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Bioremediation Role, Histology, and Mortality of *Tegillarca granosa* in Water Media of *Litopenaeus vannamei* Intensive Cultivation

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ABSTRACT

This study aimed to analyze the effectiveness of *Tegillarca granosa* at different sizes and densities on remediating the water quality of intensive *Litopenaeus vannamei* shrimp and the impact on gill damage and mortality of the shellfish themselves. This research was conducted on a laboratory scale at the Bone Marine and Fisheries Polytechnic, Bone Regency, South Sulawesi Province, Indonesia. Observations were carried out *in situ* for 7 days. Water quality parameters were analyzed at the Research Institute for Brackish Water Aquaculture and Fisheries Extension. Gill histology observations were carried out at the Main Veterinary Centre, Maros. The decrease in dissolved total organic matter indicates the effectiveness of the shellfish as filter feeders in absorbing organic matter, both particulate and suspended. Small-sized shellfish are more susceptible to damage from exposure to excessive organic and inorganic compounds, suspended and dissolved in the water, compared to the bigger ones. The deaths of shellfish are initiated by gill damage.

INTRODUCTION

Intensive cultivation of the white shrimp *(Litopenaeus vannamei)* in ponds is an effective strategy to increase shrimp production to meet human protein needs **(Muchtar *et al*., 2021)**. Intensive shrimp pond liquid waste has a high organic material content in the form of feces, residual metabolites, as well as water sterilization chemicals, residual pharmaceutical chemicals, and residual vitamins. This technique is characterized by high stocking densities and large amounts of artificial feeding **(Lananan *et al*., 2014; Emerenciano *et al*., 2022)**, which produce large amounts of organic and inorganic waste and unassimilated feed residues **(Tian *et al*., 2018)**.

The more feed used, the greater the potential for waste disposal into water bodies **(Paena *et al*., 2020)**. If discharged and accumulated in water bodies, such waste can cause environmental pollution, decreased water quality, eutrophication, loss of biodiversity, outbreaks of aquatic diseases, and habitat destruction **(Bui *et al*., 2012; Malik *et al*., 2020)**.

Eutrophication can lead to the degradation of marine ecosystems **(Tirkaso & Gren, 2017)**. In addition, it can threaten coastal water habitats **(Williamson *et al*., 2017)**. To address this issue, various wastewater treatment methods have been applied, one of which is the effort to manage wastewater through bioremediation, a natural treatment process that utilizes biological mechanisms of organisms to reduce, degrade, detoxify, or transform pollutants in water, making them harmless to the environment and reusable by communities **(Azubuike *et al*., 2016; Abatenh *et al*., 2017; Mora-Ravelo *et al*., 2017; Verma & Kuila, 2019)**.

The bivalve *T. granosa* can be utilized as bioremediators capable of improving water quality **(Brito *et al*., 2018; Nicholaus *et al*., 2019; Retnosari *et al*., 2020; Syahrir *et al*., 2021)**. The presence of shellfish populations in aquaculture environments will affect the carbon, nitrogen, and phosphorus cycles, as well as the concentration of organic matter, total suspended solid (TSS) and total dissolved solid (TDS). *T. granosa* can effectively reduce total organic matter in intensive white shrimp cultivation from 200 to 50mg/ L **(Syahrir *et al*., 2021)**. Conversely, the concentration of these inorganic and organic compounds will also affect the anatomy and physiology of the shellfish.

The gills are the main organ of shellfish used to filter water to obtain food. In addition, the gills also function as regulators of nutrient absorption concentrations needed in the metabolic process of shellfish in the form of inorganic compounds **(Vialova & Stolbov, 2022)**. The ability of shellfish to filter waste is influenced by various factors, including their size and number in certain aquatic environments **(Filippini *et al*., 2023)**. One important indicator of the efficiency of shellfish biofilters is observing changes in water quality and the impact of existing water quality containing toxic compounds on the condition of the gills and mortality of shellfish. This study aimed to analyze the effectiveness of the size and density of shellfish *T. granosa* on remediating the water quality of intensive *L. vannamei* shrimp and the impact on gill damage and mortality of the shellfish themselves.

