Zoobenthos composition in rotational rice-black tiger shrimp farming farms towards organic standards

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Abstract. The study was carried out to evaluate the species composition and abundance of zoobenthos in the rotational rice-black tiger shrimp farming model toward organic standards in Thoi Binh district, Ca Mau province. Water quality, sediment and zoobenthos samples were collected at 18 sites including 12 rice-shrimp farms and 6 canals. The rice-shrimp farms were divided into 3 groups: group N1 (conventional model), group N2 (probiotic supplement) and group N3 (probiotic supplement + additional feed). Each group of N1, N2 and N3 was repeated four times. The study was conducted from February to December 2022 with 4 sampling periods, including 2 times in the shrimp crop (beginning and end of the crop) and 2 times in the rice crop (beginning and end of the crop). The results showed that the water environment and sediment factors were suitable for the zoobenthos development. Contents of total nitrogen (TN), total phosphorus (TP) and total organic matter (TOM) in the sediment were relatively high and did not differ significantly between sampling locations. The silt and clay percentages in the sediment were 95.0±2.6% and 5.0±2.6%, respectively. The study identified a total of 36 zoobenthos species belonging to 36 genera, 26 families, 18 orders, 5 classes, and 3 phyla. At the collection sites, the zoobenthos species number ranged from 8 to 25 species. Average density and biomass of zoobenthos varied from 162±238 to 5,848±3,391 ind m^{-2}, and 8.2±11.4 to 565.0±360.8 g m^{-2}, respectively. In the rice-shrimp farms, the species composition, density and weight of zoobenthos in the shrimp crop were higher than that in the rice crop. Gastropoda dominated in all N1, N2 and N3 groups, while Bivalvia accounted for the highest proportion in canals of the study area. The average Shannon-Wiener (H'), Simpson diversity (D) and Pielou evenness (J') indices ranged from 0.3 to 1.9, 0.1 to 0.8 and 0.2 to 0.9, respectively, showing zoobenthos diversity from low to medium at the collection locations. The zoobenthos species composition and density were significantly correlated ($p < 0.05$ and $p < 0.01$) with environmental factors and sediment characteristics in the study area. The use of probiotic and feed supplement in the rotational rice-black tiger shrimp farming models contributed to creating a natural food source for shrimp to grow and develop.

Key Words: abundance, diversity indices, rotational rice-black tiger shrimp farming model, species composition, zoobenthos mass.

Introduction. Ca Mau has great potential and advantages in aquaculture development, especially brackish water shrimp farming. In which, rotational rice-black tiger shrimp (*Penaeus monodon* Fabricius, 1798) farming following organic standards is considered a sustainable, low-risk, and environmentally friendly model. In this culture system, land is used for rice cultivation in the rainy season, and when the salinity of the water increases in the dry season, it is converted to shrimp farming. Shrimp farming and rice cultivation in rotation will help improve the water quality, create a natural food source for shrimp, and increase soil fertility. In addition, the rotational rice-black tiger shrimp model can interrupt the development of pathogens on shrimp or shelter insects harmful to rice. In the shrimp farming process, natural food sources play an important role in the growth of shrimp, especially the zoobenthos population. Their lives are linked to the sediment of the water body. Benthic organisms have very important links in the food web and are capable of purifying water and being used as indicator organisms in water quality assessment (Bai 2005). Changes in organic matter content in the sediment are mainly due to the influences of agricultural production activities. This thing can significantly change the species composition and abundance of zoobenthos (Shieh et al 1999). Benthic organisms are one of the most popular indicators used in biological monitoring (Barbosa et al 2001; Thanh et al 2002). According to Allan et al (1995), shrimp have a benthic living habit, except for the larval and post-larval stages, so benthic macroinvertebrates are considered the main food source for shrimp. The main benthic groups were collected during the study period in shrimp farms and dominated by polychaetes followed by bivalves, gastropods, decapods and amphipods (Varadharajan & Soundarapandian 2013). Studies have found that the adding of probiotics to aquaculture water can reduce harmful substances in the water environment and decompose large particulate organic matter, thus purifying water quality (Mohammadi et al 2021). These things can promote the development of beneficial organisms as natural food for aquatic animals. Therefore, the use of probiotics in the rotational rice-black tiger shrimp culture model can improve the water environment and increase the live food composition for shrimp. Due to the advantages of the above-mentioned zoobenthos, this study was carried out to determine the diversity of zoobenthos species composition, contributing to managing natural food in the rotational rice-black tiger shrimp farming system following organic standards.

Materials and Methods

Sampling time and location. The study was conducted from February to December 2022. Qualitative and quantitative zoobenthos samples were collected at 12 shrimp-rice farms and 6 canals surrounding the study area in Thoi Binh district, Ca Mau province, Vietnam. The rice-shrimp farms were divided into 3 groups: group N1 (conventional model), group N2 (probiotic supplement), and group N3 (probiotic supplement + additional feed). Each group of rice-shrimp farms was repeated four times. The shrimp-rice farm areas ranged from 1.5 to 2.7 ha. Probiotics used in the shrimp-rice farms were mainly composed of *Bacillus* sp. in powder form (density of *Bacillus* sp. > 10^8 CFU g^{-1}) and incubated before fertilizing in rice-shrimp farms. The additional dosage was 20 liters ha^{-1}. Shrimp were stocked at a density of 3 shrimp m^{-2}.

In group N1 shrimps were raised by conventional culture system, not following organic standards. In group N2 shrimps were cultured following organic standards. During the farming process, shrimp farms were periodically supplemented with probiotics (5L for every 10.000 m^3 per times) every 7-15 days, and shrimp were not additionally fed. In group N3 shrimp farming procedure was carried out similarly to group N2. In addition, shrimp were supplemented with food of organic origin, such as in-farm trash fish (mainly tilapia, feeding rate depending on shrimp size) and rice germ, during the culture process. Group 4 includes water supply canals and rivers: a total of 6 sites were collected, including 5 locations in supply canals surrounding the rotational rice-black tiger shrimp area, providing water for the shrimp farms. These sites are 5-150 m away from the shrimp farms. In addition, the study also collected one location on a large river (the Trem River), about 5-6 km from the shrimp farms.

There were four sampling times in total, including two times (beginning and end of the rice crop) in the rice crop and two times (beginning and end of the shrimp crop) in the shrimp crop. Sampling locations in the rotational rice-black tiger shrimp system are shown in Figure 1.

Water quality parameters. Sampling and analyzing methods of water quality parameters are shown in Table 1.

