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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 contribute to increased phytoplankton growth in natural aquatic ecosystems. One potential approach to mitigate this issue is the use of blood cockles, which can utilize the excess nutrients in the effluent. The size of the cockles is an important factor to consider, as it may influence their capacity to assimilate available nutrients. This study aimed to evaluate the performance of blood cockles of different size groups in improving the quality of effluent from intensive shrimp farming. The experiment was conducted over 90 days at a semi-outdoor scale using shrimp pond effluent as the cultivation medium. Four treatments were applied, consisting of blood cockles with shell sizes of 25–30 mm, 30–35 mm, 35–40 mm, and a control group without cockles. Each treatment was replicated three times. The results showed that blood cockles of all size groups were capable of reducing phytoplankton abundance as well as concentrations of both inorganic and organic nutrients in the cultivation medium. The 30–35 mm size group exhibited the most effective and efficient performance. In addition, survival rates exceeded 90%, and growth increased steadily over the culture period. In conclusion, blood cockles in the 30–35 mm size group demonstrated the most efficient and optimal performance in reducing nutrient levels in shrimp pond effluent while sustaining high survival and growth throughout the 90-day culture period.

## INTRODUCTION

Blood cockles *Anadara granosa* are a type of marine bivalve found in Southeast Asia, particularly Indonesia, Malaysia, and Thailand (**Khalil *et al.*, 2017**), which live in the sediment at the bottom of muddy waters (**Rozirwan *et al.*, 2023**; **Effendi *et al.*, 2025**). This species is a bottom filter feeder and detritus feeder (**Saffian *et al.*, 2020**), with its primary nutrients derived from microalgae (**Hamli *et al.*, 2019**). Furthermore, blood cockles can be cultivated using mud substrate and relying on natural food (phytoplankton) as their main food source without being given artificial feed (**Prasetiyono *et al.*, 2022a**). The growth of blood cockles depends on the nutrients contained in the mud and the availability of natural food in the cultivation medium (**Nicholaus *et al.*, 2019**).

However, whiteleg shrimp farming produces sludge and wastewater with high nutrient content. Sludge is a waste product from feed residues or detritus in shrimp ponds (**Junda *et al.*, 2019**). The nutrient compounds in pond waste primarily contain nitrate and phosphate (**Prasetiyono *et al.*, 2022b**). Nitrate and phosphate are the main elements required for microalgae growth (**Ramli *et al.*, 2015**). The high nutrient content in whiteleg shrimp farming wastewater is due to the organic matter content from uneaten feed residues, undigested and unabsorbed feed in the form of feces, and metabolic residues from feed consumed by shrimp. This organic matter undergoes decomposition into nutrients required by microalgae for growth (**Prasetiyono *et al.*, 2024**). The nutrient content of organic matter in the sediment and wastewater from whiteleg shrimp farming activities can also be utilized by detritus feeders, one of which is the blood cockles (**Sulistiyaningsih and Arbi, 2020**).

Blood cockles have been used to improve the quality of Whiteleg shrimp pond effluent (**Prasetiyono *et al.*, 2022a**). The ability of blood cockles to live, grow, and absorb nutrients in Whiteleg shrimp pond effluent is influenced by their size. The body size (shell) of blood cockles affects their ability to survive shell damage and predation (**Mu *et al.*, 2018**; **Belgrad *et al.*, 2023**). Additionally, shell size also affects the blood cockles' response to stress (**Dethier *et al.*, 2019**), where blood cockles with smaller shells are more susceptible to abiotic stressors such as high temperatures and low oxygen levels. This is because smaller blood cockles have higher requirements for optimal temperature and dissolved oxygen compared to larger blood cockles (**Dethier *et al.*, 2019**). The body or shell size of blood cockles also affects the amount of nutrients absorbed by the cockles, with the filtration rate of food absorption in cockles increasing as the cockles' body size increases (**Qiao *et al.*, 2022**).

Blood cockles assimilate nutrients originating from microalgae, detrital organic matter, and waste generated in intensive whiteleg shrimp systems (**Ihwan *et al.*, 2025**). However, the efficiency of nutrient absorption is likely influenced by the size of the organism. This research investigates the relationship between blood cockles' shell size and their capacity to enhance the quality of whiteleg shrimp pond effluent with the goal of determining the most effective size class for maximizing biofiltration performance.