MATERIALS AND METHODS

1. Location

This research was conducted on a laboratory scale at the Bone Marine and Fisheries Polytechnic, Bone Regency, South Sulawesi Province, Indonesia. Observations were carried out *in situ* for 7 days, namely observing the condition of shellfish and water quality parameters (temperature, salinity, pH, dissolved oxygen (DO) and total dissolved solids (TDS)). *Ex situ* observations of nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), total organic matter (TOM) and total suspended solid (TSS) were carried out at the water quality laboratory of the Research Institute for Brackish Water Aquaculture and Fisheries

Extension (BRPBAP3), Maros Regency. The preparations were made and gill histology observations were carried out at the Main Veterinary Center, Maros.

2. Containers and test organisms

A series of 50cm x 50cm x 30cm tarpaulin tanks were used as an experimental container. An air pump was installed at the bottom of the tank to keep the water mixed and to prevent sedimentation. Each tank was filled with 50L of unaerated wastewater. The water used came from an intensive white shrimp pond of the Bone Maritime and Fisheries Polytechnic, pumped and stored before being distributed to the research containers. *T. granosa* were used as test biota, and the wastewater came from the intensive white shrimp *(L. vannamei)* pond culture medium. The shellfish were obtained from fishermen around the waters of Patte'ne Village, Maros Regency, and transported to the research location with the wet transport method. Before being placed in the experimental container, the shellfish were acclimated for 24 hours in aerated sterile seawater without food. In accordance with the research objectives, the test shellfishes were divided based on shell length measured using a digital caliper. As the tested factor, two sizes were used, namely small and large sizes, each measuring 2-3 and 4-5cm, with densities of 15 and 30 individuals per 10L of water for each size as another tested factor. The shellfish were acclimated for 24 hours in sterile, aerated seawater without food and then distributed into rearing containers according to size and density.

3. Research design

The experimental design used in this study was a factorial design with 2 factors and three replications. The treatments applied were size and density, as detailed in Table (1). K15 was the treatment with small-sized shellfishes (2-3cm) and a density of 15 individuals/10L, K30 was the treatment with small-sized shellfishes and a density of 30 individuals/10L, B15 was the treatment with large-sized shellfishes (4-5cm) and a density of 15 individuals/10L, and B30 referred to shellfishes with large size and a density of 30 individuals/10L.

Table 1. The treatments

Treatment	Replication 1	Replication 2	Replication 3
K15 (2-3cm, 15 individuals)	$K15_1$	$K15_2$	$K15_3$
K30 (2-3cm, 30 individuals)	$K30_1$	$K30_2$	$K30_3$
B15 (4-5cm, 15 individuals)	$B15_1$	$B15_2$	$B15_3$
B30 (4-5cm, 30 individuals)	$B30_1$	$B30_2$	$B30_3$

4. Water quality

Water quality was observed periodically for 7 consecutive days, both *in situ* and *ex situ*. *In situ* observations were carried out for temperature, salinity, pH, DO, and TDS parameters by applying a multi-parameter water quality test every 3 hours (01.00, 04.00, 07.00, 10.00, 13.00, 16.00, 19.00, 22.00). The other water quality parameters (in the form of NO_2, NO_3, PO_4, TOM and TSS) were observed once every day, namely at 10.00 AM, by taking water samples that were then analyzed at the Water Quality Laboratory of the Research Institute for Brackish Water Aquaculture and Fisheries Extension (BRPBAP3) using spectrophotometric methods.

5. Histological condition of gill and mortality of shellfish

Microanatomical observations were carried out on the gills, which were made in the form of histological preparations using the paraffin method and hematoxylin-eosin (HE) staining **(Hasan *et al*., 2017)** and were observed microscopically. Gill histology preparations were made at the Maros Veterinary Center, Maros Regency, South Sulawesi Province, Indonesia, at the end of the study. The observations conducted were detailed and thorough observations of the level of gill damage were conducted by comparing the histology preparations of shellfish gills that were acclimatized to sterile seawater and observing the level of gill damage of shellfish reared in intensive pond waste media at the end of the study. Microanatomical observations were made on gill samples prepared as histological sections using the paraffin method and stained with hematoxylin-eosin (HE) **(Hasan *et al*., 2017)**. The samples were then examined microscopically.

6. Shellfish mortality rate

Shellfish were visually observed periodically every 3 hours by checking their activity in the media during 1 week. Shellfish that were indicated to be dead showed physical characteristics in the form of shells that were open, would not close if given a shock, and emitted a more fishy smell. If dead shellfish were found, they were immediately removed from the rearing medium and the time of death was recorded.

