90.86 to 91.42% survival reported by **Saif *et al.* (2020)** in pond-cultured blood cockles.

Length growth was recorded across all treatments involving different blood cockle sizes throughout the maintenance period. Despite observable increases in length, no significant differences in growth rates were detected between treatments at each maintenance interval (Fig. 4). This lack of difference is attributed to similar nutrient utilization efficiency across size classes, as all blood cockles were in a rapid growth phase. The body length of blood cockles is determined by their shell length **(Hira *et al.,* 2025)**. Shell growth primarily requires minerals, especially calcium (Ca). According to **Sillanpaa *et al.* (2020)**, marine bivalves obtain Ca ions (Ca^{2+}) from seawater, which are absorbed mainly through the gills and mantle tissue. **Zhao *et al.* (2021)** explained that the mantle regulates calcium deposition in the biomineralization zone, facilitating the formation of new shell layers. Biomineralization—the deposition of calcium carbonate—is influenced by the availability of Ca^{2+}, carbonate ions, and the organic matrix. In addition to calcium, nutrients derived from food support growth by providing metabolic energy necessary for both soft tissue and shell development **(Checa *et al.,* 2018)**.

In terms of weight growth, no significant differences were observed among treatments during any maintenance period (Fig. 5). Biomass increase in blood cockles is closely linked to the efficient utilization of ingested food. According to **Vladimivora *et al.* (2003)**, blood cockles allocate energy derived from digestion to various physiological processes, including cell and tissue synthesis, which contributes to biomass accumulation. This energy allocation pattern is typical among bivalves receiving adequate nutrition—particularly phytoplankton—facilitating accelerated growth in biomass. Generally, the energy obtained from food is partitioned between both maintenance and the enhancement of body mass.

In this study, no significant difference in biomass weight was observed between smaller and larger blood cockles. This can be explained by the adjustment of total energy requirements for growth according to each size class's food absorption capacity. Larger cockles require more food to support the development of larger cells and tissues, while smaller cockles have a lower absorption capacity, resulting in reduced food intake—particularly phytoplankton—due to their smaller body size. As noted by **Bayne (2017)**, increased growth rates are driven by higher food consumption combined with greater growth efficiency. Blood cockles ranging from 15 to 50mm in length are typically in the rapid or exponential growth phase, during which energy from food is predominantly allocated toward biomass accumulation.

Despite observable growth, the increase in shell length of blood cockles is relatively slow. In natural habitats, they typically require about six months to grow only 4– 5mm in shell length **(Asmita & Machrizal, 2023)**. Similarly, **Saif *et al.* (2020)** reported that blood cockles cultured in pond systems exhibited average shell length growth rates of 0.66– 0.99mm/ month and weight gains of approximately 0.33g/ month. A study by **Srisomwong *et al.* (2018)** in Bang-tabun Bay, Thailand, detected weight growth ranging from 0.1 to 0.8g/ month. In the present study, shell length growth over the three-month cultivation period averaged 2.5mm, equivalent to roughly 0.83mm/ month across all size groups. Absolute weight gains during the same period were 2.720g (0.91g/ month) for the 25– 30mm group, 2.877g (0.96g/ month) for the 30– 35mm group, and 2.897g (0.97g/ month) for the 35– 40mm group.

Growth patterns within the same species can vary significantly, largely due to differences in food availability **(Alburhana *et al.*, 2023)**. In blood cockles, growth is influenced by multiple environmental factors such as seasonality, temperature, food supply, salinity, and organic matter content **(Yulinda *et al.*, 2020)**. A decline in food availability reduces the energy intake necessary for metabolism, growth, and energy storage, thereby slowing biomass accumulation **(Larsen *et al.*, 2018)**.

CONCLUSION

This study demonstrated the potential of blood cockles (*Anadara granosa*) as effective biofiltration agents in the whiteleg shrimp (*Litopenaeus vannamei*) aquaculture systems, particularly in reducing nutrient loads and phytoplankton abundance in shrimp pond effluent. Across all tested size groups, blood cockles contributed to improved water quality, highlighting their ability to assimilate both organic and inorganic nutrients.

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