## MATERIALS AND METHODS

### 1. Time and Place

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

## 2. Blood Cockles Husbandry

The experimental animals used in this study were blood cockles obtained from a blood cockles aquaculture site located in the coastal waters of Sukal Beach, West Bangka. The aquaculture containers used were tarpaulin ponds measuring 100 cm × 100 cm × 75 cm. Aeration was applied continuously throughout the maintenance period. A stocking density of 50 individuals per container was used, based on the study by **Syahira *et al.* (2021).** The maintenance period lasted for 90 days without the addition of artificial feed. During this period, regular observations and monitoring were carried out on survival rate, growth, and water quality.

The shrimp pond wastewater and sludge used in this study were collected from an intensive shrimp farming site located in the coastal waters of North Bangka (coordinates: 1°32'35.4"S 105°48'51.4" E). The effluent was taken on day 60 of the whiteleg shrimp (*Litopenaeus vannamei*) culture cycle. Before being used in the experiment, the wastewater and sludge were treated through a 14-day aeration process. After 14 days, the treated wastewater and sludge were transferred into the experimental containers of each treatment group. The thickness of the sludge layer in each container was set at 5 cm, and the water level was maintained at 65 cm.

Prior to their placement in the experimental containers, the blood cockles underwent an acclimatization process. This acclimatization was conducted simultaneously with the 14-day aeration treatment of the wastewater and sludge. The cockles were maintained in aerated media consisting of water and sediment from their original habitat. Water and sludge levels in the acclimatization containers were gradually raised (once daily for 10 days) to achieve experimental levels. Subsequently, the cockles were kept in aerated conditions for an additional 4 days without further addition of water or sludge. Following this adaptation period, the experimental animals were transferred to the experimental containers containing the pre-treated wastewater and sludge. Throughout the experiment, the cockles maintenance media were continuously aerated, with no water replacement or supplementation.

## 3. Experimental Design

An experimental approach was adopted, applying a Completely Randomized Design (CRD) with four treatments and three replications per treatment. The treatments were as follows:

Treatment A: Without blood cockles (control)
Treatment B: Blood cockles sized 25–30 mm (26.91 ± 0.24 mm)
Treatment C: Blood cockles sized 30–35 mm (33.51 ± 0.25 mm)

Treatment D: Blood cockles sized 35–40 mm (37.52 ± 0.43 mm)

The performance of blood cockles was evaluated based on their effectiveness in reducing phytoplankton abundance and nutrient concentrations, survival rates, and absolute growth in weight and shell length.

### 4. Phytoplankton Abundance

Water and sediment (sludge substrate) samples were collected five times during the study, specifically on days 0, 15, 35, 60, and 90. The analysis of phytoplankton presence and abundance in the cultivation media was conducted by collecting water samples, which were preserved using Lugol's iodine solution. Microscopic observations were carried out at magnifications ranging from 40× to 100×. Microalgae were identified using the phytoplankton identification guide by **Stafford (1999)**. Phytoplankton abundance was calculated using the formula described in **APHA (2017).**

### 5. Blood Cockles Survival and Growth

Measurements and analysis of survival and growth parameters of blood cockles were conducted five times during the study period: on days 0, 15, 35, 60, and 90. The survival rate (%) was calculated by comparing the number of live individuals at the end of the culture period to the number of individuals at the beginning, following the method of **Maoxiao *et al.* (2019)**. Growth performance parameters included absolute growth in shell length, width, and height, measured following the procedure described by **Rumondang *et al.* (2024)**. Changes in body weight were assessed by calculating the specific growth rate (SGR) based on the formula provided by **Prato *et al.* (2020).**

### 6. Data Analysis

All data were presented as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level. When a significant treatment effect was observed, post hoc comparisons were conducted using Duncan's multiple range test. Statistical analysis was performed using XLSTAT 2019 software. Differences were considered statistically significant at $p < 0.05$.