7. Data analysis

Testing of water quality parameters was carried out to determine which treatment showed the highest reduction in organic matter content; they were descriptively analyzed. The criteria for the level of gill damage were based on the percentage of gill lamella damage. The damage is characterized by microanatomical abnormalities such as edema, hyperplasia, necrosis and lamellar fusion. Changes in the microanatomical structure of the gills were calculated using the following formula :

$$\text{Gill damage (\%)} = \frac{\text{Number of damaged lamellae}}{\text{Total number of animal test}} \times 100$$

The level (criteria) of gill damage was then scored (Table 2).

Table 2. Observation of gill damage

Lamellar damage (%)	Damage criteria
0	No damage
1-25	Mild damage
26-50	Moderate level of damage
>50	Severe damage occurred (dead)

The collected data were then tabulated to determine the mortality rate of shellfish per day using the following formula **(Effendie, 1977)**:

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead animal test}}{\text{Total number of animal test}} \times 100$$

Data on shellfish mortality rates were computed at the end of the study and analyzed statistically by the ANOVA test with a significance level of 5% to determine the treatment that achieved the lowest mortality from exposure to wastewater.

RESULTS

1. Water quality

Changes in several wastewater quality parameters reflect the remediation response of *T. granosa* during the study (Table 3).

Table 3. Water quality parameters during 7 days of study

Parameters	Treatment	Result							
		D0	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**
Salinity (ppt)	K15	26.20	26.67	26.87	26.90	26.80	26.67	26.67	26.70
	K30	26.20	26.67	26.80	26.90	26.77	26.67	26.63	26.63
	B15	26.20	26.70	26.97	26.97	26.70	26.77	26.67	26.77
	B30	26.20	26.67	26.83	26.90	26.97	26.97	26.97	26.97
pH	K15	7,75	7.19	7.06	7.06	7.04	7.05	7.19	7.04
	K30	7,75	7.11	6.99	7.10	7.07	7.20	7.34	7.34
	B15	7,75	7.13	7.01	7.00	7.01	7.15	7.25	7.19
	B30	7,75	7.06	6.89	6.81	7.05	7.07	7.24	7.16
DO (mg/L)	K15	8,80	2.48	1.40	0.88	0.79	1.12	1.30	1.43
	K30	8,80	1.67	0.57	0.37	0.40	1.07	1.15	1.23
	B15	8,80	2.33	1.23	0.73	0.77	1.00	1.20	0.83
	B30	8,80	0.37	0.37	0.33	0.37	0.33	0.17	0.13
NH_3 (mg/L)	K15	1,159	0.056	0.068	1.036	1.032	1.038	1.087	1.042

