

Spice-enhanced probiotic feed additive: boosting plankton abundance and water quality for a thriving aquatic ecosystem

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Abstract. Intensification of pond businesses is generally focused on artificial feed to maximize the growth and economic value of shrimp. However, additional feed triggers the emergence of new problems, one of which is a decrease in water quality. To overcome this, spice-enhanced probiotic feed additives are used which are believed to increase plankton growth as one of the indicators of water quality. The purpose of this study is to determine the effect of spice-enhanced probiotic feed additive on pond water quality. The research was conducted on 3 types of ponds: one control pond (A) and two treatment ponds (B and C) using spice-enhanced probiotic feed additives with different doses. Pond B was treated with a formulation consisting of EM4, fermentation yeast, ripe noni fruit, and garlic. Pond C received a more complex formulation combining EM4, Yakult, skim milk, turmeric, red ginger, ripe noni fruit, garlic, and fermentation yeast. Samples were taken at 5 points in each of the three ponds 4 repeats. Water quality parameters included heavy metal content (Cd (mg kg⁻¹), Pb (mg kg⁻¹)), NH₃ (mg L⁻¹), total C-organic (%), *Vibrio* bacteria (cfu mL⁻¹), and phytoplankton and zooplankton (cells m⁻³) tested by UV Vis spectrophotometry method. Sampling was carried out 3 times, namely in the preparation of ponds (before stocking fry), shrimp in the first strong feeding phase (37 days), and shrimp in the second strong feeding phase (60 days). The data was analyzed with one-way ANOVA with Tukey's post-hoc test. The results showed a significant effect ($p < 0.000 < \alpha < 0.05$) on the use of spice-enhanced probiotic feed additive on plankton abundance. An increase in the number of plankton is linearly followed by an increase in water quality, namely a decrease in the content of heavy metals Cd and Pb, ammonia, C-organic, and *Vibrio* bacteria. Thus, the use of spice-enhanced probiotic feed additives can maintain water quality and shrimp farm productivity.

Key Words: aquaculture, feed additive, pond, quality.

Introduction. The intensive development of shrimp ponds has increased in line with the high demand for shrimp (Zein et al 2023). However, significant results from the process tend to prioritize economic commerciality, which causes aquatic vulnerability and biological disasters (Rohani et al 2022). One of the impacts is the decrease in water quality due to the use of chemical compounds (Musa et al 2021). The rest of the synthetic feed material that settles in the mud sediment contributes to the formation of ammonia. The presence of ammonia in high levels can harm and cause the death of shrimp (Koyama et al 2020; Zainul Kamal et al 2022). To overcome the problem of water quality degradation, there needs to be an effort to manage shrimp ponds that support plankton growth (Paena et al 2024). The high diversity and abundance of plankton will maintain water quality and increase the productivity of shrimp ponds (Casé et al 2008). The higher the value of diversity, uniformity, and plankton dominance index, the better the water quality and the higher the shrimp cultivation production in the pond (Farkan et al 2025). This considers that plankton abundance provided a source of nutrition for shrimps to grow (Yuniarti & Nur 2020). Moreover, shrimp larvae show a stronger relationship with plankton abundance index compared to hydrographic characteristics (Pedersen & Rice 2002). Plankton also plays a crucial role in the trophic structure of ecosystems (Panikkar et al 2024).

The application of organic fertilizers in aquaculture ponds is one of the efforts to increase plankton abundance (Ritonga et al 2023). However, along with these benefits, the use of organic fertilizers can increase ammonia and nitrites (Tie et al 2024), which are toxic to shrimp. The degradation process of organic fertilizers can also reduce oxygen levels (Rashmi et al 2020). Furthermore, nutrients from organic fertilizers that are difficult to control also cause overgrowth of algae (Tie et al 2024). In addition, existing practices in the use of manure are less acceptable due to the possibility of pathogen transmission and exposure of chemical residues to consumers (Mredul et al 2022).

Another alternative effort that can be made is to use probiotics which can increase plankton abundance, N/P ratio, and total number of bacteria (Satyantini et al 2020). Furthermore, probiotics are stimulated by an increase in the N/P ratio, producing protease enzymes that are able to neutralize toxins produced by blue-green algae and dinoflagellates (Nasution et al 2021) that are harmful to shrimp. In addition, the SYNLAB Prime probiotic studied by Cheng et al (2024) has the potential to improve shrimp growth, immune function, and metabolic pathways, as well as providing valuable insights for the development of health management strategies in shrimp farming.

In addition to probiotics, the addition of spices, such as garlic (*Allium sativum*), is also considered to increase the growth of aquatic animals. Garlic contains bioactive molecules that play an important role in fisheries production in addition to its application to humans, including promoting growth, enhancing the activity of defence systems, and conferring protection against diseases (Chen et al 2021). In addition to its easy nature to mix with feed and minimal environmental impact, garlic is an effective solution to overcome diseases, improve the health of organisms through natural ingredients, and be an alternative to antibiotics (Valenzuela-Gutiérrez et al 2021). The compounds protopine, gibberellin A4, and gibberellic acid can inhibit the activity of proteins that damage shrimp (Bari et al 2025).