## RESULTS

### 1. Phytoplankton Abundance

The results of the study on effluent from intensive shrimp farming, when cultivated with blood cockles, showed varying phytoplankton abundance across the different treatments: Treatment A (without blood cockles), Treatment B (blood cockles sized 25–30 mm), Treatment C (30–35 mm), and Treatment D (35–40 mm), as presented in Fig. 1.

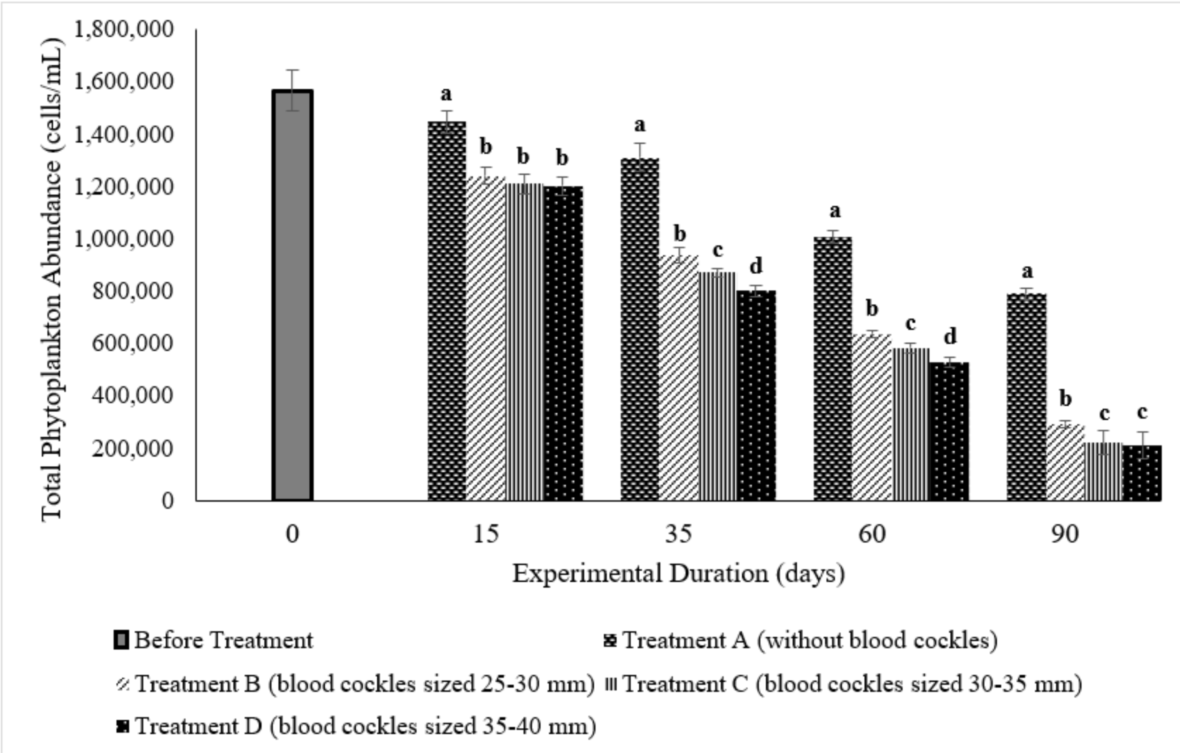


**Fig. 1.** Phytoplankton Abundance in The Culture Media of Blood Cockles with Varying Shell sizes

Based on Fig. 1, the application of blood cockles of different sizes affected phytoplankton abundance. Before treatment (day 0), phytoplankton abundance in the water was 1,567,167 ± 79,114 cells/mL. On day 15, the phytoplankton abundance in treatments B (blood cockles sized 25–30 mm), C (30–35 mm), and D (35–40 mm) did not differ significantly among treatments but differed significantly from treatment A (without blood cockles). On days 35 and 60, the total phytoplankton abundance between treatments differed significantly. On day 90, phytoplankton abundance in treatments C and D did not differ significantly between treatments but differed significantly from treatments B and A (without blood cockles). The abundance in treatments A, B, C, and D on day 90 was 789,667 ± 21,368 cells/mL, 292,500 ± 15,207 cells/mL, 220,333 ± 46,702 cells/mL, and 210,000 ± 51,061 cells/mL, respectively.

