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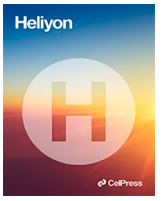

Research article

A study of bacterial community structure of shrimp farms along the Ratnagiri coast, Maharashtra

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ABSTRACT

Intensification of shrimp farming practices has increased the number and severity of disease outbreaks globally. As a result, diseases have become a significant barrier to profitable and sustainable shrimp production. Shrimp farming practices are reviving in India after its downfall in the late 90s. However, these farming practices also witness disease outbreaks due to viral and bacterial infections. Among the bacterial infections, Vibrios are the most important bacterial causative agents found in shrimp farms. They are ubiquitous and invariably seen in shrimp production conditions as opportunistic pathogens. The present study was conducted to identify the bacterial pathogens associated with the shrimp *Penaeus vannamei* farming systems along the Ratnagiri coast. In all, two farming units were selected: Varavade farm - a six-year-old farm, and Chinchkhari farm, a new virgin farm. The water and sediment samples were collected from January to May 2022 throughout culture period of one crop. The total plate count (TPC) of the shrimp farm water samples of the Varavade farm varied from 4.35 to 6.32 log10 CFU mL^{-1}. In the sediments, the minimum value of TPC was 4.99 log10 CFU g^{-1}, while the maximum value observed was 7.25 log10 CFU g^{-1}. The Total *Vibrio* count (TVC) of water samples from Varavade farm varied from 4.01 to 5.63 log10 CFU mL^{-1}. In the sediments, the minimum value of TVC was 4.64, while the maximum value observed was 6.56 log10 CFU g^{-1}. The statistical analysis showed a significant difference in TPC and TVC ($p < 0.05$) among different days of culture.

The TPC of the shrimp farm water samples of the Chinchkhari farm varied from 5.22 to 8.17 log10 CFU mL^{-1}. In the sediment, the minimum value of TPC was 5.87, while the maximum value was observed at 8.45 log10 CFU g^{-1}. The TVC of water samples from the Chinchkhari farm varied from 4.75 to 6.89 log10 CFU mL^{-1}. In the sediment, the minimum value of TVC was 5.16, while the maximum value observed was 6.70 log10 CFU g^{-1}. The statistical analysis showed a significant difference in TPC and TVC ($p < 0.05$) among different days of culture. The bacterial load was observed to increase with the progression of the culture period on both farms. The usage of probiotics, chemicals, and water exchange was observed to promote a decrease in the bacterial community.

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1. Introduction

Aquaculture is the fastest-growing food sector in the world. It has established itself as a high protein source to fulfil the food demand. The aquaculture industry has been dominated by farming of both finfish and shrimps. However, the demand for shrimp production in the global market is ever-increasing, supporting economic growth in many developing countries. In addition, it meets the growing nutritional needs and provides employment opportunities [1]. Recently, *Penaeus vannamei* (Whiteleg shrimp) is the prime species produced, accounting for ~83 % of all penaeid shrimp aquaculture [2]. India is one of the leading shrimp suppliers to Japan, Europe, Thailand, and the United States [3].

Regarding shrimp farming in India, *P. vannamei* showed a production of about 10,97,481 tons, and in the case of *P. monodon*, the estimated production was 63,041 tons in the year 2022–23 [4]. State-wise production of *P. vannamei* in 2022–23 was recorded in descending order from the states of Andhra Pradesh, Odisha, West Bengal, Gujarat, Tamil Nadu, Orissa, Maharashtra, Karnataka-Goa, Kerala and Telangana. In the case of *P. monodon*, the maximum production was reported from West Bengal, followed by Andhra Pradesh, Gujarat, Odisha, Kerala, Karnataka-Goa, Tamil Nadu and Maharashtra [4].

Globally, shrimp farms are often affected by various pathogens, which cause high mortality and bring substantial economic losses to the industry. Disease outbreaks in shrimp are the result of a complex interaction of many factors, including poor breeding environment, intensive stocking density, stress, suppression of the host immune system and the virulence of infectious pathogens such as bacteria, viruses, fungi, and parasites [5]. Recently, farmed shrimps are often seen encountered with Acute Hepatopancreatic Necrosis Disease (AHPND) caused by *Vibrio parahaemolyticus* [6], Streptococcosis, Hepatopancreatic Microsporidiosis, Necrotizing Hepatopancreatitis (NHP) caused by *Streptococcus uberis* [7], *Enterocytozoon hepatopenaei* [8], *Haptobacter penaei* [9]. The bacterial pathogens *V. harveyi*, *V. parahaemolyticus*, *V. alinolyticus*, *V. vulnificus*, *V. spelendidus*, *V. anguillarum* [10], *Mycobacterium peregrinum* [11], *Vibrio* spp., *Aeromonas* spp., *Flavobacterium* spp [12]. caused White gut disease, Mycobacteriosis, Brown spot Disease/Shell Disease, respectively.