Considering the benefits of using probiotics and spices, the combination of the two is considered to increase effectiveness (Prakasita et al 2019). Thus, more in-depth research is needed to prove that spice-enhanced probiotic feed additives can increase plankton abundance as natural feed and shrimp pond water quality. The findings of this study are expected to provide natural alternative feed for shrimp pond farmers to not have to solely intensify the provision of expensive synthetic supplementary feed and can threaten the sustainability of shrimp pond productivity and quality. Thus, research questions include:

- RQ1: what is the diversity of plankton that are given spice-enhanced probiotic feed additives?
- RQ2: is there an effect of the use of spice-enhanced probiotic feed additive on plankton abundance?
- RQ3: is an increase in plankton counts linearly followed by an increase in water quality (NH₃, Cd, Pb, ammonia, C-organic, and *Vibrio* bacteria)?

Material and Method

Field experimental sites. This research was conducted from May to September 2022. The research was conducted on 3 ponds located in Purworejo village, Pasir Sakti District, East Lampung Regency, Indonesia. The 3 intensive shrimp ponds include: 1) one pond owned by one of the farmers, named Pond A; 2) one pond belonging to one of the farmers who is a member of the "Mina Bahari" Pond Farmer Group, which is named Pond B; and 3) one pond belonging to one of the farmers who is a member of the Pond Farmer Group "Sido Makmur", which is named Pond C (Figure 1). Pond A is not given spice-enhanced probiotic feed additive while Pond B is given spice-enhanced probiotic feed additive with limited amounts of spices; and Pond C by being given spice-enhanced probiotic feed additive with a larger number of spices. Ponds B and C were designated as experimental ponds because the farmers who manage the two ponds have used spice-enhanced probiotic feed additives. Each pond has an area of 1200 m², with a water depth of 100-120 cm. At the time of seed sowing each pond receives as many as 50,000 heads/pond. The type of water in these ponds is brackish water.

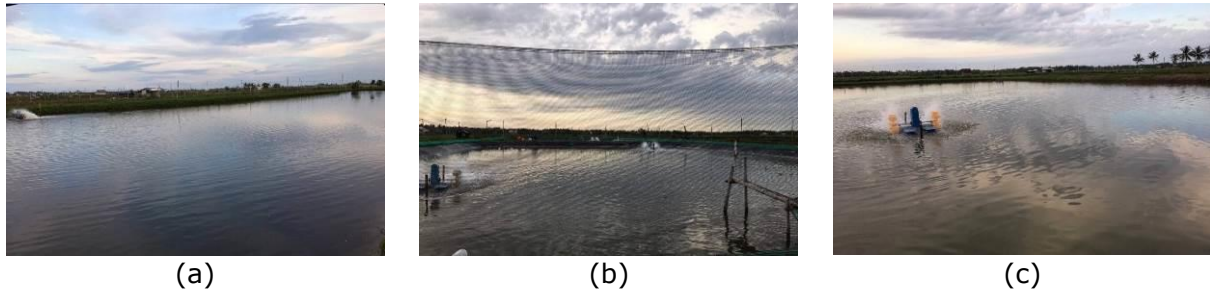


Figure 1. a) pond A; b) pond B; c) pond C.

Preparation of spice-enhanced probiotic feed additive. The research design used a complete random design. Pond A as a control pond was not given a spice-enhanced probiotic feed additive. Ponds B and C were fed with the addition of different spice-enhanced probiotic feed additives, whereas C had a greater variety of spices (Figure 2). This herb is the result of innovations carried out by shrimp pond farmers at the research site. Both herbs were selected based on the best yield, where they minimized the percentage of shrimp mortality by more than 50% before harvest time and increased the weight of the shrimp by more than 25%. Please note that the farmers involved in this study are not authors in this article. Spice-enhanced probiotic feed additives are only added to daytime feeding, where the shrimp are actively eating and the weather is usually not rainy, so it does not affect the condition of the pond water. In detail, the probiotic ingredients and spices in each pond are presented in Table 1.

Table 1

Composition of spice-enhanced probiotic feed additive formulations

Pond A	Pond B	Pond C
Not supplemented with probiotics or spices	EM4* – 250 mL Molase – 1000 mL Fermentation yeast (5 tablets, 2.5 g each) Ripe noni fruit – 2.5 kg Garlic – 250 g Well water as needed**	EM4 50 mL Molase 200 mL Fermentation yeast (1 tablet, 2.5 g) Ripe noni fruit – 200 g Garlic – 50 g Yakult*** (1 bottle, 65 mL) Skim milk – 50 g Turmeric – 50 g Red ginger – 50 g Well water as needed*

Note: * EM4 (Effective Microorganisms 4) is a liquid probiotic product used in aquaculture and fish farming. Each liter of EM4 contains *Lactobacillus casei* (10^6 CFU mL⁻¹), *Saccharomyces cerevisiae* (10^6 CFU mL⁻¹), 50 mL of molasses, and water up to 1,000 mL; ** well water was used to dilute the mixture to a total volume of 5000 mL; *** Yakult is a fermented milk drink containing the probiotic strain *Lactobacillus casei* Shirota at a concentration of approximately 10^8 CFU per 65 mL bottle. It is formulated with skimmed milk, glucose, sucrose, and water, and is intended to support digestive health by enhancing the balance of gut microbiota.



Figure 2. Spice-enhanced probiotic feed additive formulations: 1) formulation applied in pond B; b) formulation applied in pond C.