Based on Fig. 1, there was a decrease in phytoplankton abundance in all treatments, including treatment A, B, C, and D. The decrease in phytoplankton abundance in treatments with blood cockles cultivation (treatments B, C, and D) compared to the treatment without blood cockles (treatment A) illustrates the effectiveness of using blood cockles of different sizes compared to without blood cockles. The percentage decrease in total phytoplankton abundance in the blood cockles treatments (sized 25–30 mm, 30–35 mm, and 35–40 mm) compared to the control treatment (without blood cockles) is presented in Fig. 2.

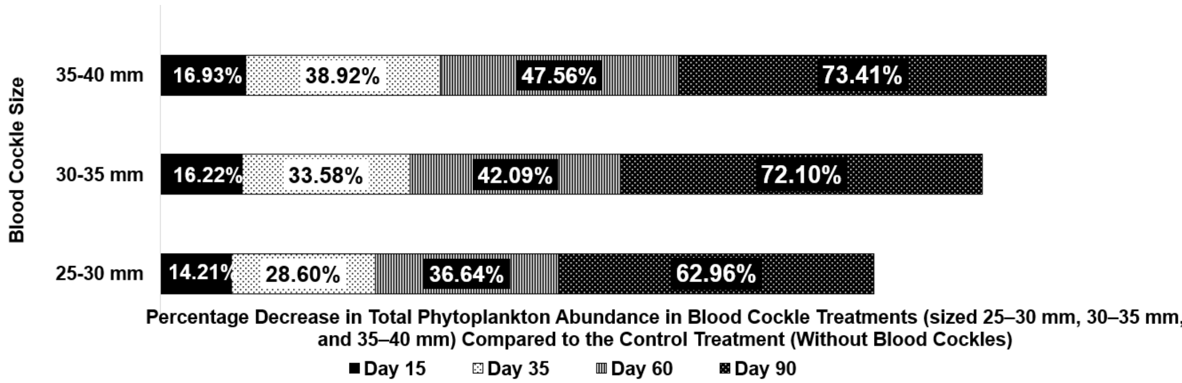


**Fig. 2**. Percentage Decrease in Total Phytoplankton Abundance in Culture Media With Blood Cockles of Various Sizes Compared to The Control Treatment (Without Blood Cockles) in Each Culture Period.

The percentage decrease in total phytoplankton abundance in the blood cockles treatment (sized 25–30 mm, 30–35 mm, and 35–40 mm) compared to the control treatment (without blood cockles) showed that the percentage decrease increased with increasing cultivation period (Fig. 2). The highest percentage decrease in phytoplankton abundance was achieved on day 90 of cultivation. The highest percentage decrease was achieved in treatment C (blood cockles sized 30-35 mm) and D (blood cockles sized 35–40 mm) treatments, with reduction percentages of 72.10% and 73.41%, respectively, which were significantly different from treatment B (blood cockles sized 25–30 mm) with a percentage of 62.96%.

Six phytoplankton groups were found in the experimental whiteleg shrimp pond waste media. These are Chlorophyta, Cyanophyta, Cryptophyta, Bacillariophyta, Euglenophyta, and Pyrrophyta. Chlorophyta is a group of phytoplankton that has chlorophyll a and b, giving it a distinctive green color. This group of primary producers is commonly found in both freshwater and marine environments. Meanwhile, Cyanophyta, or blue-green algae (BGA), is a group of phytoplankton capable of photosynthesis but with bacterial-like cell structures. Some species within this group can cause harmful algal blooms (HABs). Cryptophyta are phytoplankton that contain plastids with chlorophyll a and c, as well as additional pigments such as phycoerythrin and phycocyanin. The Bacillariophyta or diatom group is a phytoplankton with box-shaped cell walls composed of silica. Euglenophyta have distinctive plastids capable of photosynthesis and flagella for movement. Pyrrophyta, or dinoflagellates, have two flagella that aid in movement, and some species can produce harmful toxins.