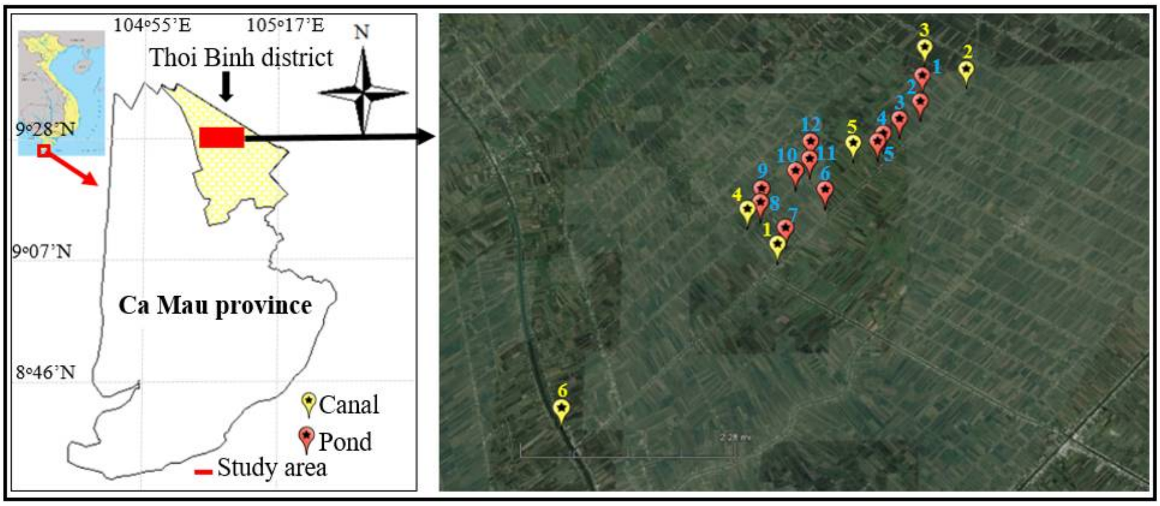

Figure 1. Sampling sites in the study areas.

Table 1

Sampling method and analyzing of water quality parameters

No.	*Parameters*	*Sampling method and preservation*	*Analysis method*
		Water sample	
1	Temperature (°C)	Direct measure	Hana Multiparameter HI9828
2	pH	Direct measure	Hana Multiparameter HI9828
3	Salinity (‰)	Direct measure	Hana Multiparameter HI9828
4	Transparency (cm)	Direct measure	Secchi disk
		Bottom sediment sample	
5	% TOM	500 g of sediment, preservation at 4°C for TOM, soil texture, TN, and TP parameters	Loss-on-ignition method (Mudroch et al 1997)
6	Soil texture		Estimating soil texture (Whiting et al 2016)
7	TN ($mg\ g^{-1}$)		4500-N C-persulphate method (APHA 2017)
8	TP ($mg\ g^{-1}$)		4500-P B-persulphate method (APHA 2017)

Note: TOM = total organic matter; TN = total nitrogen; TP = total phosphorus.

Qualitative and quantitative sampling methods of zoobenthos. Qualitative and quantitative samples of zoobenthos were collected using a Petersen grab (MRC 2010; Nocentini 1989) with a grab mouth area of 0.03 m^2. At each location, zoobenthos samples were collected from a total of 10 grabs at various locations in rotational rice-black tiger shrimp farms, rivers, and supply canals. Then, the sample was put into a bottom sieve with a mesh size of 0.5 mm and screened to remove impurities and trash. After being collected, the sample was placed in a nylon bag and fixed with formalin at a concentration of 8-10%.

Analysis method. The species composition of zoobenthos in rice-shrimp farms was identified by the morphology analysis following publication of taxonomic documents, namely Yunfang (1995), Sangpradub & Boosoong (2006), Bouchard (2012) and Madsen & Hung (2014). Zoobenthos abundance was calculated according to the following formula:

$$D = X / S$$

where: D is the zoobenthos density (ind m^{-2}) or biomass (g m^{-2}), X is the number of individuals counted, S (S = n x d) is the sampling area (m^2), n is the grab number and d is the area of the grab mouth.

Biological indices. Biological indices, including the Shannon-Weiner diversity index (H'), Simpson diversity index (D), and Pielou evenness index (J), were calculated using PRIMER 6.1.5 software:

$$\text{Shannon-Weiner diversity index: } H' = -\Sigma Pi \, . \, LnPi$$

where: Pi = ni/N (ni is the individual number of species i, and N is the total number of all individuals in the sample).

$$\text{Simpson diversity index: } D = 1 - (\Sigma n(n-1))/N(N-1)$$

where: n is the number of individuals of one species, and N is the total number of all individuals in the sample.

$$\text{Pielou's evenness index: } J' = H'/(LnS)$$

where: S is the total number of zoobenthos species, and H' is the Shannon-Wiener diversity index.

Data analysis. Relationships (Pearson) between water quality parameters with species composition and density of zoobenthos were analyzed by SPSS 22.0 software.

Results

Zoobenthos in the rotational rice-black tiger shrimp farms. The zoobenthos species composition in the study area recorded 36 species belonging to 36 genera, 26 families, 18 orders, 5 classes, 3 phyla. In which, the molluscs phylum had the highest species number with 25 species (69%), followed by the annelid phylum with 6 species (17%) and the lowest one was the arthropod phylum identified 5 species (14%) (Figure 2). The total number of benthic fauna species in the shrimp crop and rice crop reached 29 species and 26 species, respectively. The species number of Bivalvia, Polychaeta and Insecta in the shrimp crop was higher than that in the rice crop. In contrast, Gastropoda and Malacostraca had lower species numbers in the shrimp crop than that in the rice crop (Figure 3). The study revealed that out of a total of 36 identified species, there were 19 species distributed in both shrimp and rice crops in the study area. In addition, the study also found that there were 10 benthic macroinvertebrates species distributed in the shrimp crop without in the rice crop, such as Libellulidae larvae, *Anadara granosa*, *Limnoperna supoti*, *Arcuatula senhousia*, *Marphysa* sp., *Nucula nitidosa*, *Capitella capitata*, *Margarya* sp., *Barbatia* sp. and *Namalycastis longicirris*. Zoobenthos species, such as *Gomphina melanaegis*, *Exopalaemon styliferus*, *Lymnaea swinhoei*, *Haloa yamaguchii*, *Anomalocardia squamosa*, *Indoplanorbis exustus* and *Filopaludina sumatrensis* were only distributed in the rice crop and they were not found in the shrimp crop through sampling period.

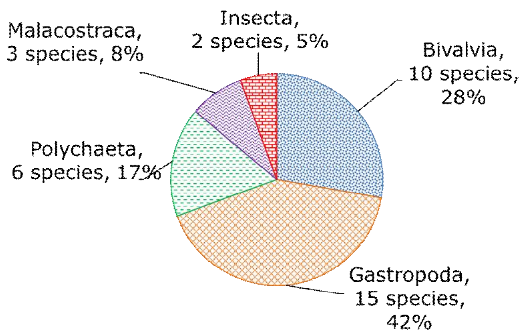

Figure 2. Structure of zoobenthos species composition in the rotational rice-black tiger shrimp farming farms.