Plankton collection. Plankton sampling was conducted between 10:30 AM and 12:00 PM, which is the optimal time for plankton to conduct photosynthesis (Nashriah et al 2024). Samples were randomly collected from five points in each of pond's A, B, and C. Sampling was repeated four times at each point, resulting in a total of 20 samples. Water samples were collected using a 10-liter bucket, and from the 10 liters, 10 mL was extracted and placed into a sample bottle for laboratory analysis. Observations were made at three intervals: before stocking (W1), when the shrimp were 37 days old (first feeding phase, W2), and when the shrimp were 60 days old (second feeding phase, W3). The samples were analyzed for heavy metal concentrations (Cd in mg kg⁻¹, Pb in mg kg⁻¹), ammonia (NH₃ in mg L⁻¹), total C-organic (in %), *Vibrio* bacteria (cfu mL⁻¹), and the number of phytoplankton and zooplankton (cells m⁻³) using UV-Vis spectrophotometry.

The equipment used in this study included a plankton net, buckets, sample bottles, cool boxes, writing materials, documentation tools, droppers, adhesive tape, markers, object glass, Sedgwick-Rafter counter cell (SRC), microscope, computer, and a plankton identification book. The materials used included plankton samples, distilled water (aquades), tissue paper, and Lugol's solution.

Plankton samples were taken by taking 100-liter pond water using a 10-liter bucket 10 times. The pond water was then filtered with a plankton net and the filtered water was stored in a plankton net bucket. From the plankton net bucket, 100 mL of filtered water was taken to be put into the sample bottle and given 3 drops (3 mL) of lugol. Lugol was added to preserve plankton samples so that they are not damaged before the analysis process. The sample bottles are tucked into a cool box and taken to the laboratory for analysis and identification.

Plankton identification. The plankton identification and analysis process were carried out in the water quality laboratory at the University of Muhammadiyah Malang. Plankton were observed under Olympus BX40 microscopes using 40× magnification and the Sedwick Rafter Counter (SRC) was used solely for this purpose (e.g., Fahrur et al 2024).

Statistical analysis. The abundance data of zooplankton and phytoplankton (cells m⁻³) were analyzed using SPSS 22 to determine whether there was a significant effect, and which type of spice-enhanced probiotic was more effective in influencing plankton density. Data were analyzed using one-way ANOVA with Tukey's post-hoc test for parameters with normally distributed and homogeneous data, such as C-organic, and Kruskal-Wallis test for other parameters with non-homogeneous and non-normally distributed data.

Results. The results of plankton identification are presented in Table 2, which shows that the identified genera of phytoplankton and zooplankton each consisted of 12 genera. The total number of phytoplankton and zooplankton in pond A was lower than in pond B, with pond C showing the highest abundance (A < B < C), with even distribution across the first (W1), second (W2), and third (W3) sampling times. Furthermore, no single genus was found to be dominant. Additionally, the number of phytoplankton was greater than that of zooplankton (Table 3).

Water quality measurements of the shrimp ponds were also conducted at three different sampling times. The parameters measured included NH₃, total C-organic, Pb, Cd, and *Vibrio* spp. to assess water quality. Plankton abundance was also tested by measuring phytoplankton and zooplankton parameters (Table 3).

Based on the data in Table 3, the levels of NH₃, C-organic, Pb, Cd, and *Vibrio* spp. in pond C were always the lowest at all times of intake. This shows that spice variety influences reducing the amount of harmful substance levels. On the other hand, the number of phytoplankton and zooplankton in pond C was always higher compared to the other two ponds. Thus, it can be stated that the use of more spices is more effective in increasing plankton growth.

Table 2

Types of plankton identified in shrimp ponds A, B, and C at different sampling times (W1, W2, and W3)

Plankton	Genus	Sampling time		
		W1	W2	W3
Diatom phytoplankton	<i>Bacteriastrum</i>	A < B < C	A < B < C	A < B < C
	<i>Biddulphia</i>	A < B < C	A < B < C	A < B < C
	<i>Chaetoceros</i>	A < B < C	A < B < C	A < B < C
	<i>Eucampia</i>	A < B < C	A < B < C	A < B < C
	<i>Hemiaulus</i>	A < B < C	A < B < C	A < B < C
	<i>Nitzschia</i>	A < B < C	A < B < C	A < B < C
	<i>Rhizosolenia</i>	A < B < C	A < B < C	A < B < C
	<i>Stephanopyxis</i>	A < B < C	A < B < C	A < B < C
	<i>Thalassiothrix</i>	A < B < C	A < B < C	A < B < C
Dinoflagellate phytoplankton	<i>Ceratium</i>	A < B < C	A < B < C	A < B < C
	<i>Dinophysis</i>	A < B < C	A < B < C	A < B < C
	<i>Pyrocystis</i>	A < B < C	A < B < C	A < B < C
Zooplankton	Trocophore	A < B < C	A < B < C	A < B < C
	<i>Acartia</i>	A < B < C	A < B < C	A < B < C
	<i>Alpheus</i>	A < B < C	A < B < C	A < B < C
	<i>Balanus</i>	A < B < C	A < B < C	A < B < C
	<i>Calanus</i>	A < B < C	A < B < C	A < B < C
	<i>Corycaeus</i>	A < B < C	A < B < C	A < B < C
	<i>Evadne</i>	A < B < C	A < B < C	A < B < C
	<i>Microstellaria</i>	A < B < C	A < B < C	A < B < C
	<i>Mysis</i>	A < B < C	A < B < C	A < B < C
	<i>Oithona</i>	A < B < C	A < B < C	A < B < C
	<i>Photonectes</i>	A < B < C	A < B < C	A < B < C
	<i>Zoea</i>	A < B < C	A < B < C	A < B < C