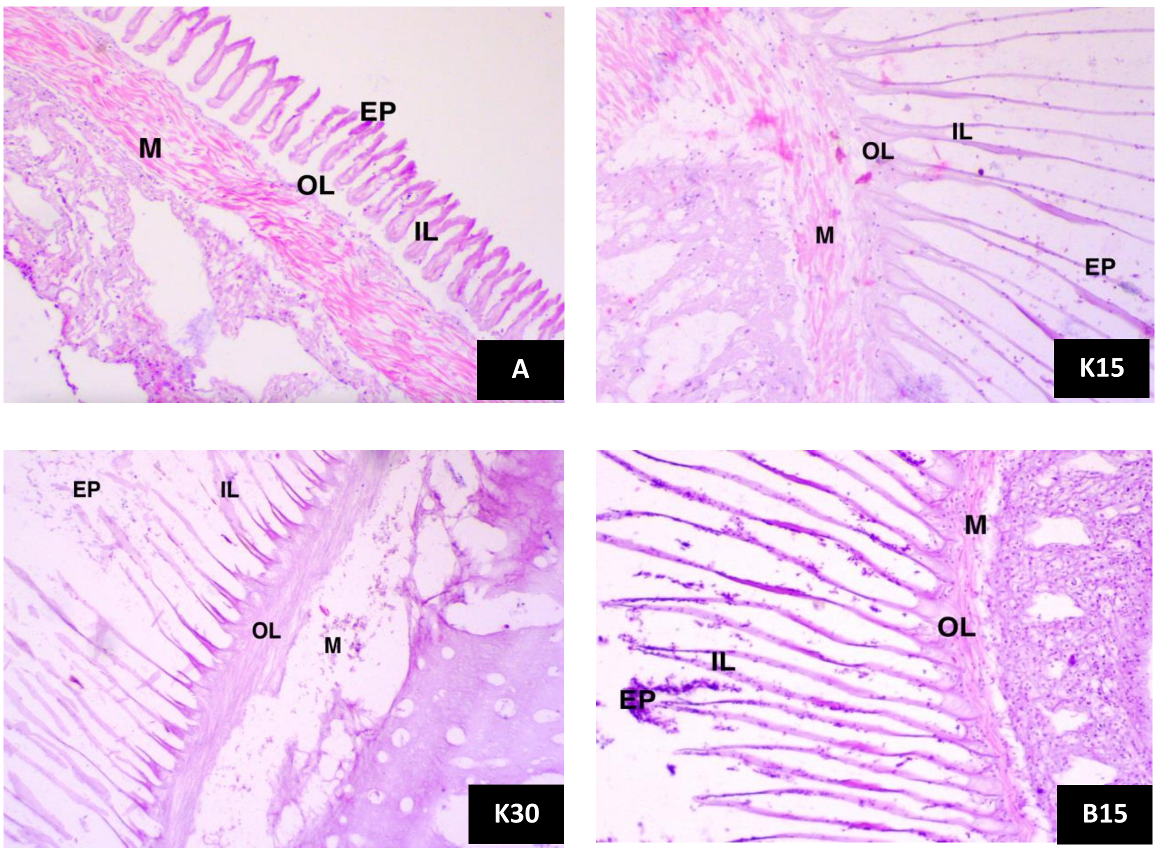
	K30	1,159	0.046	0.577	3.157	2.609	3.135	3.447	3.447
	B15	1,159	0.068	0.518	2.816	3.034	3.085	3.136	3.292
	B30	1,159	0.090	0.725	3.130	2.927	3.028	2.978	2.933
NO_2 (mg/L)	K15	7,232	3.97	5.73	6.22	6.54	5.74	6.62	6.33
	K30	7,232	5.65	5.92	3.32	3.51	2.58	3.44	2.98
	B15	7,232	5.70	6.90	5.40	5.85	2.57	1.30	1.52
	B30	7,232	6.73	3.69	1.05	1.58	2.95	1.35	1.94
NO_3 (mg/L)	K15	0,338	23.65	32.59	32.12	30.34	27.93	25.47	24.89
	K30	0,338	29.59	33.18	24.61	26.85	18.39	21.62	16.17
	B15	0,338	29.25	22.41	18.46	17.08	12.51	14.31	21.20
	B30	0,338	5.21	10.25	15.02	20.75	20.53	19.80	22.69
PO_4 (mg/L)	K15	0,021	0.03	0.03	0.05	0.04	0.04	0.04	0.05
	K30	0,021	0.04	0.04	0.06	0.06	0.06	0.07	0.06
	B15	0,021	0.02	0.02	0.03	0.05	0.05	0.06	0.05
	B30	0,021	0.03	0.04	0.05	0.05	0.05	0.05	0.05
TOM (mg/L)	K15	70,400	55.54	56.10	54.20	52.12	54.18	56.47	57.03
	K30	70,400	66.75	58.18	54.51	57.10	54.70	54.82	54.82
	B15	70,400	60.76	52.85	57.37	56.82	54.87	54.82	55.00
	B30	70,400	61.03	57.82	60.49	56.17	56.32	54.39	53.16
TSS (mg/L)	K15	18,00	43.333	53.333	57.000	60.867	50.667	47.333	55.333
	K30	18,00	37.000	62.333	71.667	74.000	77.667	60.667	67.833
	B15	18,00	47.667	37.333	56.333	39.000	47.333	36.333	39.340
	B30	18,00	16.667	68.000	69.667	66.000	61.000	65.000	61.667
TDS (mg/L)	K15	19,90	20.24	20.39	20.40	20.29	20.24	20.19	20.29
	K30	19,90	20.21	20.32	20.41	20.27	20.18	20.22	20.22
	B15	19,90	20.26	20.40	20.44	20.28	20.27	20.20	20.26
	B30	19,90	20.21	20.32	20.36	20.31	20.37	20.43	20.46

Description: D0 (initial day), D1 (1 day after stocking), D2 (2 day after stocking), D3 (3 day after stocking), D4 (4 day after stocking), D5 (5 day after stocking), D6 (6 day after stocking), D7 (7 day after stocking).