The most abundant group was Chlorophyta, while Cyanophyta was the least abundant. The abundance of phytoplankton groups in each treatment and the percentage of decrease are presented 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 25-30 mm) | C (blood cockles sized 30-35 mm) | D (blood cockles sized 35-40 mm) |
| 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 tended to decrease from the initial measurement (day 0) to the end of the culture period (day 90) in all treatments. On day 15, the abundance of the phytoplankton groups Chlorophyta, Cyanophyta, Cryptophyta, and Bacillariophyta in treatments B (25–30 mm), C (30–35 mm), and D (35–40 mm) did not differ significantly between treatments but differed significantly from treatment A (without blood cockles). This condition was different from the Euglenophyta and Pyrrophyta groups, which did not differ significantly across all treatments on day 15.

On day 35, the abundance of Chlorophyta groups differed significantly between treatments. Treatment D had the lowest abundance, while treatment A had the highest. In other phytoplankton groups, treatments B, C, and D did not differ significantly between treatments but differed significantly from treatment A (without blood cockles).

On day 60, the abundance of Chlorophyta groups differed significantly between treatments. Treatment D had the lowest abundance of Chlorophyta groups compared to the others. The abundance of the Cryptophyta, Bacillariophyta, and Pyrrophyta groups did not differ significantly among treatments B, C, and D, but was significantly lower and differed significantly from treatment A. The Cyanophyta group was not found in treatments C and D. The Euglenophyta group was also not found in the blood cockles culture medium in treatments B, C, and D.

On day 90, the abundance of the Chlorophyta group in treatments C and D did not differ significantly between treatments and was significantly lower than in treatments B and A. However, treatments B and A differed significantly between treatments. The Chlorophyta, Cyanophyta, and Bacillariophyta groups in treatments B, C, and D were not found in the culture medium, while the Pyrrophyta group was not found in treatment D.

## 2. Survival and Growth of Blood Cockles

The survival rate serves as a key indicator of the adaptability and resilience of blood cockles during the 90-day cultivation period. Survival reflects not only the organisms' capacity to withstand environmental conditions but also their continued ability to utilize available natural food sources and thus reduce the nutrient load of effluent water. In this study, survival was quantified as the percentage of individuals that remained alive at the end of the cultivation period. Variations in survival across different size groups are depicted in Fig. 3.

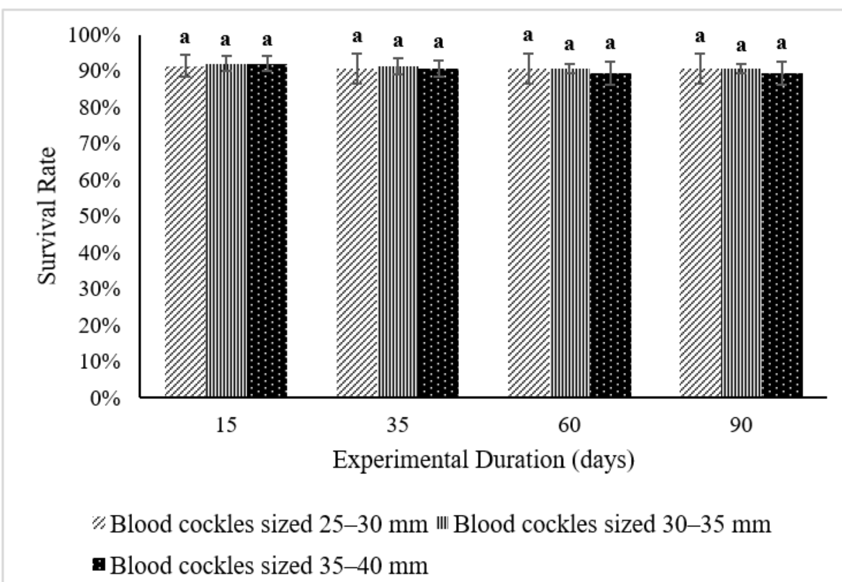


**Fig. 3.** Survival of Blood Cockles Cultivated at Different Sizes in Sludge and Wastewater from Whiteleg Shrimp Ponds (25–30 mm, 30–35 mm, and 35–40 mm).