Table 3

Water quality parameters of shrimp ponds at three sampling intervals

Time	Tested parameters	Pond		
		A	B	C
W1	NH ₃ (mg L ⁻¹) (values multiplied by 10 ⁴)	1072	1080	982
	C-organic (%) (values multiplied by 10 ²)	2000.7	1821.1	1605.6
	Pb (mg L ⁻¹) (values multiplied by 10 ⁵)	960	750	570
	Cd (mg L ⁻¹) (values multiplied by 10 ⁵)	550	400	330
	<i>Vibrio</i> spp. (cfu mL ⁻¹) (values multiplied by 10)	873	608	555.25
	Phytoplankton (cells m ⁻³)	1219.620	2874.188	3472.088
	Zooplankton (cells m ⁻³) (values multiplied by 10)	350.980	529.430	633.330
W2	NH ₃ (mg L ⁻¹) (values multiplied by 10 ⁴)	1184	1050	1008
	C organic (%) (values multiplied by 10 ²)	2259.34	1566.1	1289.51
	Pb (mg L ⁻¹) (values multiplied by 10 ⁵)	980	720	540
	Cd (mg L ⁻¹) (values multiplied by 10 ⁵)	580	390	300
	<i>Vibrio</i> spp. (cfu mL ⁻¹) (values multiplied by 10)	945	424	379
	Phytoplankton (cells m ⁻³)	829.772	4296.363	5183.342
	Zooplankton (cells m ⁻³) (values multiplied by 10)	402.38	720.33	885.79
W3	NH ₃ (mg L ⁻¹) (values multiplied by 10 ⁴)	1196	1032	981
	C Organic (%) (values multiplied by 10 ²)	2385.1	1285.9	1066.8
	Pb (mg L ⁻¹) (values multiplied by 10 ⁵)	1030	670	480
	Cd (mg L ⁻¹) (values multiplied by 10 ⁵)	580	350	270
	<i>Vibrio</i> spp. (cfu mL ⁻¹) (values multiplied by 10)	1292.5	395	357.75
	Phytoplankton (cells m ⁻³)	755.238	6015.442	7228.196
	Zooplankton (cells m ⁻³) (values multiplied by 10)	480.29	1094.62	1326.16

Furthermore, the levels of harmful substances in the pond without the spice-enhanced probiotic feed additive tended to increase (Figure 3). This contrasts with the levels of harmful substances in ponds treated with the spice-enhanced probiotic feed additive, where a decline was generally observed. However, Pond C, which received a higher dose of the spice-enhanced probiotic feed additive, was more effective in controlling harmful substances. Conversely, phytoplankton growth decreased in Pond A, while it increased in Ponds B and C (Figure 4). The increase in phytoplankton was significantly greater in Ponds B and C compared to Pond A. Nonetheless, zooplankton growth increased in all ponds, although the growth in Pond A was relatively minimal.