Among the bacterial pathogens, *Vibrio* spp. the gram-negative, opportunistic bacteria are ubiquitous in marine and estuarine ecosystems. These species are natural inhabitants of aquatic environments with high salinity and temperatures, accounting for 38–81 % of the total bacterial biota in shrimp farms [13,14]. The larval, post-larval and juvenile stages of shrimps are more susceptible to *Vibrio* diseases and cause very high mortality among shrimps [15].

Stress factors such as physicochemical and microbial quality of culture water, poor nutritional status and high stocking density can cause infection by opportunistic pathogens. There are known and unknown pathogens in the environment that pose severe risk factors to aquaculture organisms, leading to the onset of mortality [16]. Poor water and soil quality parameters could accelerate the abiotic stress in the culture system and make the animals susceptible to opportunistic pathogens, leading to various disease outbreaks [17]. Numerous studies have attempted to identify the biotic and abiotic factors that influence the abundance of *Vibrio* spp. in the environment and also to understand their association [18]. The abundance of vibrios in the shrimp culture system may vary depending on environmental and geographical factors. It is evident that the diseases are often encountered due to inadequate husbandry practices. In recent years, the shrimp farming activities are steadily growing along the Konkan coast. Thus, viral and bacterial disease outbreaks are often observed in the region. In order to assess the epidemiology of some bacterial pathogens, the study was undertaken on the shrimp farms of Ratnagiri.

In aquatic ecosystems, water and sediment are two different but closely related environments. The bacterial communities that live in water and sediment habitats have different origins, levels of diversity, and influencing variables [19]. The biogeochemical cycle of elements in aquaculture environments and the health of aquatic products are both greatly influenced by the microbial communities of water and sediment [20,21]. Bacteria in sediments are crucial for promoting biogeochemical cycles and have the ability to affect water quality due to their high metabolic activity, which includes the breakdown of organic matter, the storage and release of nutrients, and the dissolution of chemicals [22]. Therefore, it's critical to comprehend the patterns that exist between the microbial populations in sediments and water of shrimp culture.

The significance of this study lies in its investigation of the impact of bacterial diseases like vibriosis on shrimp farming in the Konkan region, a burgeoning industry with immense economic potential. The narrow strip of water bodies in the region has limited water exchange during most of the time of the farming activities, which can result in the accumulation of waste products, proving the development of pathogenic bacteria, viruses, and other pathogens. This increases the risk of disease transmission, including vibriosis. Unlike the peninsular Indian topography, the porous laterite acidic soil of the Konkan region leads to acidic soil and water conditions, stressing the cultivable species. The porous soil can also lead to increased bacterial loads in the water, as bacteria can thrive well in the porous soil. By comparing the scale of intensity of vibriosis in newer and older farms, the research aims to provide valuable insights into the dynamics of this bacterial disease and its effects on the sustainability of shrimp farming operations. The findings of this study can contribute to the development of effective disease management strategies, enabling farmers to mitigate the risks associated with vibriosis and optimize their yields. The research can also contribute to the improvement of shrimp farming practices, ultimately enhancing the overall productivity and profitability of the industry in the region to minimize the risk of disease transmission and to promote ecosystem health.

2. Materials and methods

2.1. Chemicals and culture media

Different microbiological culture media and chemicals such as Plate count agar (PCA), Thiosulphate citrate bile sucrose (TCBS), Alkaline peptone water, Normal saline (0.85 %), Peptone water, etc (HiMedia, Mumbai, India) were used for microbial analysis.

2.2. Farm locations

The study was carried out for a period between Jan 2022 and May 2022. A total of two shrimp farms in Ratnagiri district, Maharashtra, India, were selected for the study (Fig. 1). The farm locations were as follows: Varavade farm, 17°20′18.6″ N, 73°25′33.6″ E and Chinchkhari farm, 16°96′89.9″ N, 73°33′01.6″ E. The total water spread area of the Varavade farm was 1.8 ha, and that of the Chinchkhari farm was 1.0 ha. Varavade farm was established six years ago, and Chinchkhari farm was built in 2022, a virgin new farm.

2.3. Collection of samples

2.3.1. Water samples

Water samples were collected from all ponds of each farm and five different locations of each pond, viz. near the inlet, outlet, feed tray, aerators, and at the centre of the pond about 30 cm depth below the surface using a sterile glass 250 ml glass bottle. Five samples were then mixed to form one composite sample as per standard methods given by the American Public Health Association (APHA) [23] for further analysis. A sterilized glass bottle (250 ml) was utilized to take water samples. After being sterilized for an hour at 160 °C in a hot air oven, 200 ml of water samples were taken from the composite sample and transported in an insulated container. To prevent contamination, proper aseptic techniques were employed during sample collections, and the samples were handled and transported to laboratory carefully. The samples were kept at temperatures 4 °C in an insulated container, and bacteriological work was done within 6 h of the sample collection.