Salinity levels remained relatively stable across all treatments, with minimal daily fluctuations. pH values decreased significantly on day one in all treatments, then stabilized at around 7 for the subsequent seven days. Dissolved oxygen concentrations declined sharply from day one until the end of the study across all treatments. The decrease in ammonia concentration only occurred on day-1 and day 2; afterwards, it continuously increased until the end of the study with all sizes and densities of shellfish. A difference in the decrease of nitrite concentration was observed due to the influence of shellfish size and density factors. With bigger shellfish size, both high-density (B30) and low-density (B15) treatments showed a consistent decrease in nitrite concentration until the end of the study. In contrast, smaller shellfish showed a decrease in nitrite concentration until the end of the study only at high density (K30), whereas at low density (K15), the decrease in nitrite concentration occurred from day 2 to 5, followed by an increase in nitrite concentration (day 7). The increase in nitrate concentrations began on day 1 of the study. Although the daily fluctuations in concentration were too pronounced, the range of concentrations exceeded the tolerance limit of the survival of the shellfish. Phosphate concentrations varied slightly between treatments, with increases observed mainly in the later days. Total organic matter decreased with both densities of shellfish (K30 and K15) until the end of the study. Overall, total dissolved and total suspended solids concentration increased until the end of the study in all treatments, either with bigger or shellfish, both at high and low densities.

2. Histological condition of gill

Shellfish that were intensively exposed to white shrimp pond waste experienced damage (microanatomical structural abnormalities) to the gills as an organ that filtered water containing food for shellfish (Fig. 1).