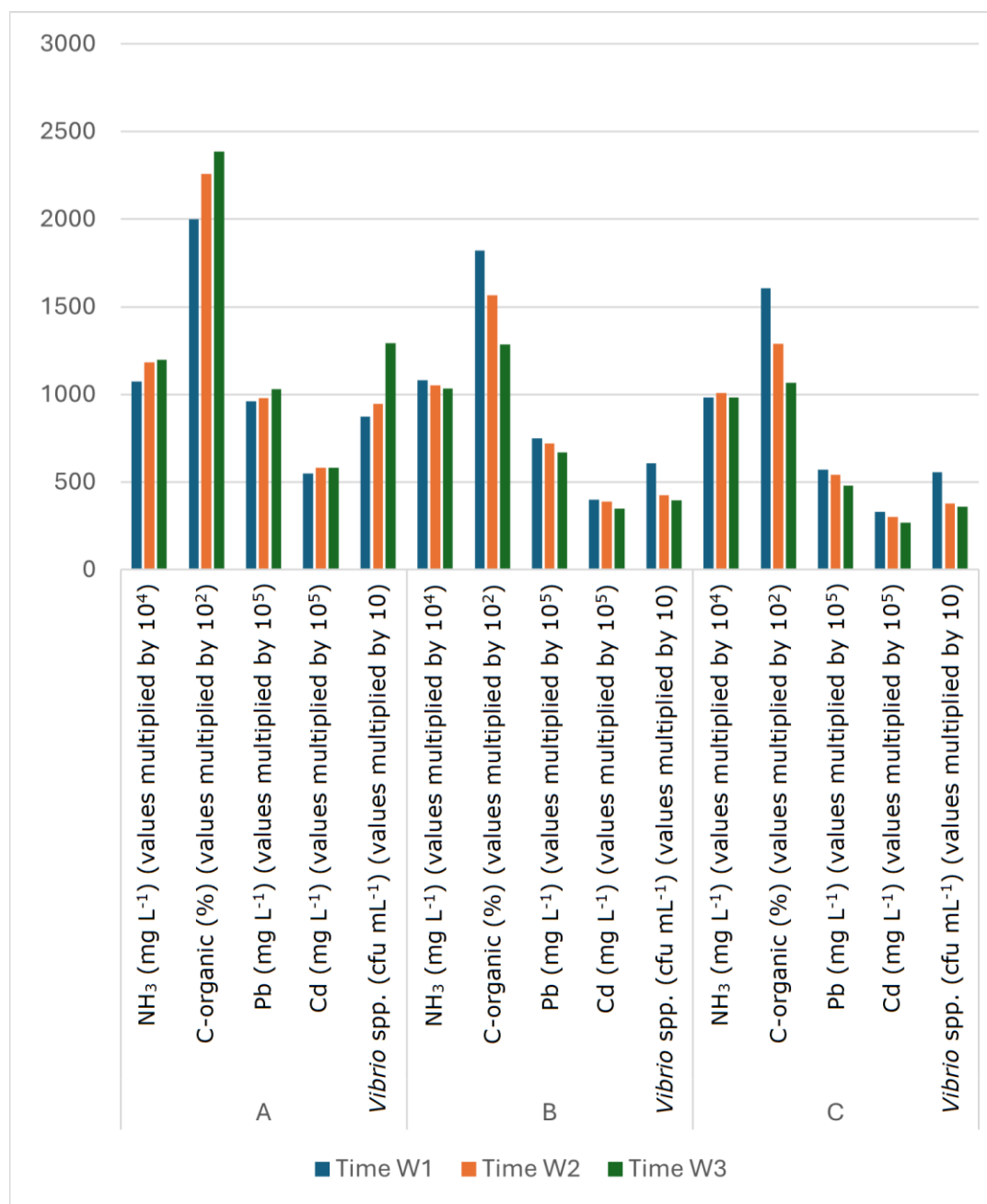


Figure 3. Comparison of the increase in harmful substance levels in ponds A, B, and C.

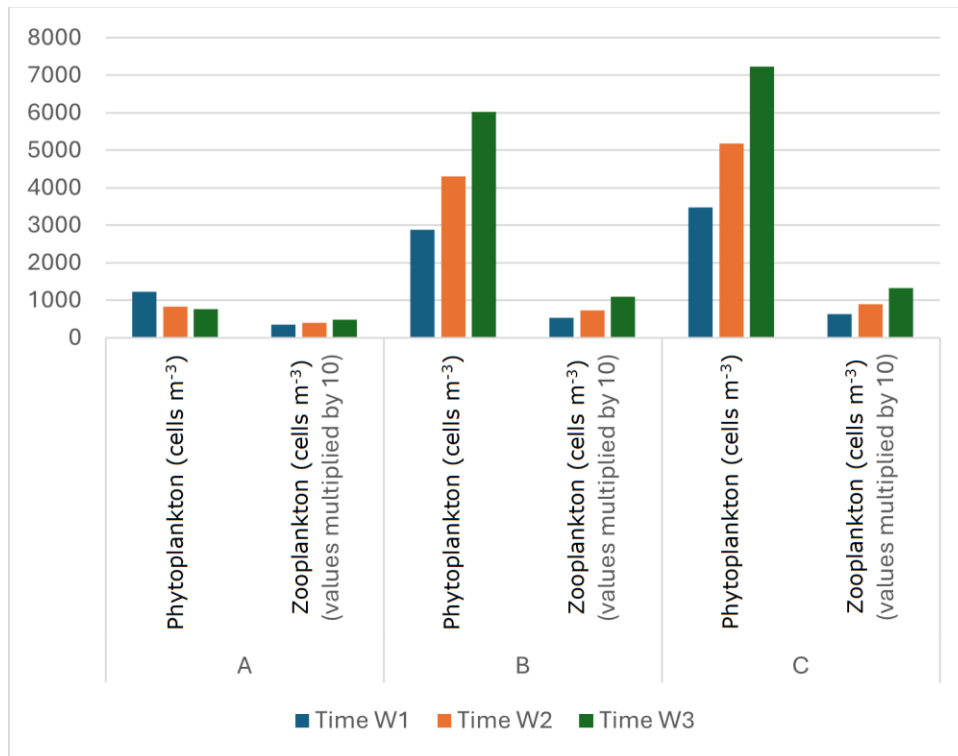


Figure 4. Comparison of phytoplankton and zooplankton in ponds A, B, and C.

Phytoplankton in pond with spice-enhanced probiotic feed additive. Based on the prerequisite test, the data was found to be non-homogeneous and non-normal, so the statistical test proceeded with the non-parametric Kruskal-Wallis test. The results of the statistical test are presented in Table 4. According to Table 4, the significance value obtained is 0.000, which is smaller than 0.05, indicating that there is a significant difference in the total abundance of phytoplankton in the water between pond A, pond B, and pond C, which differ in the use of spice-enhanced probiotic feed additive.

Table 4
Results of the non-parametric Kruskal-Wallis test: the effect of spice-enhanced probiotic feed additive on phytoplankton abundance

Phytoplankton abundance (cells m ⁻³)	Ranks		
	Pond treatment	N	Mean rank
	Pond A	60	30.50
	Pond B	60	112.14
	Pond C	60	128.86
	Total	180	
Phytoplankton abundance (cells m ⁻³)	Test statistics ^{a,b}		
	Chi-Square		122.425
	df		2
	Asymp. Sig.		0.000

a. Kruskal Wallis test; b. grouping variable: pond treatment.

Based on the results of the post-hoc test for total phytoplankton abundance in the water, treatments B and C both had a significant effect on the total phytoplankton abundance. The effect size estimation was performed by comparing the averages of B-A and C-A (Table 5). Furthermore, the data analysis from the effect size estimation indicates that treatment C had the most significant effect on the total phytoplankton abundance in the water.