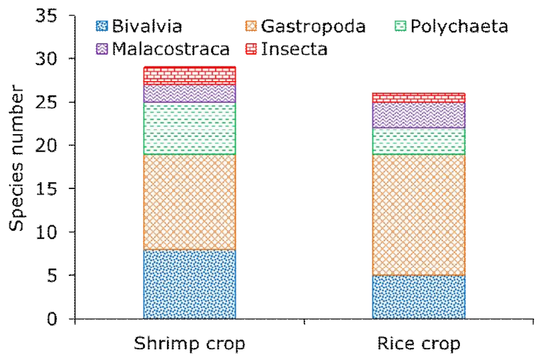

Figure 3. Zoobenthos species composition in the rice and shrimp crops.

Zoobenthos species composition of rice-shrimp groups and canals surrounding study area. Zoobenthos species composition varied relatively high between the rice-shrimp farm groups and the canals, ranging from 13 to 33 species. The highest number of zoobenthos species was found in the canal whereas the lowest one was in group N1. In most rice-shrimp farms, Gastropoda class had the highest percentage, ranging from 8 to 15 species, followed by Bivalvia (3-10 species). The remaining classes, including Polychaeta, Malacostraca and Insecta, varied from 1 to 2 species (Figure 4).

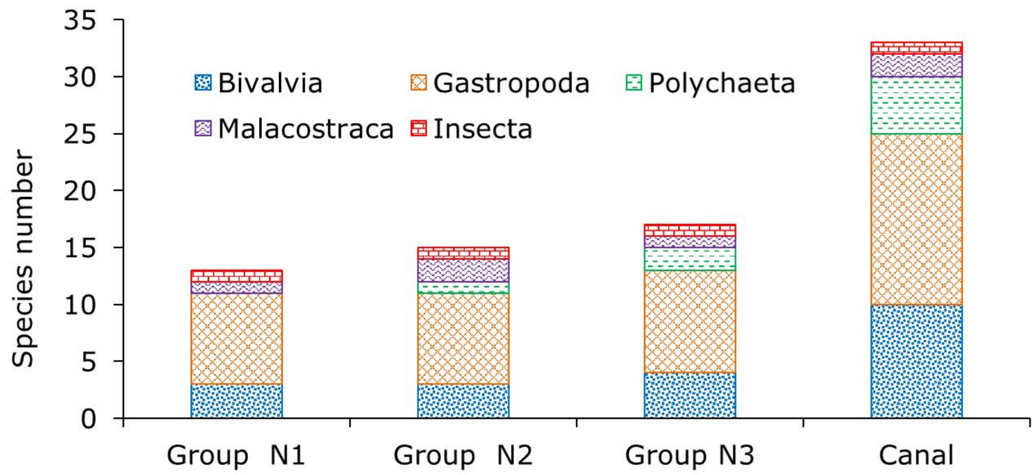

Figure 4. Total number of zoobenthos species in the rice-shrimp groups and canals in the study area.

In general, the species number belonging to classes of the Bivalvia, Gastropoda and Polychaeta in the surrounding area were higher than that in the rice-shrimp farm groups. Furthermore, the Malacostraca species composition was higher in groups N2 and canal than those in groups N1 and N3. Insecta, on the other hand, had a similar number of species across all survey groups. Each group only had one species recognized. The canals had more species of the Bivalvia, Gastropoda, Polychaeta, and Malacostraca classes than that in all groups N1, N2, and N3.

Variations of zoobenthos species composition during the sampling period. The total number of benthic macroinvertebrates through sampling periods in the study area ranged from 8 to 25 species. In group N1, the number of zoobenthos species increased the most at the beginning of the shrimp crop (13 species) and decreased the least at the end of the rice crop (8 species). Among them, Gastropoda accounted for the highest ratio in all the sampling times of both the shrimp and rice crops, and ranging from 5 to 8 species. The other groups, including Bivalvia, Malacostraca, and Insecta, were relatively few, changing from 1 to 3 species, while Polychaeta did not find any species during the sampling period (Figure 5).

In the group N2, the species number of zoobenthos varied from 9 to 13 species. Gastropoda species were more diverse than the others through sampling periods in both shrimp and rice crops, ranging from 5 to 8 species. The other classes, including Bivalvia, Malacostraca, and Insecta, had species numbers varying from 2 to 3 species, 1 to 2 species, and 0 to 1 species, respectively. In addition, Polychaeta only found one species at the end of the shrimp crop (Figure 5). Similarly, in group N3, the number of zoobenthos species across survey stages ranged from 8 to 14 species. Zoobenthos species composition was the highest at the end of the shrimp crop and the lowest at the end of the rice crop. Gastropoda also had the highest number of species in both shrimp and rice crops, ranging from 5 to 8 species. The species number of the Bivalvia class changed from 2 to 3 species. The remaining groups, such as Malacostraca, Polychaeta, and Insecta, each recorded only 1 species during the survey (Figure 5).

The species number of zoobenthos in the canals through sampling periods was higher than that in the rice-shrimp farms, ranging from 15 to 25 species. Their species composition tended to rise at the beginning of the shrimp crop and decline at the end of the rice crop. The Gastropoda population accounted for the highest proportion across sampling periods, varying from 10 to 13 species. The classes of Bivalvia, Polychaeta, and Malacostraca had species numbers changing from 1 to 6 species. In addition, Insecta only detected one species at the beginning of the shrimp crop (Figure 5).

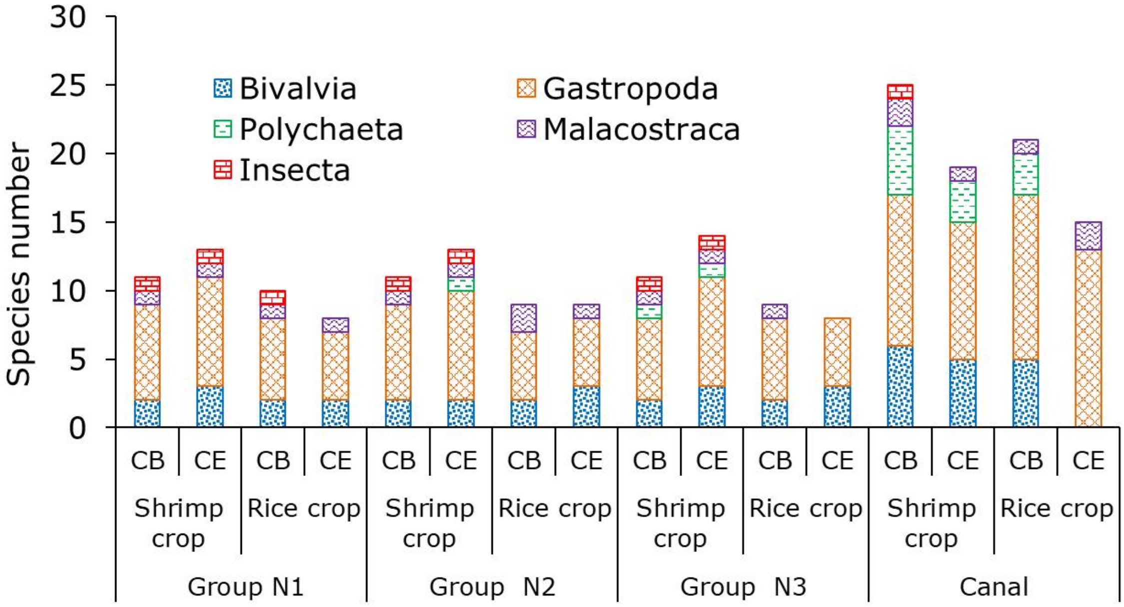

Figure 5. Varying on zoobenthos species composition during the sampling periods (CB: crop beginning; CE: end of the crop).