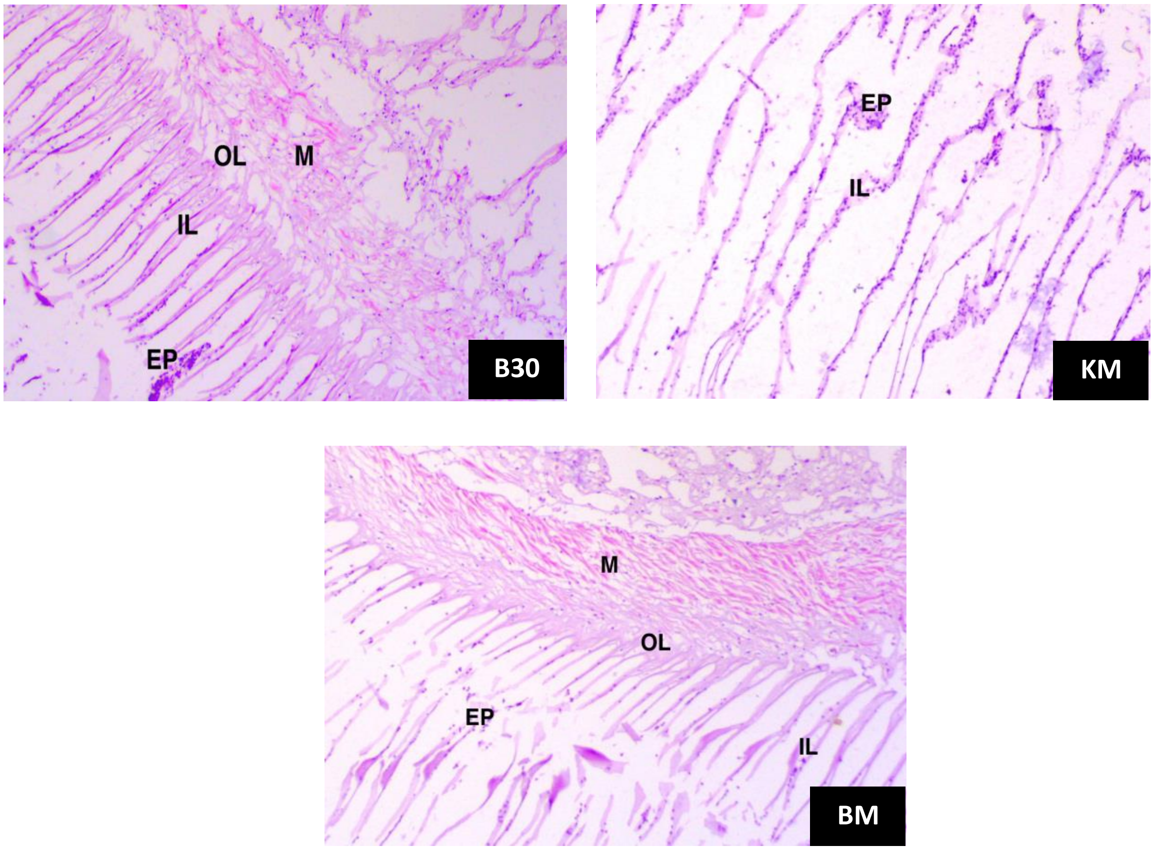


Fig. 1. Microanatomic structure of the gills

Description. IL: inner lamella, OL: outer lamella, EP: epithelium, M: muscle, A: initial shell condition (healthy or no change); B15 (living bigger living shellfishes with density 15 individuals) mild gill damage with swelling in the inner lamella and the epithelium degeneration; B30 (living bigger shellfishes with density 30 individuals) mild damage with epithelium hyperplasia; K15 (living smaller shellfishes with density 15 individuals) moderate gill damage with swelling in the inner lamella, enlargement of blood vessels occurred in the outer lamella and the epithelium necrosis; K30 (living smaller shellfishes with density 30 individuals) with moderate damage of with the epithelium was desquamated (necrotic) and there were some fusions in the inner lamella; BM (dead bigger shellfish), moderate damage with the epithelium was desquamated (necrotic) and there were some fusions in the inner lamella; KM (dead smaller shellfish)with dead shellfish with severe damage), epithelium and inner lamella were completely necrotic. The histological conditions describing the anatomical damage of the gill parts in each treatment with scores indicating the level of damage can be seen in Table (4).

Table 4. Histological condition of gills in each treatment

Treatment	**Histological condition of gills**				**Lamellar damage (%)**
	Necrotic	**Edema**	**Hyperplasia**	**Lamellar Fusion**	
A	-	-	-	-	0
B15	-	+	-	-	1 - 25
B30	-	-	+	-	1 - 25
K15	+	+	-	-	26 -50
K30	+	-	-	+	26 -50
BM	+	-	-	+	26 -50
KM	+++	-	-	-	>50

Description. (+): occurred; (-) : not occurred.

Gill damage was evident in various tissue components of the organ, notably hyperplasia, which is a thickening of the epithelial tissue at the ends of the filaments that shows a bile-like shape, which indicates swelling of the epithelium **(Maftuch *et al*., 2015)**. Hyperplasia is a condition characterized by an abnormal increase in cell proliferation within gill tissues. Severe hyperplasia is characterized by the formation of spaces between secondary lamellae, which are filled with new cells, which is also accompanied by epithelial rupture (tearing), which is characterized by the detachment of the cells from their supporting poles **(Alvarado *et al*., 2006)**. Necrotic refers to a condition where tissue or cells have undergone cell death (necrosis). Edema is a condition characterized by tissue swelling, often caused by infection. Lamellar fusion is the merging or fusion of lamellae within tissues.

No gill damage was observed in shellfishes placed in sterilized standard shrimp pond seawater (A). Small dead shellfish usually have gills with severe gill damage (Fig. 1. KM), while bigger shellfish only experience moderate gill damage (Fig. 1. BM). Gill damage in small-sized shellfish was found to range from 26 to 50%, which is characterized as moderate damage in surviving shellfish, both at low (Fig. 1. K15) and high density (Fig. 1. K30). Severe damage (exceeded 50% lamellar damage) was observed in dead smaller shellfishes (Fig. 1. KM). Histopathological changes observed in the lamellae included necrosis, edema, and lamellar fusion. There was no hyperplasia that occurred in the lamellae in this small-sized shellfish. Gill damage in bigger shellfish was observed in a range from 1 to 25%, which is characterized as wild damage in surviving shellfish, both at low (Fig. 1. B15) and high density (Fig. 1. B30). Histopathological changes observed in the lamellae included edema and hyperplasia. Moderate damage was observed in living dead bigger shellfishes (Fig. 1. BM). There was no necrosis or fusion in the lamellae in these bigger-sized shellfish.

3. Mortality of shellfish

Observations of shellfish mortality showed an increase from day to day, each of which varied in the four treatments (Table 5).

Table 5. Mortality rate of shellfish during 7 days of maintenance

Treatment	Mortality (%) +standard deviation						
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**
K15	0.44±0.77	18.67±2.31	37.78±4.07	44.00±8.74	51.11±7.58	55.11±9.46	56.44±9.08
K30	0.00±0.00	24.22±0.38	66.67±25.98	79.56±19.89	96.44±1.68	97.56±1.39	97.78±1.02
B15	0.44±0.77	9.33±1.33	18.22±5.05	24.44±8.04	34.22±10.1	36.00±9.24	40.44±7.34
B30	0.00±0.00	23.78±4.02	51.56±7.34	61.78±7.73	74.89±4.68	91.56±2.14	98.22±1.02

Description: D1 (1 day after stocking), D2 (2 days after stocking), D3 (3 days after stocking), D4 (4 days after stocking), D5 (5 days after stocking), D6 (6 days after stocking), D7 (7 days after stocking).