Based on Fig. 3, blood cockles of different sizes cultured in sludge and wastewater from intensive whiteleg shrimp ponds showed no significant difference in survival rates among those sized 25–30 mm, 30–35 mm, and 35–40 mm on days 15, 35, 60, and 90. On day 15, the survival rates for blood cockles sized 25–30 mm, 30–35 mm, and 35–40 mm were 91.33 ± 3.06%, 92.00 ± 2.00%, and 92.00 ± 2.00%, respectively. On day 35, the survival rates in all size groups remained above 90%, with respective values of 90.67 ± 4.16%, 91.33 ± 2.31%, and 90.67 ± 2.31%. On day 60, the survival rates were 90.67 ± 4.16%, 90.67 ± 1.15%, and 89.33 ± 3.06% for the 25–30 mm, 30–35 mm, and 35–40 mm groups, respectively. The survival rate percentages on day 90 were the same as on day 60 for each treatment.

Blood growth over the experimental period, both in terms of shell length and body weight, is one indicator that the nutrients entering the body are digested by the individual. Length growth in blood cockles is depicted in Fig. 4.

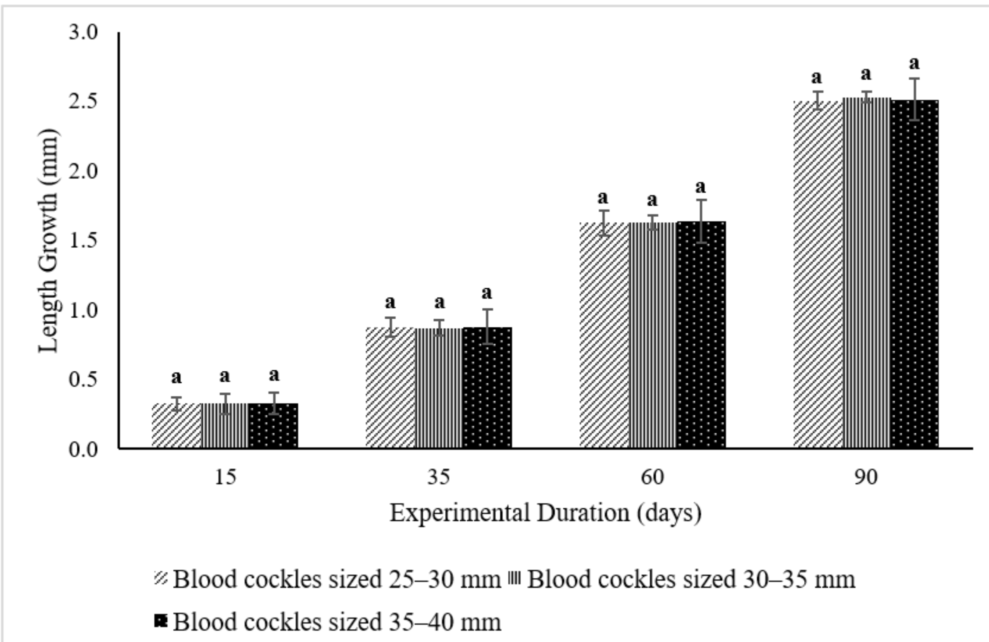


**Fig. 4**. Length Growth of Blood cockles of Different Sizes Cultivated in Sludge and Pond Wastewater: 25–30 mm, 30–35 mm, and 35–40 mm.

Based on Fig. 4, an increase in body length occurred during each culture period. Blood cockles of different initial shell lengths cultured in shrimp pond effluent showed no significant differences in growth between size groups at any culture period (days 15, 35, 60, and 90). Length growth on day 15 for the 25–30 mm, 30–35 mm, and 35–40 mm groups was 0.327 ± 0.048 mm, 0.325 ± 0.075 mm, and 0.328 ± 0.074 mm, respectively. By day 35, length growth in these groups was 0.874 ± 0.068 mm, 0.871 ± 0.053 mm, and 0.879 ± 0.125 mm, respectively. By day 60, the increases were 1.624 ± 0.092 mm, 1.626 ± 0.051 mm, and 1.637 ± 0.153 mm, respectively. On day 90, the total length increases were 2.504 ± 0.060 mm, 2.528 ± 0.040 mm, and 2.510 ± 0.150 mm, respectively.

The weight growth of blood cockles was measured as the total weight of the blood cockles (shell and internal organs) over the cultivation period. The absolute weight growth of blood cockles in each treatment is presented in Fig. 5.