Table 5

The effect size estimation results for phytoplankton

Comparison	Cohens d effect score	Effect size criteria
cohens d effect size treatment pond B-A	3.302	Small
cohens d effect size treatment pond C-A	3.341	Small

Zooplankton in pond with spice-enhanced probiotic feed additive. Based on the prerequisite tests, it was found that the data were not homogeneous and not normally distributed, so the statistical test continued with the non-parametric Kruskal-Wallis test. The results of the statistical test are presented in Table 6. Based on Table 6, the significance value obtained was 0.000, which is smaller than 0.05, indicating that there is a significant difference in the total zooplankton abundance in the water of ponds A, B, and C, which differ in the use of spice-enhanced probiotic feed additives.

Table 6

Results of the non-parametric Kruskal-Wallis test: the effect of spice-enhanced probiotic feed additive on zooplankton abundance

Zooplankton abundance (cells m ⁻³)	Ranks		
	Pond treatment	N	Mean rank
	Pond A	60	42.60
	Pond B	60	104.06
	Pond C	60	124.84
	Total	180	
Zooplankton abundance (cells m ⁻³)	Test statistics ^{a,b}		
	Chi-Square		80.832
	df		2
	Asymp. Sig.		0.000

a. Kruskal Wallis test; b. grouping variable: pond treatment.

Based on the post-hoc test results for the total zooplankton abundance in the water, both treatments B and C significantly affected the total zooplankton abundance. The effect size estimation was carried out by comparing the mean values of B-A and C-A (Table 7). Furthermore, data analysis from the effect size estimation shows that treatment C had the most significant effect on the total zooplankton abundance in the water.

Table 7

The effect size calculation results for zooplankton

Comparison	Cohens d effect score	Effect size criteria
cohens d effect size treatment pond B-A	1.559	Ignored
cohens d effect size treatment pond C-A	1.950	Ignored

Discussion

Plankton diversity in ponds A, B, and C. The results of the study (Table 2) showed that each treatment pond contained 12 phytoplankton genera and 12 zooplankton taxa (including both genera and developmental stages such as trochophore). Abundance was ranked from highest to lowest in ponds C, B, and A. These organisms were evenly distributed across water layers W1, W2, and W3, with no single genus dominating. Overall, phytoplankton counts exceeded those of zooplankton. The composition indicates that the ecosystem of ponds A, B, C is good, because there is no dominating genus, so it can be said to be dynamically stable/balanced. Khalik (2021) studied the brackish-water rice cultivation system in Maros and found that *Coscinodiscus* sp., *Ceratium* sp., and *Oscillatoria* sp. were numerically dominant at several observation stations. These

conditions affect the balance of plankton populations in the community so that there is a need to handle the abundance of certain types of plankton that are detrimental, such as *Oscillatoria* sp., *Navicula* sp., and *Nitzschia* sp. in rice and tiger shrimp cultivation ponds operated under a brackish-water, integrated rice-shrimp farming system. The dominance of some types of plankton in a community can suppress other species, which can be catastrophic for the loss of preyed species and disrupt the stability and dynamics of ecosystems.

The abundant and balanced availability of plankton is also highly beneficial for the availability of natural feed for shrimp farming products. This considers that shrimp consume natural feed more intensively during the early stages of growth, which has entered a ketogenic diet (Roy et al 2022). In the mid-growth phase, shrimp enter a balanced diet, where the introduction of cereal-based feed as an additional feed source is necessary. In the later stages of growth, it is more appropriate to conclude with a diet high in starch (cereal-based). Thus, the excess nitrogen (N) from proteins and amino acids, which are relatively easier to digest, and the insufficient energy from carbohydrate sources, are effectively met. Furthermore, most of the digested N is pumped back into the environment as reactive nitrogen (NH₄-N). The excess phosphorus (P) that is digestible per unit of digested N will trigger the elimination of digestible P by the kidneys, which is then pumped back into the environment as PO₄.

Furthermore, the presence and abundance of plankton are also influenced by the eutrophic conditions of the environment (Roy et al 2022). In eutrophic conditions, the environment is high in nutrients and supportive of the aquatic plants and animals that live in it. On the other hand, the environment in oligotrophic waters is generally clear, deep and not found with an abundance of aquatic plants and algae. The findings of this study show that the waters are in eutrophic conditions so that they support life in ponds. Thus, the waters provide great potential for the availability of natural feed for shrimp as a result of increased plankton abundance (Bi et al 2022). Furthermore, natural feed is more profitable because it guarantees better and healthier digestibility to feed. In addition, the abundance and diversity of plankton genera produced by the addition of spice probiotic feed can provide a better environment, so that the plankton in ponds B and C was more abundant than in pond A.

The influence of spice-enhanced probiotic feed additive on plankton abundance.