Abundance of zoobenthos in the rotational rice-black tiger shrimp farming areas. Zoobenthos density in rice-shrimp farms through sampling periods was generally much lower than that in canals, ranging from 162±238 to 5,848±3,391 ind m^{-2} (Figure 6). In group N1, the density ranged from 267±194 to 928±631 ind m^{-2}. Gastropoda dominated the study stages in both shrimp and rice crops, varying from 255±183 to 844±541 ind m^{-2}. Zoobenthos abundance was the highest at the beginning of the shrimp crop and the lowest at the end of the rice crop. The Bivalvia, Malacostraca, and Insecta classes had densities ranging from 1 to 35 ind m^{-2} across surveys. Similarly, zoobenthos abundance in group N2 tended to decrease from the beginning of the shrimp crop to the end of the rice crop, fluctuating from 162±238 to 1,098±1,138 ind m^{-2}. The study found that zoobenthos reached the highest density at the starting shrimp crop, with a predominance of Gastropoda (1,052±1,093 ind m^{-2}). The classes of Bivalvia, Polychaeta, Malacostraca, and Insecta were not abundant during the survey; their densities ranged from 0 to 25 ind m^{-2} (Figure 6).

In the group N3, zoobenthos densities varied from 310±256 to 767±885 ind m^{-2} and tended to decline from the start of the shrimp crop to the end of the rice crop. Throughout the shrimp and rice crop sampling periods, Gastropoda accounted for the largest proportion among them, with a range of 284±243 to 638±691 ind m^{-2}. The groups of Bivalvia, Polychaeta, Malacostraca, and Insecta had densities changing from 0 to 103 ind m^{-2} during the survey (Figure 6). The density of insects obtained a high value in shrimp crop in group N3 (Figure 9). The densities of zoobenthos in the canals were significantly higher than their abundance in the rice-shrimp farms. The benthic macroinvertebrates abundance in canals ranged from 2,919±2,135 to 5,848±3,391 ind m^{-2}. In which, Bivalvia, with a range of 1,498±1,106 to 3,543±2,867 ind m^{-2}, dominated during sampling periods. The density of the Gastropoda was also exceptionally high in canals, ranging from 1,392 to 2,327 ind m^{-2}. The densities of Polychaeta, Malacostraca, and Insecta groups fluctuated between 0 and 26 ind m^{-2} over the course of the study, which was considered as relatively low densities (Figure 6, Figure 7). In addition, the present study also found that Polychaeta presented in groups of N2 and N3, but their density was relatively low (Figure 8).

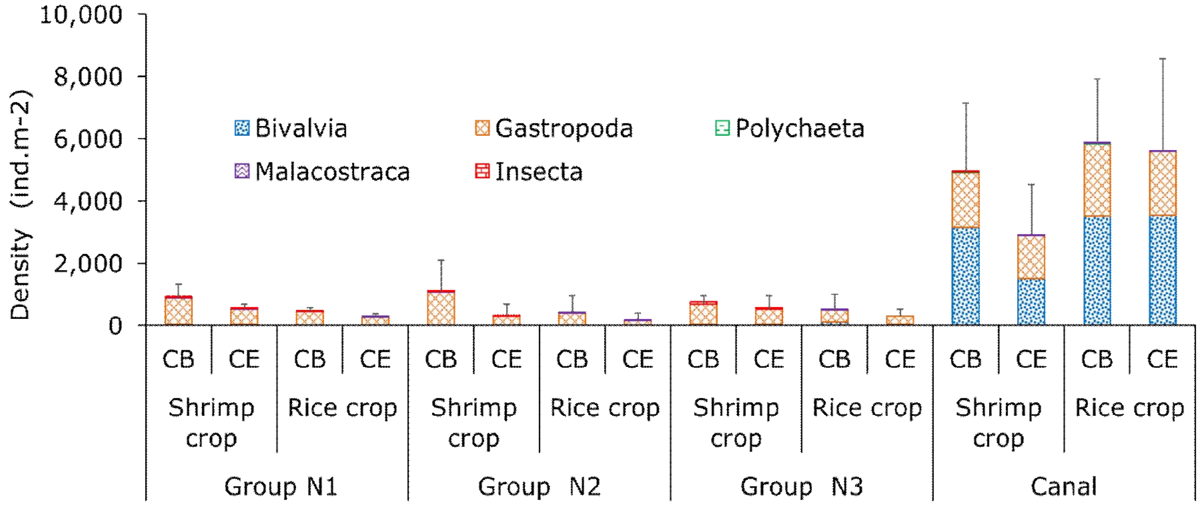

Figure 6. Variation of zoobenthos abundance during the sampling periods.

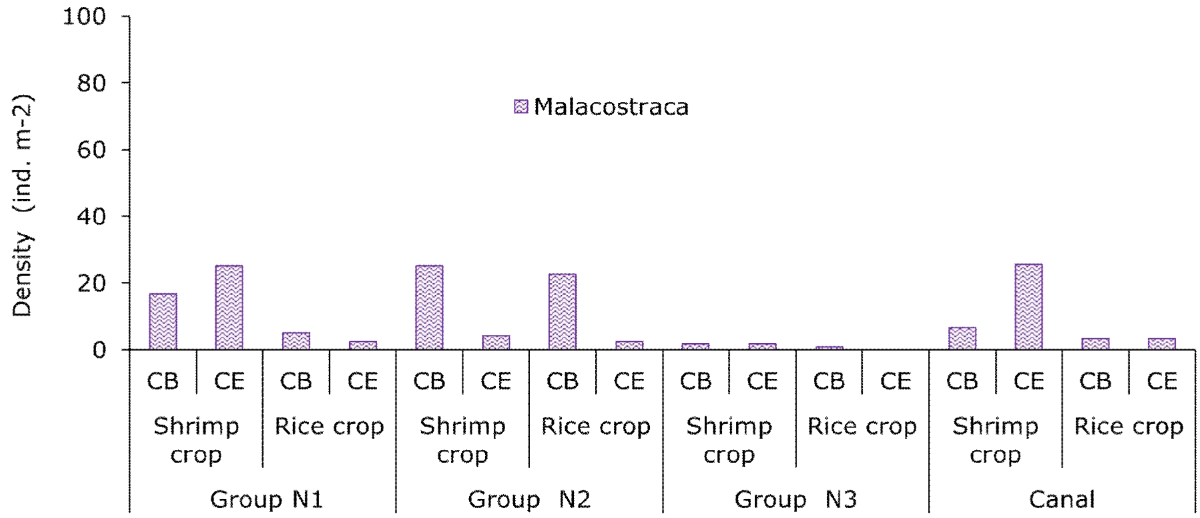

Figure 7. Variation of Malacostraca abundance during the sampling periods.