2.3.2. Sediment samples

Sediment samples were collected by using a box sampler from four places in each pond, viz. near the inlet, outlet, feed tray, and centre of the pond. The samples were then mixed to get one composite sample as per standard methods given by APHA [23]. The samples were transferred to U-V sterilized polythene bags for further analysis. To prevent contamination, proper aseptic techniques were employed during sample collections, and the samples were handled and transported to laboratory carefully. The samples were kept at temperatures 4 °C in an insulated container, and bacteriological work was done within 6 h of the sample collection.

2.4. Preparation of the sample

The water and sediment samples collected were serially diluted in a sterile diluent of 0.85 % saline solution. Dilution stages of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared by transferring 1 mL of the previous dilution into 9 mL sterile saline solution and so on (Figs. 2 and 3). Mixing of the sample at each dilution was done by a cyclomixer.

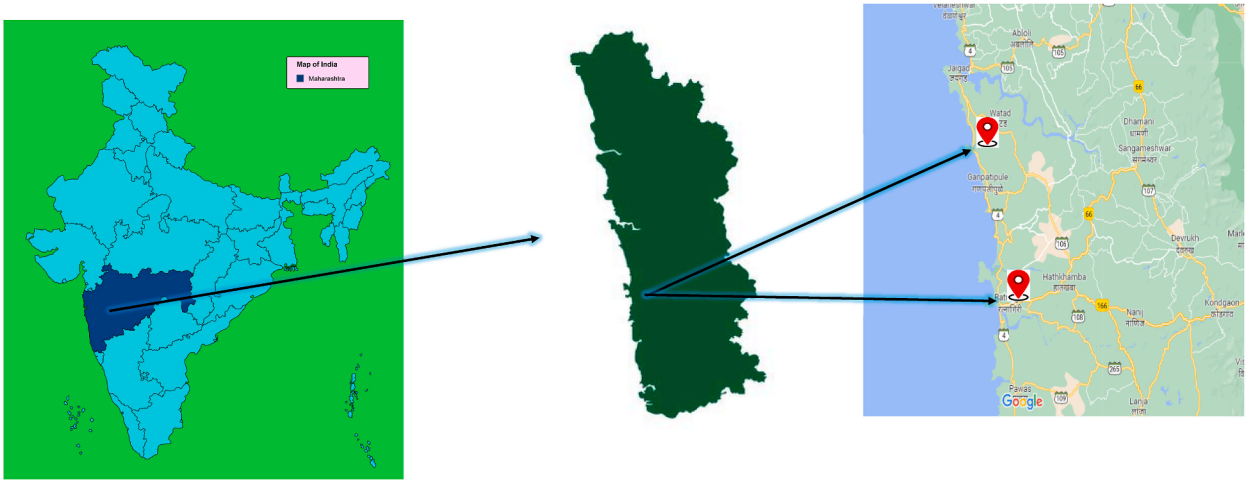

Fig. 1. Experimental location (Map lines delineate study areas and do not necessarily depict accepted national boundaries.).