The results of statistical analysis (Tables 4, 5, 6 and 7) showed that there was a significant difference ($p = 0.000 < \alpha 0.05$) in shrimp ponds fed with spice probiotic supplements. This proves that spice probiotics have a good influence on the availability of plankton as natural feed and the stability of the pond environment. Natural ingredients in spices and natural microbes provided in feed create better water conditions and can even control pathogenic bacteria that can attack pond products (Nurhayati et al 2021). The findings of this study are very important to be applied considering the many cases of vibriosis found in shrimp ponds caused by *Vibrio harveyi* bacterial infection. Various efforts have been made to prevent vibriosis disease attacks, one of which is the application of technology carried out by continuously administering antibiotics to inhibit the growth of pathogenic bacteria (Okeke et al 2022) and increase shrimp immunity. However, in practice, the prolonged use of antibiotics can lead to increased bacterial resistance (Baran et al 2023). Moreover, the presence of antibiotics - whether direct or indirect - can negatively affect shrimp growth and quality (Suzuki et al 2023).

Given the various disadvantages that have caused significant losses, alternative approaches to controlling vibriosis are necessary. One promising alternative is the use of natural herbal extracts containing antibacterial compounds. *In vitro* research conducted by Nurhayati et al (2021) demonstrated the inhibition of *Vibrio harveyi* growth through a bacterial inhibition zone test using natural materials such as mangrove extract, turmeric, and garlic. This method involved measuring the clear zone around paper discs placed on a bacterial culture. The study observed that the inhibition zone diameter for turmeric was 8.68 mm, mangrove extract was 8.91 mm, and garlic extract exhibited the widest inhibition zone at 26.20 mm. All three natural extracts showed greater inhibitory effects compared to the negative control. However, the inhibition zones of mangrove and

turmeric extracts were narrower than that of the positive control (20.68 mm). These findings indicate that garlic extract is more effective in inhibiting *V. harveyi* growth than both mangrove and turmeric extracts. As reflected in the current study, differences were also observed among shrimp ponds A, B, and C. Compared to pond A, ponds B and C had a higher abundance of plankton. Meanwhile, Pond C was most overgrown with plankton because it utilized more and more varied types of spice probiotics, so that there was a synergistic mechanism in providing a plankton living environment. The existence of various kinds of spices and probiotics will cause synergistic work, mutual support in antioxidant mechanisms, as well as environmental conditions that ensure plankton life (Helmalia et al 2019).

The influence of plankton abundance on shrimp pond water quality. A linear increase in the number of plankton was also followed by an increase in pond water quality, with a decrease in the level of heavy metals Cd, Pb, ammonia, C-organic levels, and even the number of vibrio pathogenic bacteria (see Figures 3 and 4). The decrease in ammonia is strongly related to the decrease in the C-organic of the pond water. Generally, ponds with high C-organic will increase ammonia, thus making pond water growing products unhealthy, because the availability of oxygen is reduced. Probiotics in supplemental feed in pond treatment B and C have been proven to degrade C-organic, so that they can suppress ammonia which has the potential to pollute the pond environment and even kill pond product organisms. This is also in line with opinion of Koyama et al (2020) which stated that maximizing the recovery of NH₃ gas during thermophilic composting of shrimp pond sludge by bacteria provided in probiotics is an excellent and advantageous approach to producing clean nitrogen used for the cultivation of high-value microalgae, which in principle has improved the water quality of ponds. The improved quality of pond water indirectly allows the growth and development of plant and aquatic animal biota including plankton. In addition, this effort is important considering that the thermophilic bacterial communities involved in composting have a low ability to secrete enzymes that can increase the hydrolysis of shrimp pond sludge (Zainul Kamal et al 2022). Cultivated sludge, including shrimp pond sludge, is a solid waste that accumulates at the bottom of the pond and must be disposed of consistently. This thick sludge disposal creates an increased environmental burden. The recovered sludge is often dumped directly into the environment, contaminating the soil and contaminating water bodies.

The improvement in shrimp pond water quality enriched with probiotic herbs was also marked by a significant decrease in heavy metals Cd and Pb concentrations, particularly in Ponds B and C. The incorporation of garlic as an herbal additive in feed (Prasanto et al 2017) provides a range of therapeutic effects, including antibacterial, antiviral, antifungal, antithrombotic, antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, and hypoglycemic properties. The organosulfur compounds and phenolic antioxidants present in garlic play a crucial role in preventing oxidative damage to cells and organs. The phenolic compounds in garlic, which contain one or more hydroxyl groups, act as hydrogen proton donors that neutralize free radicals. Antioxidants in garlic and other herbs such as turmeric (*Curcuma longa*), red ginger (*Zingiber officinale* Roscoe var. *rubrum*), noni (*Morinda citrifolia*), and temulawak (*Curcuma xanthorrhiza*), help protect aquatic biota in pond ecosystems from oxidative stress caused by reactive oxygen species (ROS), including superoxide anions (O₂⁻), hydroxyl radicals (-OH), peroxy radicals (ROO⁻), alkoxy radicals (RO⁻), and hydrogen peroxide (H₂O₂). These ROS can damage proteins, lipids, and DNA, leading to disease and impaired growth in aquatic organisms.

The use of natural herbal ingredients rich in antioxidants can detoxify ROS generated by heavy metals through hydrogen proton donation, thereby stabilizing their reactivity. This is particularly relevant in the current global context, where heavy metal pollution - especially Cd and Pb - is of increasing concern due to its potential to pose ecological risks to aquatic organisms, communities, and ecosystems (Bai et al 2022). While the toxic effects of metals at the individual organism level are well documented, their broader ecological impacts, particularly under eutrophic conditions, are still poorly

understood (Oros 2025). Cd-induced pH reductions were found only under high nutrient conditions, suggesting that eutrophication may amplify the toxic effects of Cd on community structure and metabolic function. Shrimp ponds contaminated with heavy metals are therefore at significant risk of bioaccumulation and biomagnification, with plankton serving as the primary accumulators of heavy metals in the aquatic food web (Jana-Marini et al 2020; Wang et al 2023). Therefore, controlling plankton in the right amount and composition and is environmentally friendly, it is very important to be strived, including by providing spice probiotics, especially in ponds located in downstream areas, bordering the sea as a reservoir for pollutants.