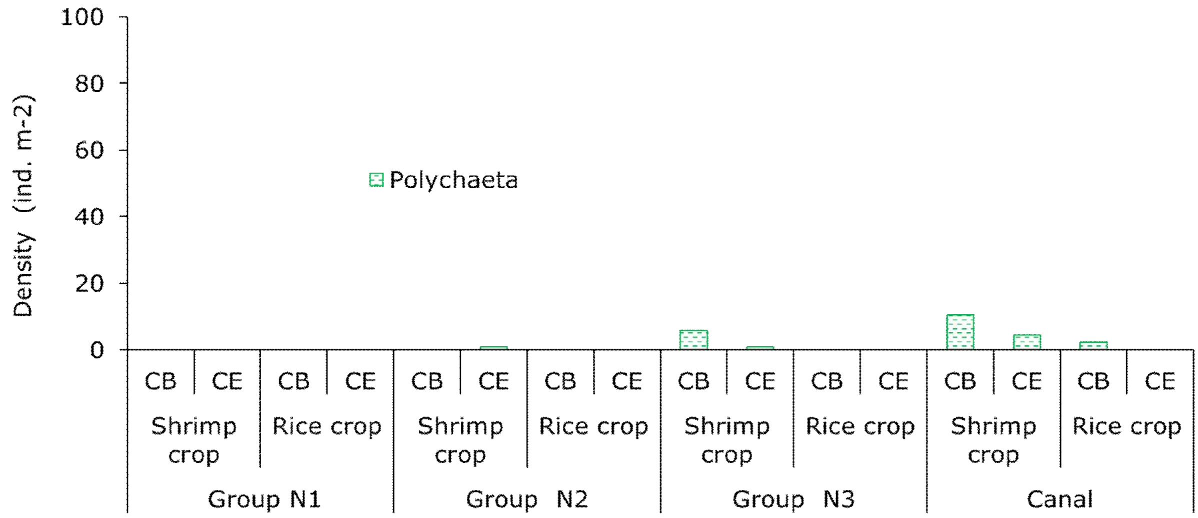

Figure 8. Variation of Polychaeta abundance during the sampling periods.

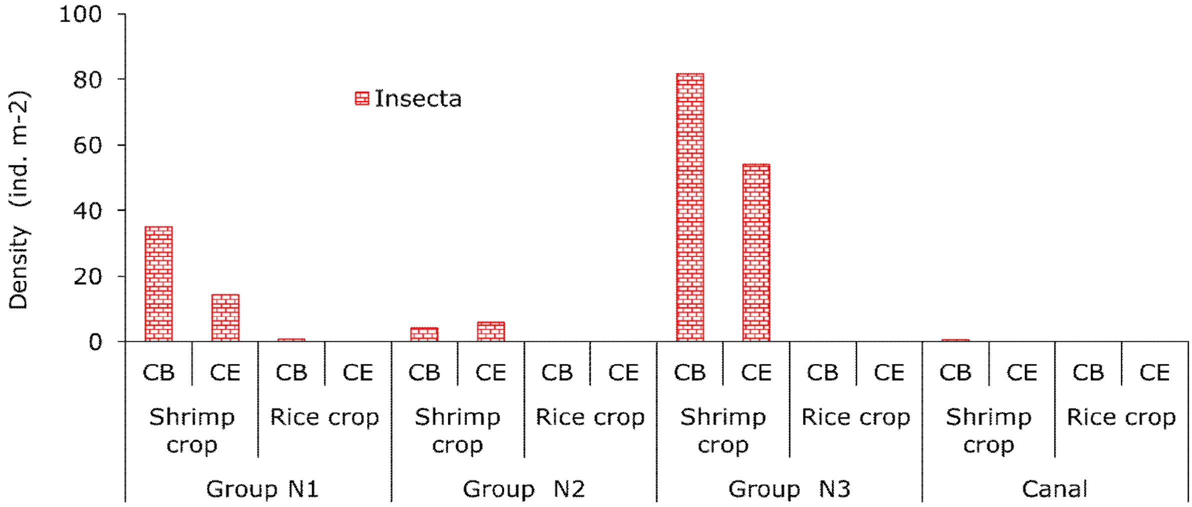

Figure 9. Variation of Insecta larvae abundance during the sampling periods.

Biomass of zoobenthos in the rotational rice-black tiger shrimp farming areas. The weight of zoobenthos through sampling periods in the study area ranged from 8.2±11.4 to 565.0±360.8 g m^{-2}. The mass of zoobenthos was the highest in the canal and the lowest in the group N2 at the end of the rice crop (Figure 10). In the group N1, the weight of zooplankton tended to decrease from the shrimp crop to the rice crop and ranged from 19.0±10.3 to 55.1±36.5 g m^{-2}. In which, the Gastropoda mass accounted for the highest proportion over the sampling periods, ranging from 18.2±9.7 to 55.1±36.5 g m^{-2}. The Bivalvia, Malacostraca, and Insecta classes had very low masses, ranging from 0 to 1.5 g m^{-2} during the survey (Figure 10). Similarly, the zoobenthos weight in group N2 reduced from the beginning of the shrimp crop to the end of the rice crop and ranged from 8.2±11.4 to 81.8±85.6 g m^{-2}. Among them, the mass of Gastropoda was higher than other groups in most sampling periods of both shrimp and rice crops, ranging from 8.0±11.1 to 81.7±85.5 g m^{-2}. Their density was the highest at the beginning of the shrimp crop and the lowest at the end of the rice crop. The groups of Bivalvia, Polychaeta, Malacostraca, and Insecta had very low masses, varying between 0 and 1.0 g m^{-2} during the sampling periods (Figure 10).

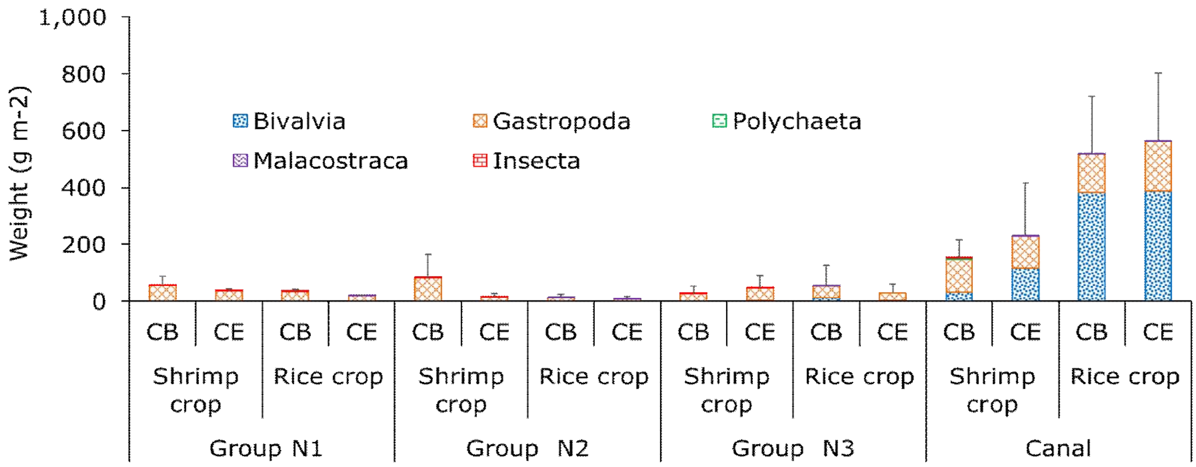

Figure 10. Biomass of zoobenthos during the sampling periods.