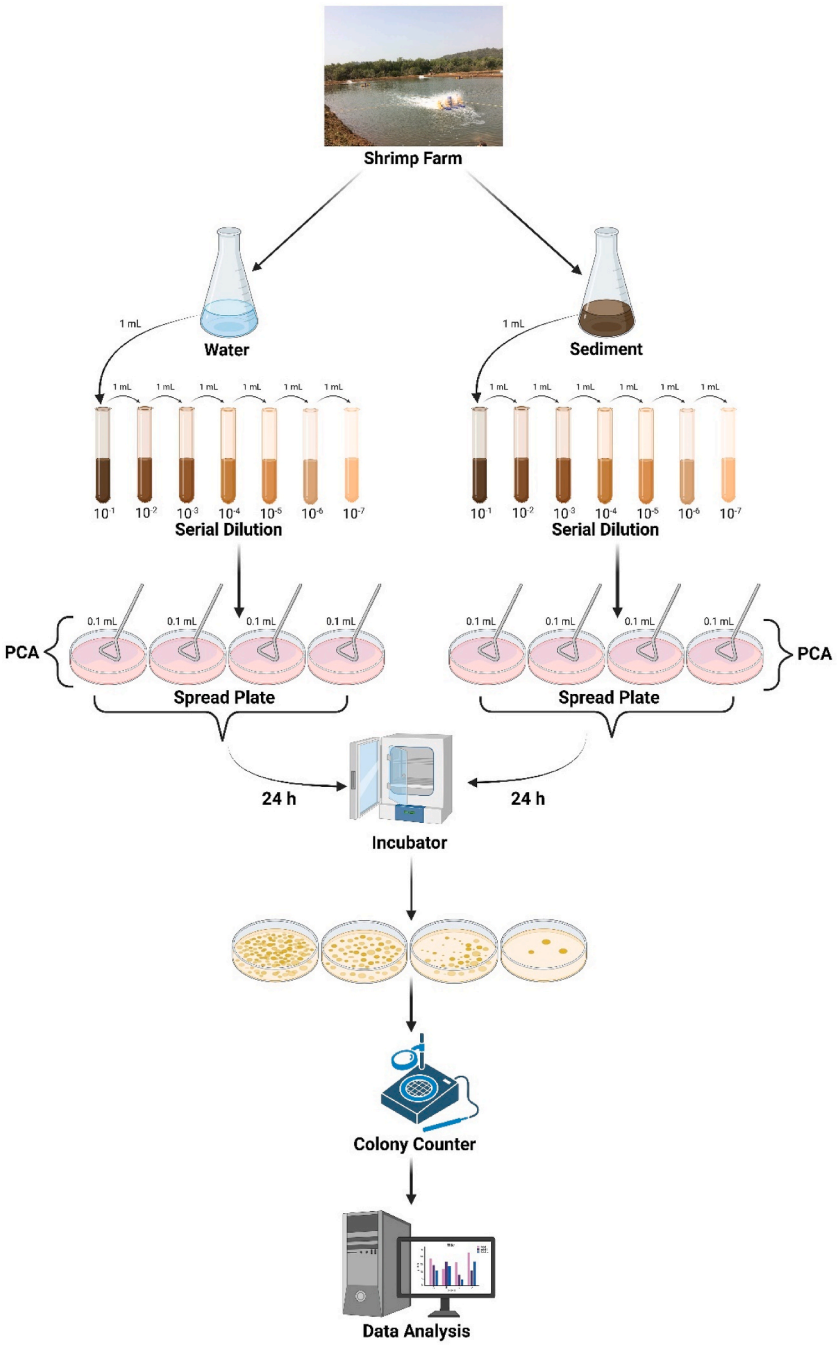

Fig. 2. Pictorial representation of experimental design for enumeration of TPC on plate count agar.

2.5. Preparation of culture media

Plate count agar (PCA) and thiosulphate citrate bile salt sucrose (TCBS) were prepared using the standard method given by APHA [23].

2.6. Sterilization

All the glassware was sterilized in an oven at 180 °C for 1 h. Bacteriological culture media, diluents and autoclavable labware were sterilized in an autoclave at 121 °C for 15 min unless otherwise specified. Polythene bags were sterilized by UV rays for 30 min.