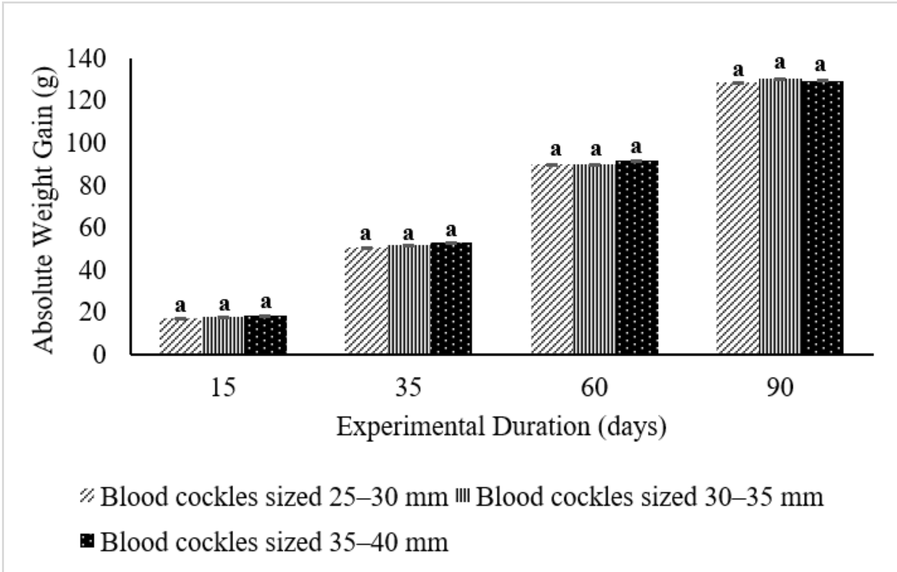


**Fig. 5.** Absolute Weight Growth of Blood Cockles of Different Sizes Cultivated in Pond Water and Sludge: 25–30 mm, 30–35 mm, and 35–40 mm.

Fig. 5 shows that the absolute weight of blood cockles increased with the length of the cultivation period. The cultivation of blood cockles sized 25–30 mm, 30–35 mm, and 35–40 mm showed no significant differences in absolute weight growth among the size groups on days 15, 35, 60, and 90 of the cultivation period. The absolute weights on day 15 for the 25–30 mm, 30–35 mm, and 35–40 mm groups were 16.733 ± 0.663 g, 17.630 ± 0.682 g, and 18.257 ± 1.285 g, respectively. On day 35, the weights were 50.550 ± 0.875 g, 51.570 ± 1.326 g, and 52.753 ± 2.523 g, respectively. On day 60, the weights were 89.753 ± 8.854 g, 89.743 ± 0.864 g, and 91.597 ± 4.879 g, respectively. The highest absolute weights were recorded on day 90, with the 25–30 mm, 30–35 mm, and 35–40 mm groups showing respective weights of 128.397 ± 4.377 g, 130.417 ± 2.670 g, and 129.370 ± 5.084 g.

## 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 (25–30 mm), C (30–35 mm), and D (35–40 mm) 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 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 (25–30 mm), C (30–35 mm), and D (35–40 mm), 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 (35–40 mm) 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. As biomass grows, so do 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 (30–35 mm) and treatment D (35–40 mm), 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 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' presence 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 (25–30 mm), C (30–35 mm), and D (35–40 mm) 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., 2020**).

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 cockles 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)** explain 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 **Vladimirova *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 50 mm 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 4–5 mm in shell length (**Asmita and Machrizal, 2023**). Similarly, **Saif *et al.* (2020)** reported that blood cockles cultured in pond systems exhibited average shell length growth rates of 0.66–0.99 mm/month and weight gains of approximately 0.33 g/month. A study by **Srisomwong *et al.* (2018)** in Bang-tabun Bay, Thailand, observed weight growth ranging from 0.1 to 0.8 g/month. In the present study, shell length growth over the three-month cultivation period averaged 2.5 mm, equivalent to roughly 0.83 mm/month across all size groups. Absolute weight gains during the same period were 2.720 g (0.91 g/month) for the 25–30 mm group, 2.877 g (0.96 g/month) for the 30–35 mm group, and 2.897 g (0.97 g/month) for the 35–40 mm 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 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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