Although the mortality rate of the shellfish had already occurred on the first day of the study with a very low percentage (0.44%±0.77), and continuously increased until the end of the study (day 7), statistical analysis showed there was no significant effect of the interaction of size with density ($P< 5\%$) on the mortality rate of the shellfish. However, size and density singly had a significant effect on the mortality rate of shellfish on the sixth day ($P< 5\%$). The single effect of shellfish size on the mortality rate of shellfish is clearly seen, where the mortality rate is higher (55.11%±9.46- 97.56%±1.39) compared to 36.00%±9.24 - 91.56%±2.14 of the larger size. While for the single effect of shellfish density, it is apparent that higher density causes a significantly higher mortality rate (91.56%±2.14- 97.56%±1.39) compared to 9.24 - 55.11% ± 9.46 mortality at low density. On the seventh day, the mortality rates were relatively the same as those that occurred on day-6.

DISCUSSION

1. Water quality

The concentration of dissolved oxygen (DO) drastically decreased from day 1 until the end of the study, indicating a rapid decomposition process of organic matter consisting of unconsumed and undigested feed residues, feces, and other metabolic waste products contained in the intensive white shrimp pond liquid waste by decomposer microorganisms. This process continuously occurred until the end of the study. With high densities, namely 30 individuals (treatments K30 and B30), the rate of DO concentration decrease was faster compared to treatments with low densities, namely 15 individuals (treatments K15 and B15). The continued decrease in DO concentration throughout the study period until it reached a concentration below the tolerance threshold of shrimp indicates a decomposition process of organic matter in the form of uneaten and undigested shrimp feed residues in large quantities.

The concentration of NH_3, which is a product of the decomposition of feed residues and other organic matter containing protein, decreased until day 2. The concentration of NH_3 increased from day 3 until the end of the study, in line with the increasing decomposition process of dead shellfish meat, which also continued to increase during the study period. Until the end of the study, this gas continued to be oxidized through the nitrification process by nitrifying bacteria, as indicated by the increasing concentration of nitrate (the final product of nitrification) until the end of the study. A consistent decrease in nitrite concentration until the end of the study with bigger shellfish size indicates greater absorption of this organic compound into the bodies of bigger shellfish, either through the gills or through the skin.

The decrease in nitrite concentration can also be caused by the biological oxidation process of ammonia by *Nitrosomonas* bacteria in the early stages of the nitrification process before being further oxidized by Nitrobacter into nitrate. PO_4 concentrations varied mildly between treatments, with increases observed mainly in the later days across all treatments, likely due to the increased breakdown of organic matter. TOM tended to decrease in most treatments, except for B30, which showed greater fluctuations, possibly due to higher density and increasing shellfish mortality. Total organic matter decreased with both densities of shellfish until the end of the study. This decrease indicates the effectiveness of the shellfish as filter feeders in absorbing organic matter, both particulate and suspended. The process of injection of organic matter by shellfish is an active one, namely through the use of energy **(Beecham, 2008)**.

For the TDS (composed of minerals, salt, metal, and organic compounds), there was a consistent mild increase throughout the observation period, although the differences between treatments were not significant. The increase in TSS (composed of inorganic particles, e.g., sand, silt, and clay; organic particles, e.g., plant material and microorganisms; pollutants; and contaminants) can be caused by the addition of shellfish tissue, including gills and damaged shellfish meat that breaks off and releases into the water. The excess organic matter ingested and not utilized by the shellfish also will come out into the water as pseudofeces **(Gosling, 2015)**. The increase in TDS and TSS may also be contributed to by the release of organic and inorganic compounds resulting from the decomposition of dead shellfish tissues and organs.

2. Histological condition of gill

Shellfish have limited mobility for filtered water, pumping it through the mantle cavity, then using inhalant siphons to obtain food and absorb whatever is dissolved and suspended in the water and their surrounding environment **(Sekarwardhani *et al*., 2022)**, including toxic compounds, causing damage to the microanatomy of the gills. Shellfish that live in the white shrimp water media experience mild, moderate, and severe damage to the gills. Meanwhile, shellfish placed in unpolluted brackish water do not experience damage to their gills.