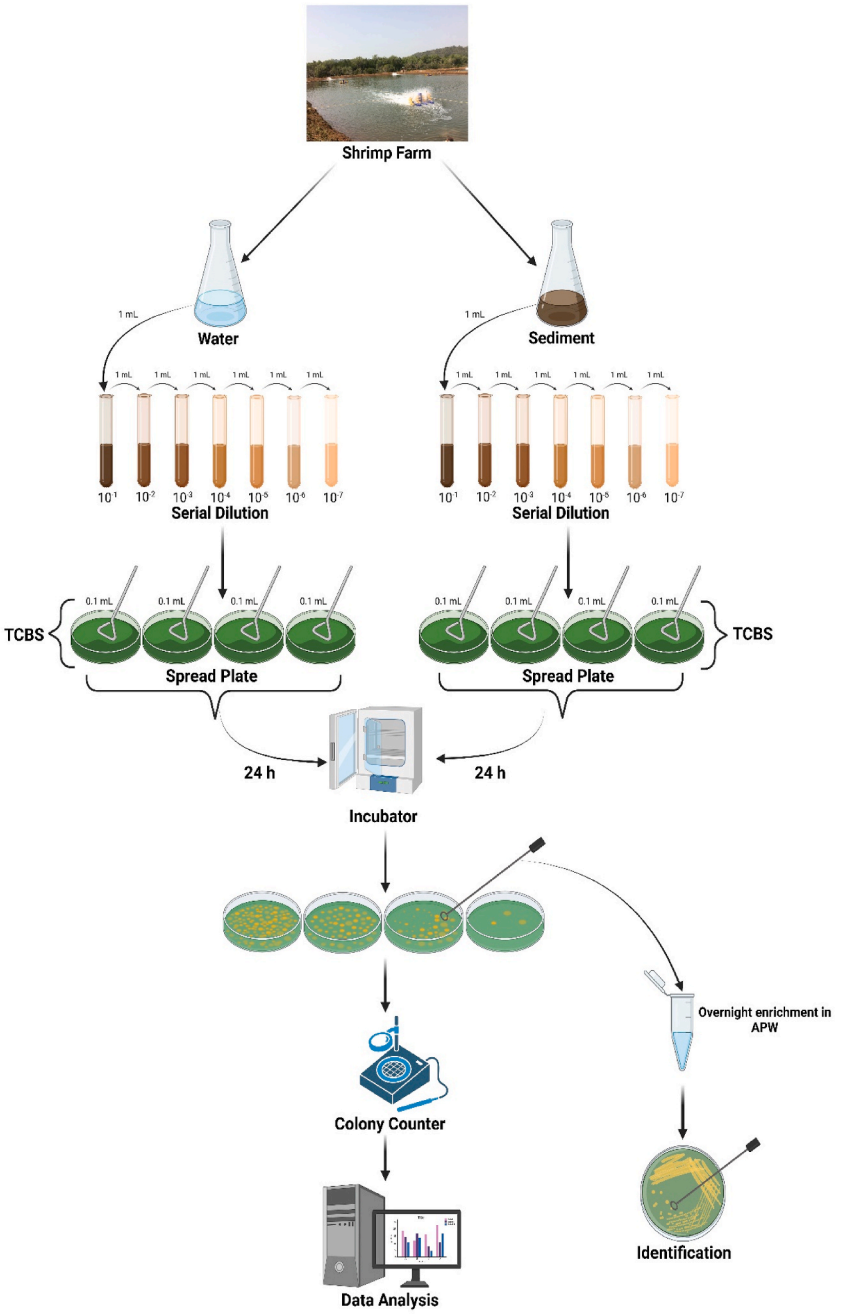

Fig. 3. Pictorial representation of experimental design for enumeration and identification of TVC on TCBS agar. (TCBS – Thiosulphate Citrate Bile Salt Sucrose, APW – Alkaline Peptone Water)

2.7. Quantitative bacteriological analysis

The serially diluted water samples and sediment samples were used for analysis. The total plate count (TPC) of water and sediment samples was assessed by plating with selected serial dilutions of the samples on PCA (Fig. 2). The total *Vibrio* count (TVC) of the water and sediment samples was estimated by plating the samples on TCBS agar [23] (Fig. 3). The count was taken as the mean value of the viable colonies, which appeared in the triplicate agar plates made per each sample.

2.8. Enumeration of total plate count (TPC)

- 1 mL sample is mixed with 9 mL of normal saline solution for serial dilution of the sample and mixed well after each serial dilution by a cyclo mixer.
- Then, 0.1 mL of sample was spread on PCA and incubated at 37 °C for 24 h.
- After incubation, the colonies were counted in a colony counter (Fig. 2).

2.9. Isolation, enumeration and characteristics of typical colonies of Vibrio spp

The diluted test sample was spread on TCBS and incubated at 37 °C for 24 h. After incubation, yellow colonies of 2–3 diameter with a bluish-green centre were obtained; the colonies were counted in a colony counter as *Vibrio* count (Figs. 3 and 4).

2.10. Enumeration

The TPC and TVC were calculated using this formula.
Bacteria count (CFU mL^{-1} and CFU g^{-1}) = No. of colonies × Dilution factor % Weight of the sample.

2.11. Estimation of water quality parameters

Water temperature, salinity, water pH, dissolved oxygen and total alkalinity were determined as per the standard method given by APHA [23].

2.12. Statistical analysis

Mean values of TPC and TVC were analysed by one-way analysis of variance (ANOVA) using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Statistical differences were considered significant at $p < 0.05$. When the difference was significant, the means were compared using the Tukey post-hoc test. Principal component analysis was also conducted to compare the similarities between TPC, *Vibrio* population and water quality parameters among different days of culture (DOCs) using OriginPro 10.0 software (Originlab Co., Northampton, MA, USA).

3. Results

3.1. Bacterial load

3.1.1. Varavade farm

3.1.1.1. Total plate count (TPC). Results of the quantitative estimation of the total heterotrophic bacterial count in shrimp pond

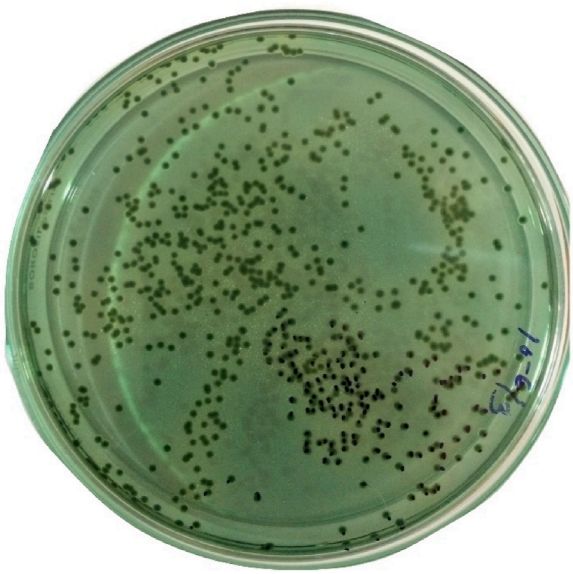

Fig. 4. Vibrio bacteria on TCBS agar.