In the group N3, the mass of benthic macroinvertebrates ranged from 27.8±28.8 to 54.5±72.1 g m^{-2}. In which, the Gastropoda weight had the highest percentages across the sampling periods of both crops, ranging from 26.7±30.8 to 44.4±50.2 g m^{-2}. The mass of the Bivalvia, Polychaeta, Malacostraca, and Insecta classes changed between 0 to 12.7 g m^{-2} (Figure 10). In the canal, the mass of benthic community in the shrimp crop was lower than in the rice crop, ranging from 153.2±95.9 to 565.0±360.8 g m^{-2}. In which, the weight of Bivalvia was the highest in the rice crop, ranging from 31.3±21.3 to 387.6±230.0 g m^{-2}. Gastropoda also had quite a high mass, fluctuating from 113.4 to 176.8 g m^{-2}. Gastropoda had a relatively high mass, ranging from 113.4 to 176.8 g m^{-2}. Gastropoda in the canals weighed more than Bivalvia at the start of the shrimp culture. During the survey, the Polychaeta, Malacostraca, and Insecta groups had relatively low masses, ranging from 0 to 3.7 g m^{-2} (Figure 10).

***Biological indices**.* Benthic macroinvertebrates species composition diversity was shown by the Shannon-Weiner diversity (H'), Simpson (D), and Pielou evenness (J) indices (Figure 11). The H' index across sampling periods in the study area ranged from 0.3 to 1.9. The H' index was the highest at the beginning of the shrimp crop in group N2, and the lowest in group N2 at the beginning of the rice crop and group N1 at the end of the rice crop. The average H' index over the sampling stages in group N1 varied from 1.0±0.3 to 1.4±0.2. Similarly, the average H' index in groups N2, N3, and canals fluctuated from 0.8±0.4 to 1.4±0.3, 1.0±0.3 to 1.2±0.3, and 1.1±0.2 to 1.2±0.2, respectively (Figure 11). During the sampling periods, the H' index in group N1 was higher than in groups N2, N3, and canals.

The J' index in the rotational rice-shrimp system across sampling periods ranged from 0.2 to 0.9. The average J index values fluctuated from 0.5±0.1 to 0.7±0.1; 0.6±0.3 to 0.8±0.1, 0.6± 0.1 to 0.6±0.2, and 0.4±0.1 to 0.5±0.1 for groups N1, N2, N3 and canals, respectively (Figure 11). The higher the J' index, the more evenly distributed the zoobenthos species density. As a result, during the study periods, the evenness of zoobenthos species density in rice-shrimp farms and canal sample locations was rather high. In addition, the Simpson diversity index (D) across sampling periods in the study area ranged from 0.1 to 0.8, an average of 0.6±0.2. The D index was the highest in groups N1 and N2 at the start of the shrimp crop and lowest at the end of the rice crop in the group N2. The average D index for groups N1, N2, N3, and canals ranged from 0.50.1 to 0.70.1, 0.40.3 to 0.70.1, 0.50.1 to 0.60.1, and 0.50.1 to 0.60.1, respectively (Figure 11).

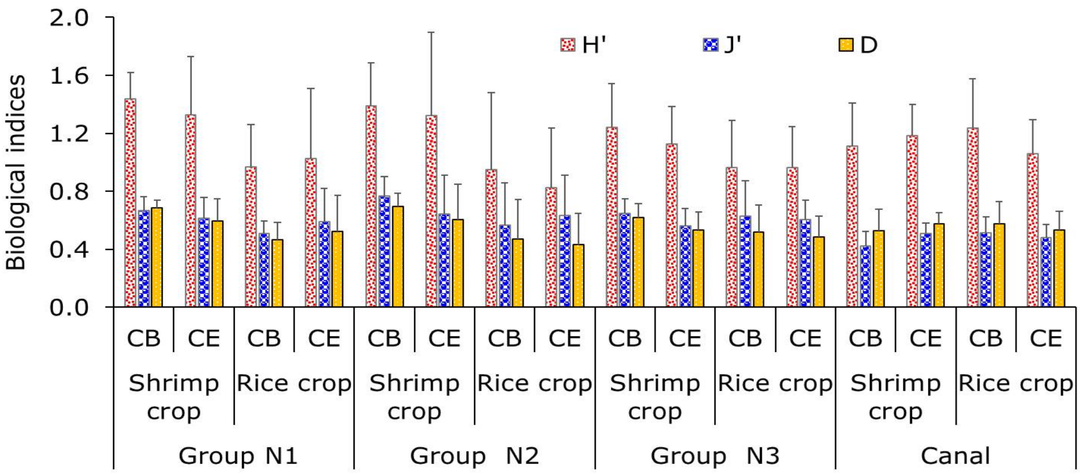

Figure 11. Biological indices on zoobenthos during sampling periods.

***Relationship between water environment parameters and species composition and density of zoobenthos in the rotational rice-shrimp culture area**.* Based on Pearson correlation analysis, the results showed that sediment characteristics, water quality factors, and diversity indices were significantly correlated with the species composition and density of benthic macroinvertebrates in the study area. The percentage of clay in shrimp-rice pond sediment had a significant negative correlation ($p < 0.05$) with the total abundance of benthic organisms. On the contrary, the silt proportion was significantly positively correlated ($p < 0.05$) with the total density of benthic fauna (Table 2).