In addition, there are continuous reports that acid waste is released into the ocean waters (Picone et al 2023). In addition, the use of water scrubber technology containing high-concentration metals, polycyclic aromatic hydrocarbons (PAHs), and alkylated PAHs, has the potential to affect plankton in recipient waters, including ponds. Toxicity tests prove a significant decrease in plankton. The decrease in plankton has many influences on the bioluminescence of bacteria (*Aliivibrio fischeri*), the growth of algae (*Phaeodactylum tricornutum*, *Dunaliella tertiolecta*), and the survival of copepods (*Acartia tonsa*). This indicates the risk of severe impacts on copepod populations which in turn can result in disruption to the overall food web.

The reduction of heavy metal levels of Cd and Pb by the effect of supplemental feeding of spice probiotics is important to consistently continue to be given that Cd and Pb pose a strong ecological risk and little is known about their adsorption mechanisms and composite risks (Liu et al 2022). To support this, Bi et al (2022) developed the Data Plankton Scope, which revealed that diurnal cycles, rainy seasons, and episodic events such as typhoons significantly affect plankton dynamics - factors that must be carefully monitored. These environmental variables not only affect the behavior and composition of plankton communities but may also influence the bioavailability and distribution of heavy metals in aquatic systems (Manic et al 2024). Consequently, any mitigation strategy involving feed additives must consider the timing and environmental conditions to ensure optimal effectiveness. Furthermore, integrating ecological data with toxicological assessments can enhance our ability to predict and manage heavy metal exposure in aquatic food webs (Sultana et al 2022). This integrated approach will be crucial in developing sustainable, ecologically responsive interventions for heavy metal pollution.

On the other hand, Gilles et al (2013) demonstrated the concept of Integrated Multi-Trophic Aquaculture, which leverages mutualistic interactions between various detritivorous species (in this context, including shrimp) and phytoplankton. Shrimp recycle nutrients by consuming live (and dead) algae, thereby providing inorganic carbon that promotes the growth of living algae. In turn, algae purify the water and produce oxygen required by shrimp. This mutualistic mechanism helps stabilize the recycling functions of artificial pond ecosystems. Hence, a symbiotic relationship is formed between the primary aquaculture product (shrimp) and surrounding biota, reinforcing ecological food chains and webs. Furthermore, the study by Martinez-Cordova et al (1998) confirmed that supplementing commercial white shrimp (*Penaeus vannamei*) cultures with natural feed sources is a more effective feeding strategy than the use of synthetic commercial feed. This approach not only supports healthier shrimp growth but also contributes to better water quality and environmental sustainability.

The reduction of pathogenic *Vibrio* bacteria is a critical factor that contributes to the increased plankton population in shrimp pond waters during observation. As noted by Dawood & Koshio (2016), intensive shrimp farming systems have evolved and are considered one of the most practical and promising tools to meet shrimp productivity demands. However, in practice, intensive shrimp farming often exposes the animals to stressful conditions that weaken their immune systems (Emerenciano et al 2022), thus increasing susceptibility to diseases. These diseases have led to significant production losses for shrimp farmers. Consequently, farmers, relying on traditional methods, treat their shrimp as extensions of themselves by using herbal probiotic and prebiotic supplements to maintain their health and vitality. Natural probiotic herbs have been shown to improve the immune responses of shrimp (Wei et al 2022), enhancing their

tolerance to various stressors and minimizing the risks associated with chemical products such as vaccines, antibiotics, and chemotherapy.

Conclusions. Based on the findings of this study on plankton abundance influenced by the application of probiotic and spice additives in shrimp pond management, the following conclusions can be drawn: 1) the phytoplankton and zooplankton found in each treatment pond consist of 12 phytoplankton genera and 12 zooplankton taxa, with no dominant genus, and the number of phytoplankton is higher than that of zooplankton; 2) the addition of spice-enhanced probiotic feed additive significantly ($p = 0.000 < \alpha = 0.05$) increased the abundance and diversity of plankton, resulting in the presence of 12 genera of phytoplankton and 12 taxa of zooplankton in a balanced composition without dominance by any particular genus. This indicates the formation of a dynamic and stable pond ecosystem; and 3) the increase in plankton abundance contributes to improved water quality by reducing the levels of heavy metals (Cd and Pb), NH_3 , total C-organic, and pathogenic *Vibrio* bacteria. Moreover, the use of more diverse and abundant probiotic and spice additives proved to be more effective in enhancing pond water quality.

Recommendations. Based on the findings of this study, the following recommendations are proposed: 1) to ensure sustainable, healthy, and productive shrimp aquaculture, it is advisable to explore and utilize local wisdom by incorporating locally sourced probiotics and spices, which have been shown to contain active compounds capable of improving and enhancing pond water quality; and 2) it is necessary to conduct laboratory analyses to determine the content and concentration of active compounds in both probiotics and spices, in order to establish accurate dosage levels for optimal efficiency and effectiveness in their application.

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