waters and sediments are presented in Table 1. The total plate count of bacteria in shrimp farm waters and sediments from Varavade farm ranged between 4.35 and 6.32 log10 CFU mL^{-1} and 4.99 to 7.25 log10 CFU g^{-1}, respectively. Results showed an increasing trend (Fig. 5) of TPC of pond water samples from the start of culture to the days of culture (DOC) 150. The statistical analysis showed that there was a significant difference ($p < 0.05$) in total plate count values of water samples. The Tukey post hoc test showed a significant difference ($p < 0.05$) in TPC from DOC 45 to DOC 135. There was no significant difference ($p > 0.05$) for TPC between DOC 0 to DOC 30 and DOC 135 to DOC 150. In sediment samples, the counts were increased from DOC 0 to DOC 60, after which they were slightly reduced to DOC 75 and increased till the end of the culture. The statistical analysis showed that there was a significant difference ($p < 0.05$) in total plate count values of sediment samples from DOC 15 to DOC 30, DOC 45 to DOC 60, and DOC 75 to DOC 120. The test showed no significant difference ($p > 0.05$) in TPC values of DOC 0 to DOC 15, DOC 30 to DOC 45, DOC 60 to DOC 75, and DOC 120 to DOC 150.

3.1.1.2. Total Vibrio count (TVC). The total *Vibrio* count of shrimp pond waters and sediments ranged between 4.01 and 5.63 log10 CFU mL^{-1}, and 4.64 to 6.56 log10 CFU g^{-1}, respectively (Table 1). Results showed (Fig. 5) an increasing trend of TVC of pond waters sample from the start of culture to the DOC 135; afterwards, a slight decrease in the count was noticed. The statistical analysis showed a significant difference ($p < 0.05$) in TVC values of the pond water samples at DOC 0–DOC 15, DOC 75–DOC 90, and DOC 105–DOC 135. The test showed no significant difference ($p > 0.05$) between DOC 15–DOC 75 and DOC 135–DOC 150. In the sediment sample, the count increased from the start to the end of the culture. The statistical analysis showed a significant difference ($p < 0.05$) in TVC values of pond sediment samples at DOC 0–DOC 30, DOC 75–105, and DOC 120–DOC 135. The test showed no significant difference ($p > 0.05$) in TVC values in DOC 30–DOC 75, DOC 105–DOC 120, and DOC 135–DOC 150.

The principal component analysis indicated that the first three principal components accounted for 98.6 % variability of the Varavade farm (Fig. 7). Principal component analysis revealed that the first component (PC1) explained about 82.8 %, the second component (PC2) 10.7 %, and the third component (PC3) accounted for 5.1 % of the total variance. Principal component analysis loading plots depicted the relationship among TPC, TVC, and water quality parameters of different DOCs. In the plot, an acute angle (<90°) was observed between water and sediment samples of TPC and TVC, and water quality parameters showed a close positive relationship between these parameters in the shrimp farm.

3.1.2. Chinchkhari farm

3.1.2.1. Total plate count (TPC). The total plate count of bacteria in shrimp pond waters and sediments from Chinchkhari farm was varied between 5.22 and 8.17 log10 CFU mL^{-1} and 5.87 to 8.45 log10 CFU g^{-1}, respectively (Table 2). Results showed an increasing trend (Fig. 6) of TPC of pond water samples from the start of the culture to DOC 135; there was a slight decrease in count in the later duration of the culture. The statistical analysis showed a significant difference ($p < 0.05$) in TPC values of the pond water samples at DOC 15 to DOC 60, and DOC 75 to DOC 135. The test showed no significant difference ($p > 0.05$) from DOC 0 to DOC 15, DOC 60 to DOC 75, and DOC 135 to DOC 150. In the sediment samples, the count increased from DOC 0 to DOC 60, after which there was a slight reduction at DOC 75, an increase again to DOC 135, and a slight reduction at DOC 150. The analysis showed a significant difference ($p < 0.05$) in total plate count values of pond sediment samples at DOC 0–DOC 15, DOC 30–DOC 60, DOC 90–DOC 105, and DOC 135–DOC 150. The test showed no significant difference in the samples from ($p > 0.05$) DOC 15 to DOC 30, DOC 60–DOC 90, and DOC 105–DOC 135.

3.1.2.2. Total Vibrio count (TVC). The total *Vibrio* count of shrimp pond waters and sediments varied between 4.75 and 6.89 log10 CFU mL^{-1} and 5.16 to 6.70 log10 CFU g^{-1}, respectively (Table 2). Results showed an increasing trend (Fig. 6) in TVC of pond water samples from the start of culture to the DOC 135, a reduction in the count afterwards. The statistical analysis showed a significant

Table 1
Bacterial Count ((log10 CFU mL^{-1}/CFU g^{-1}) of Varavade farm.