Table 2

Correlation between water environment parameters and species composition and density of zoobenthos in the rotational rice-shrimp culture areas (n = 72)

Species composition and density of zoobenthos, and biological indices	*Temp.*	*pH*	*Salinity*	*Trans.*	*TN*	*TP*	*TOM*	*Clay*	*Silt*
Bivalvia species number	-0.073	0.035	0.389**	0.235*	0.331**	-0.100	0.158	-0.027	0.027
Gastropoda species number	0.010	0.060	0.351**	0.607**	0.404**	-0.085	0.068	-0.213	0.213
Polychaeta species number	0.016	0.052	0.504**	0.266*	0.290*	-0.065	0.186	-0.098	0.098
Malacostraca species number	-0.098	0.022	0.223	-0.019	0.059	0.021	0.124	-0.015	0.015
Insecta species number	-0.100	0.197	0.257*	-0.035	-0.240*	0.029	-0.148	-0.067	0.067
Total number of zoobenthos species	-0.039	0.086	0.510**	0.508**	0.397**	-0.090	0.130	-0.176	0.176
Bivalvia density	0.125	-0.152	0.127	0.486**	0.289*	-0.322**	0.030	-0.226	0.226
Gastropoda density	-0.104	-0.075	0.148	0.516**	0.319**	-0.050	-0.021	-0.211	0.211
Polychaeta density	-0.002	0.010	0.543**	0.221	0.257*	0.036	0.148	-0.111	0.111
Malacostraca density	-0.131	0.094	0.125	-0.104	0.073	0.082	0.082	0.067	-0.067
Insecta density	-0.294*	0.080	0.203	0.078	-0.068	0.037	-0.089	0.029	-0.029
Total density of zoobenthos	0.045	-0.134	0.151	0.537**	0.323**	-0.246*	0.013	-0.238*	0.238*
J'	-0.127	0.091	-0.068	-0.218	-0.094	-0.020	0.027	0.146	-0.146
D	-0.207	0.135	0.178	0.132	0.087	-0.086	0.064	0.068	-0.068
H'	-0.210	0.187	0.227	0.138	0.118	-0.018	0.035	0.015	-0.015

Note: ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); Trans. = transparency.

pH and TOM levels were not significantly correlated ($p > 0.05$) with species composition, density, and diversity indices. A significant negative correlation ($p < 0.05$) between temperature and the density of insects was found in the study area. Similarly, salinity was significantly positively correlated with the species composition of Bivalvia ($p < 0.01$), Gastropoda ($p < 0.01$), Insecta ($p < 0.05$), and total zoobenthos species ($p < 0.01$). In addition, salinity was also positively correlated with the density of Polychaeta ($p < 0.01$). Transparency had a significant positive relation with the species composition of Bivalvia ($p < 0.05$), Gastropoda ($p < 0.01$), Polychaeta ($p < 0.05$), and total zoobenthos species ($p < 0.01$). In addition, transparency was also strongly correlated with the density of Bivalvia ($p < 0.01$), Gastropoda ($p < 0.01$), and total zoobenthos species ($p < 0.01$).

The TN concentration in the sediment was significantly positively correlated with the species composition and density of Bivalvia, Gastropoda, Polychaeta, and even zoobenthos. However, TN content was negatively correlated with the species composition of insects. Similarly, the TN content in the substrate was negatively correlated with the bivalvia abundance, and thus this parameter was also negatively correlated with the zoobenthos density. The results also revealed that most water environment factors in general had insignificant correlations with H', D and J' indices in the survey area. In addition, the TP parameter in the sediment had a significant negative correlation ($p < 0.01$) with the Bivalvia abundance ($p < 0.05$), and the total density of benthic macroinvertebrates ($p < 0.05$) during the study period.

Disscusion. The zoobenthos population found in this study was consistent with the findings of Hoa et al (2022), who found that 34 species from 31 genera, 24 families, 16 orders, 5 classes, and 3 phyla made up the benthic fauna in the shrimp-cultivated area of Ca Mau province during the rainy season. Among them, the phylum Mollusca accounted for the highest proportion with 22 species (64.8%), the phyla Annelida and Arthropoda had an equal number of species; each phylum had 6 species (17.6%). In addition, according to Thai et al (2017), benthic macroinvertebrate communities in aquaculture farms in Nam Can district, Ca Mau province, recorded 22 species belonging to 15 families, 5 classes including Polychaeta and Oligochaeta (Annelida), Gastropoda and Bivalvia (Mollusca), and Crustacea belonging to the phylum Arthropoda. In this study, the distribution of benthic macroinvertebrates was not the same among their groups between the shrimp and the rice crops. This was due to differences in the sediment characteristics, the cultivated species, and the salinity. During the study periods, salinity in the shrimp crop ranged from 7.0 to 26.8‰ and in the rice crop from 0.5 to 1.6‰. The zoobenthos species number in the shrimp crop (29 species) was higher than that in the rice crop (26 species). In which case, the species number of Polychaeta and Bivalvia in the shrimp crop was higher than that of the rice crop. Nonetheless, compared to the shrimp crop, there were more species of Gastropoda and Malacostraca in the rice crop. These results showed that the salinity factor significantly affected the distribution of zoobenthos in the rotational shrimp-rice model. According to McLusky & Elliott (2004), benthic communities varied significantly depending on environmental conditions, especially due to sediment characteristics, salinity, food availability, and hunting ability of their prey.

The average zoobenthos density in shrimp farms ranged from 495 to 532 ind m^{-2}, remarkable lower than that in the area around shrimp-rice farming areas (4,823 ind m^{-2}). According to Tho et al (2012), zoobenthos was poor in the improved extensive shrimp farming system in the Mekong Delta of Vietnam. There were 11 species categorized into two phyla, with a density ranging from 7 to 1,971 ind m^{-2}. In total, seven Mollusca species were present, belonging to the Gastropoda and Bivalvia classes, while four Polychaeta species represented the Annelida. Zoobenthos are one of the major food sources for the cultured shrimp system. Furthermore, juvenile shrimps feed mainly on algal material, while adults feed on crustaceans, annelids, algae, mud, and unidentified matter (El Hag 1984). The impoverished zoobenthos community raised the concern of a lack of natural food sources for the cultured shrimps.

The species composition, density, and biomass of zoobenthos in rice-shrimp farms were generally much lower than in canals. This is because the sediment in the canal had a higher proportion of silt, and the TN content was appropriate for the development of benthic organisms. The average silt percentage and TN content were 94.7±2.31% and 1.32±0.11 mg g^{-1}, 95.7±1.1% and 1.47±0.07 mg g^{-1} for shrimp farms and canals, respectively. Gastropoda accounted for the highest proportion through samplings in rice-shrimp farms, with the predominance of snails *Melanoides tuberculata*, *Sermyla riqueti*, *Tarebia granifera*, *Thiara scabra* belonging to the Thiaridae family, and *Clea helena* (Nassariidae family) indicating the organic pollution sediment. In addition, Karadede-Akin & Unlu (2007) showed that the appearance of *M. tuberculata* indicates that the sediment is contaminated with heavy metals. This is a freshwater snail species adapted to nutrient-rich water environments but can also be found in estuaries (Bolaji et al 2011). Gastropoda species *Sermyla tornatella* was dominant with a large number of individuals in the organic shrimp farming farms in Nam Can district, Ca Mau province (Thai & Quang 2018). The occurrence of *Stenothyra* spp. in shrimp-rice farms with a relatively high density (113-223 ind m^{-2}). This snail species is small in size and has a thin and soft shell, so it is a useful natural food source for shrimp. The rice-shrimp farms (groups N2 and N3) supplemented with probiotic products in the shrimp farming crop promoted the process of organic matter decomposition on the sediment. A small particle size of detritus in the bottom sediment would be an essential food source for small benthic fauna such as *Stenothyra* spp. and Polychaeta species. Therefore, when the rice-shrimp farms were supplemented with probiotics, *Stenothyra* spp. and Polychaeta species had higher densities than those in shrimp farms without probiotic use, especially at the end of the shrimp crop. The addition of probiotics (mostly *Bacillus* spp.) to the shrimp farms enhanced the rate of organic matter decomposition in the sediment, thereby creating favorable conditions for useful zoobenthos groups to develop, providing natural food for shrimp to grow and develop.