These gill damages can be caused by various factors, such as injury, infection, toxins, lack of oxygen and lack of nutrients. fluid imbalance, the abnormal accumulation of fluid within the gill's tissues, or environmental changes. Secondary lamella swelling (Fig. 1. K15) could be associated with lamella edema and changes in the basic mast cell architecture **(Roberts, 2002)**. Necrosis as a condition of excessive secretion (hypersecretion), which caused oxygen and nutrients to not be able to reach the epithelial cells as experienced in the K15 and K30 treatments (Fig. 1. KM), would result in gill tissue death **(Maftuch *et al*., 2015)**. The gills of shellfish measuring 2-3cm tended to suffer serious damage compared to shellfish that were bigger (4-5cm). This was thought to be because small shellfish filtered relatively more water in their feeding activities compared to large shellfish. Small-sized shellfish tended to require more food, which was followed by more water media filtration activity in the form of white shrimp pond waste. This caused small shellfish to have a higher risk of exposure to waste. Small shellfish tend to have a higher filtration frequency than large shellfish, so small shellfish have a higher level of food absorption compared to large shellfish **(Tantanasarit *et al*., 2013)**. Organic and inorganic particles will be absorbed into their bodies when shellfish filter water during their feeding activities. Thus, the more food that is filtered, the more toxic materials will enter the shellfish's body **(Sauvey *et al*., 2021)**. The compounds and substances involved were TSS and TDS, as well as ammonia gas. Ammonia levels continued to increase due to the decomposition of organic matter containing protein present in the water, in the form of uneaten and undigested shrimp feed, as well as from dead shellfish biomass. Ammonia is a water quality parameter that influences shellfish gill damage, especially in high concentrations **(Doyle *et al*., 1985; Gladwin & Kim-Shapiro, 2008; Lv *et al*., 2022)**. High ammonia concentration (exceeding 0.1mg/ L) exceeding the tolerance limit and lower DO concentration (below 2 mg/L) for survival of shellfish began to occur on day 2 of the study **(Syamsuddin, 2014)**.

3. Mortality

Significant mortality began to appear from day 2 to day 7. Several factors suspected to cause mortality which includes differences in the ability of shellfish to tolerate the organic and inorganic compounds that are absorbed into their bodies. These factors include density factors in limited water volume in addition to shellfish size **(Tan & Ransangan, 2019)**. Small-sized shellfish are more susceptible to experience damage from exposure to excessive organic and inorganic compounds suspended and dissolved in the water compared to the bigger ones.

The death of shellfish is initiated by damage to the filtering gills, which absorb compounds that are absorbed during feeding **(Panigoro & Astuti, 2007; Harith *et al*., 2016)**. In this case, these compounds are ammonia and nitrite. As a result of exposure to high concentrations of ammonia **(Doyle *et al*., 1985; Gladwin & Kim-Shapiro, 2008; Lv *et al*., 2022)** and nitrite **(Ibarrola *et al*., 2008)** exceeding the tolerance limit of shellfish, gill damage occurs as a respiratory organ, leading to death. Nitrite ions are absorbed during respiration, and then they are diffused throughout the body through the blood **(Ibarrola *et al*., 2008)**. In the blood, nitrite oxidizes hemoglobin to methemoglobin. This reduces the ability of hemoglobin to bind oxygen **(Doyle *et al*., 1985; Gladwin & Kim-Shapiro, 2008)**. Another causal factor of this mortality is the condition of hypoxia (oxygen deficiency) experienced by shellfish during the respiratory process **(Hashim *et al*., 2020; Zhan *et al*., 2022)** due to very low concentrations below the optimal concentration for shellfish survival, which is 2mg/ L **(Syamsuddin, 2014)**, and the impact is further exacerbated by damage to the gill tissues. Hypoxic conditions have occurred since the first day of exposure, especially in high-density shellfish.

CONCLUSION

The concentration of dissolved oxygen drastically decreased, and the concentration of ammonia increased until the end of the study, indicating a rapid decomposition process of organic matter consisting of unconsumed and undigested feed residues. TOM decrease indicates the effectiveness of the shellfish as filter feeders in absorbing organic matter, both particulate and suspended. Small-sized shellfish are more susceptible to damage from exposure to excessive organic and inorganic compounds suspended and dissolved in the water compared to the bigger ones. The death of shellfish is initiated by the gill damages (necrotic, edema, hyperplasia, and lamellar fusion) to the gills. These damages are due to the absorption exposure of high concentrations of ammonia and nitrite. Another causal factor of this mortality is the condition of hypoxia (oxygen deficiency) experienced by shellfish during the respiratory process.

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