Varavade farm				
Bacterial Count (log10 CFU mL^{-1}/CFU g^{-1})				
	TPC (Water)	TPC (Sediment)	TVC (Water)	TVC (Sediment)
DOC 0	4.35 ± 0.04^{a}	4.99 ± 0.10^{a}	4.01 ± 0.03^{a}	4.64 ± 0.05^{a}
DOC 15	4.44 ± 0.03^{a}	5.18 ± 0.04^{a}	4.24 ± 0.02^{b}	5.02 ± 0.02^{b}
DOC 30	4.56 ± 0.07^{a}	5.45 ± 0.02^{b}	4.28 ± 0.11^{bc}	5.19 ± 0.07^{c}
DOC 45	4.93 ± 0.11^{b}	5.68 ± 0.15^{b}	4.41 ± 0.01^{bcd}	5.29 ± 0.03^{cd}
DOC 60	5.05 ± 0.10^{bc}	6.30 ± 0.03^{cd}	4.47 ± 0.01^{cd}	5.40 ± 0.01^{de}
DOC 75	5.15 ± 0.06^{c}	6.12 ± 0.08^{c}	4.58 ± 0.04^{d}	5.46 ± 0.01^{e}
DOC 90	5.40 ± 0.05^{d}	6.42 ± 0.02^{d}	4.90 ± 0.03^{e}	5.85 ± 0.04^{f}
DOC 105	5.66 ± 0.10^{e}	6.73 ± 0.12^{e}	5.03 ± 0.02^{e}	6.07 ± 0.02^{g}
DOC 120	6.05 ± 0.06^{f}	7.09 ± 0.10^{f}	5.26 ± 0.02^{f}	6.19 ± 0.02^{g}
DOC 135	6.29 ± 0.10^{g}	7.17 ± 0.02^{f}	5.63 ± 0.14^{g}	6.47 ± 0.08^{h}
DOC 150	6.32 ± 0.02^{g}	7.25 ± 0.02^{f}	5.60 ± 0.10^{g}	6.56 ± 0.07^{h}

Values are expressed as mean ± standard deviation.
[a,b,c,d,e,f,g,h,i]The mean values in a column with different superscripts differ significantly ($p < 0.05$).

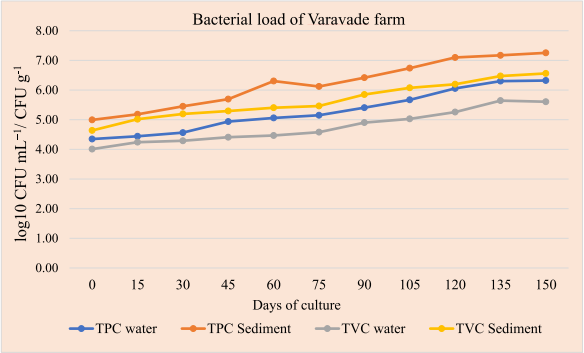

Fig. 5. Bacterial load of Varavade farm.

Table 2
Bacterial Count (log10 CFU mL^{-1}/CFU g^{-1}) of Chinchkhari farm.

Chinchkhari farm				
Bacterial Count (log10 CFU mL^{-1}/CFU g^{-1})				
	TPC (Water)	TPC (Sediment)	TVC (Water)	TVC (Sediment)
DOC 0	5.22 ± 0.06^{a}	5.87 ± 0.18^{a}	4.75 ± 0.03^{a}	5.16 ± 0.03^{a}
DOC 15	5.33 ± 0.03^{a}	6.19 ± 0.08^{b}	5.14 ± 0.11^{b}	5.27 ± 0.06^{a}
DOC 30	5.75 ± 0.12^{b}	6.43 ± 0.04^{b}	5.31 ± 0.04^{c}	5.41 ± 0.02^{b}
DOC 45	6.15 ± 0.04^{c}	6.94 ± 0.04^{c}	5.91 ± 0.04^{d}	5.52 ± 0.05^{b}
DOC 60	6.40 ± 0.02^{d}	7.29 ± 0.06^{d}	5.85 ± 0.03^{d}	5.90 ± 0.04^{c}
DOC 75	6.45 ± 0.01^{d}	7.25 ± 0.05^{cd}	6.08 ± 0.03^{e}	6.16 ± 0.02^{d}
DOC 90	6.93 ± 0.08^{e}	7.46 ± 0.19^{d}	6.20 ± 0.01^{e}	6.28 ± 0.01^{e}
DOC 105	7.23 ± 0.06^{f}	8.06 ± 0.19^{e}	6.38 ± 0.02^{f}	6.40 ± 0.02^{f}
DOC 120	7.70 ± 0.02^{g}	8.30 ± 0.05^{ef}	6.45 ± 0.01^{f}	6.70 ± 0.07^{g}
DOC 135	8.17 ± 0.04^{h}	8.45 ± 0.06^{f}	6.89 ± 0.04^{g}	6.15 ± 0.02^{d}
DOC 150	8.03 ± 0.02^{h}	8.07 ± 0.05^{e}	6.34 ± 0.03^{f}	6.41 ± 0.02^{f}

Values are expressed as mean ± standard deviation.
^{a,b,c,d,e,f,g,h,i}The mean values in a column with different superscripts differ significantly ($p < 0.05$).