The study also discovered that Bivalvia had quite low densities and weights in rice-shrimp farms, but a high abundance and biomass in canals. This result was because Bivalvia is a passive filter feeder, adapted to flowing water and a muddy bottom with a high organic matter content in the canal, which are suitable conditions for Bivalvia growth. The *Mytilus edulis* (Bivalvia) species dominated the canal with densities ranging from 77 to 8,720 ind m^{-2}. *M. edulis* species is eurytopic and can tolerate wide fluctuations in salinity, desiccation, temperature, and oxygen tension, enabling it to withstand the wide range of conditions found in high intertidal, estuarine, oceanic, warm, and ice-scoured environments (Seed & Suchanek 1992).

As for Polychaeta, they were found in rice-shrimp farms of groups N2 and N3, but at very low density. However, Polychaeta was not recorded in group N1 (Figure 8); this is the difference in species composition and density of zoobenthos between groups N1 and groups N2 and N3. According to Varadharajan & Soundarapandian (2013), the main benthic groups collected during the study period in the shrimp farming area were dominated by Polychaeta, followed by Bivalvia, Gastropoda, Decapoda, and Amphipoda. In addition, the Polychaeta were reported to be the most prominent zoobenthos in shrimp farming systems and have been recognized as the main prey item of several penaeid species (Nunes et al 1997). In this study, species of the Polychaeta class were recorded, including *Sabella penicillus*, *Nephthys* sp., and *C. capitata*.

Malacostraca had a fairly low density, fluctuating from 0 to 25.6 ind m^{-2}, and was mostly a *Gammarus locusta* species from the Amphipoda order observed in this model. They had a higher abundance in shrimp-rice farms in group N1 at the beginning and end of the shrimp crop. In contrast, Malacostraca tended to have a lower number of individuals at the end of the shrimp crop in groups N2 and N3 (Figure 7). Amphipods are a major order of crustaceans that stands out for their local abundance (both in terms of density and biomass), diversity, ecological importance and role as hosts for parasites (Giari et al 2020). Additionally, marine amphipods have recently gained attention as alternative live feed in aquaculture, since they are an important natural diet of many marine fish of commercial interest (Amara et al 2001). Natural food items like amphipods in bioflocs have been shown to provide nutrient-rich live feed for aquaculture species. They have lipid content making them suitable as live food in shrimp culture, potentially replacing fishmeal (Promthale et al 2021). However, Amphipoda can cause problems for shrimp growth. Within the food web, parasite Amphipoda can co-occur at different levels, depending on the trophic level of their host and their developmental stage, and play different roles: parasites can function as consumers, feeding on and acquiring energy from their hosts (Giari et al 2020). In addition, Insecta (mainly larvae of the Chironomidae family) had a higher abundance in the shrimp crop than in the rice crop. The individual amount of Insecta rose the greated in group N3, ranging from 51 to 82 ind m^{-2} (Figure 9). Chironomidae larvae play an important role in a wide range of aquatic ecosystems. The development of Chironomidae indicates organic pollution in sediment. The addition of trash fish as shrimp food was one of the reasons contributing to increasing the level of pollution in the sediment.

Assessing the diversity of the zoobenthos community in the study area was expressed through the H', J', and D indices. The result showed that the H', J', and D indices ranged from 0.3 to 1.9, 0.2 to 0.9, and 0.1 to 0.8, respectively. Based on the H' and D indices, it is possible to evaluate the diversity of the benthic macroinvertebrate population between sampling locations. Higher values of these indices indicated higher zoobenthos diversity in the study area. In addition, the J' index represented the uniformity of the number of individuals of different species in the same sampling location. In this study, the diversity of the zoobenthos community at most collection sites in group N1 tended to be higher than that in groups N2, N3, and canal. The relationship between H', D, and J' indices with parameters of water environment and bottom sediment was not found in the rotational black tiger shrimp-rice model. Species composition diversity of benthic fauna through the sampling stages in the shrimp-rice farms and canals reached from low to medium in the study zone.

Water environment parameters, including temperature, salinity, transparency, TN and TP concentrations in bottom mud, and sediment properties, had a statistically significant influence on the zoobenthos development. According to Aura et al (2011), the distribution of benthic animals not only depends on the nutritional conditions in the water environment but also depends on the organic matter content and bottom characteristics of the water body. The sediment with a high organic matter content created a diverse food source that contributed to the richness of benthic organism groups. In this study, substrate properties had a significant influence on the abundance of benthic macroinvertebrates. Zoobenthos abundance increased at sites with high silt percentage. Research by Subramanian & Sivaramakrishnan (2007) showed that water environmental factors also significantly affected the fluctuation of the benthic macroinvertebrate population. In addition, Ruggiero & Merchant (1979) revealed that the benthic fauna composition correlated more closely with substrate properties than water quality parameters. The individual amount of benthic organisms reached low values in water bodies with clay sediments (Strayer & Smith 2001). Furthermore, Yamamuro (2004) suggested that the zoobenthos composition depends mainly on ecological changes such as food sources, sediment properties, pH, temperature, dissolved oxygen, flow velocity, and interactions between organisms in the aquatic environment. Similarly, Hussein et al (2011) also identified some ecological factors affecting the development of zoobenthos as temperature, pH, DO, conductivity, total dissolved salt concentration, depth and percentage of higher plant.

In general, the number of species, density, and weight of zoobenthos in the canal were higher than those in groups N1, N2, and N3 in the study area. The Gastropoda community occupied a high proportion of rice-shrimp farms, while Bivalvia dominated in canals. The development of zoobenthos was strongly impacted by factors such as temperature, salinity, transparency, TN and TP contents in bottom sediment, and the sediment properties of the water body. Shrimp farms were supplemented with probiotics that contributed to the increase of beneficial benthic species by providing live food for shrimp and helping shrimp grow better.

Conclusions. The study identified 36 species of benthic organisms. Gastropoda had a high species number, density, and mass in the rice-shrimp farms, while Bivalvia had a high density and mass in the canal. Zoobenthos diversity ranged from low to moderate in the rotational shrimp-rice system in the study area. The abundance of benthic fauna was affected by the sediment characteristics and the nutrient content of the bottom mud. Small snails, *Stenothyra* spp. and Polychaeta, tended to grow well in rice-shrimp farms supplemented with probiotics, while they were not recorded in shrimp farms with conventional farming techniques. The use of probiotic products in rice-shrimp farms contributed to improving the sediment and creating appropriate conditions for benthic macroinvertebrates development, providing a natural food source for shrimp growth.

Further studies about the use of higher probiotic doses in the rotational black tiger shrimp-rice farming farms should be conducted in order to contribute to developing this model following organic standards in Ca Mau province.

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Conflicts of interest. The authors declare that there is no conflict of interest.

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