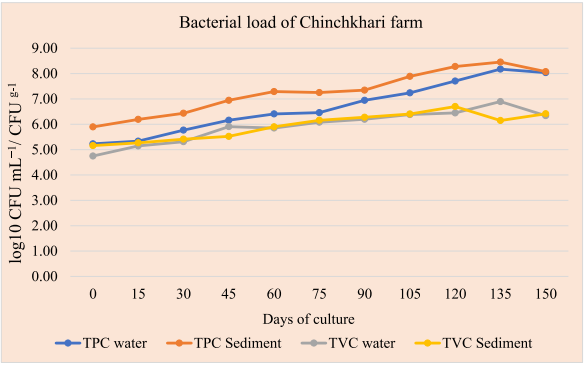

Fig. 6. Bacterial load of Chinchkhari farm.

difference ($p < 0.05$) in TVC values of pond water samples of DOC 0–DOC 45, DOC 60–DOC 75, DOC 90–DOC 105, DOC 120–DOC 135, and DOC 135–DOC 150. The test showed no significant difference ($p > 0.05$) from DOC 45 to DOC 60, DOC 75 to DOC 90, and DOC 105 to DOC 120. In the sediment samples, the count increased from the start of culture DOC 120 and later decreased till the end. The statistical analysis showed a significant difference ($p < 0.05$) in TVC values of pond sediment samples at DOC 15–DOC 30, and DOC 45–DOC 150. The test showed no significant difference for TVC values in DOC 0 to DOC 15, and DOC 30 to DOC 45.

The principal component analysis indicated that the first three principal components accounted for 96.1 % variability of the Chinchkhari farm (Fig. 8). Principal component analysis revealed that the first component (PC1) explained about 80.9 %, the second component (PC2) 10.8 %, and the third component (PC3) accounted for 4.4 % of the total variance. Principal component analysis loading plots depicted the relationship among TPC, TVC, and water quality parameters of different DOCs. In the plot, an acute angle

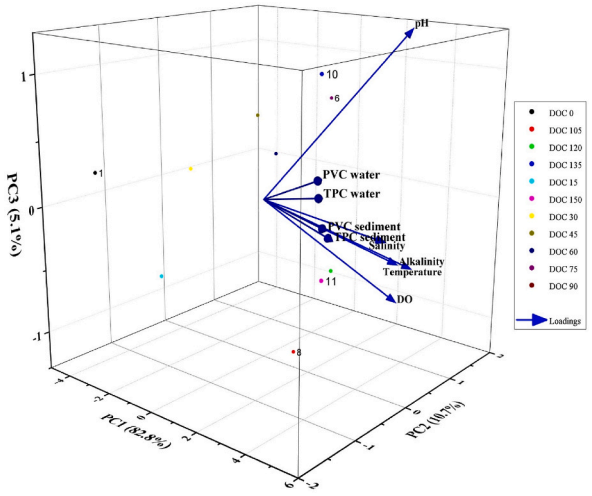

Fig. 7. Principal component analysis of Bacterial load of Varavade farm.

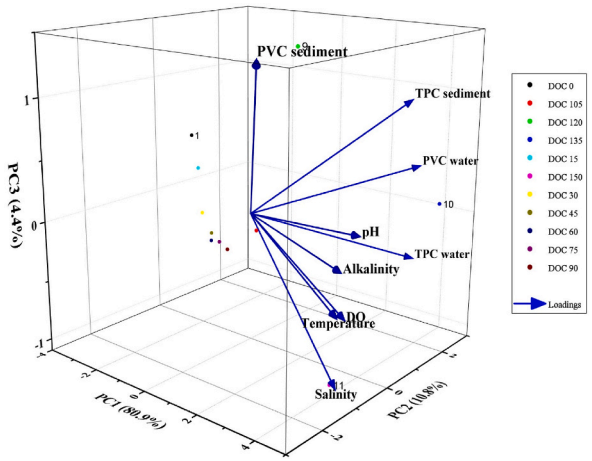

Fig. 8. Principal component analysis of Bacterial load of Chinchkhari farm.

(<90°) was observed between water and sediment samples of TPC and TVC, and water quality parameters showed a close positive relationship between these parameters in the shrimp farm.

3.2. Managemental aspects

Different managemental aspects data is given in Table 3.

3.3. Water quality parameters

The water temperature, salinity, water pH, dissolved oxygen, total alkalinity was ranged from 26 to 30.5 °C and 27.5–33 °C, 26–33 psμ and 27–35 psμ, 7.8–8.1 and 7.7–8.3, 4.5–6 mg L^{-1} and 5–6.5 mg L^{-1}, 119–160 mg L^{-1} and 102–170 mg L^{-1} in Varavade and Chinchkhari farm, respectively. (Figs. 9–14).

4. Discussion

The study envisaged a pattern of bacterial communities present in two shrimp farming systems. A comparative investigation was conducted on the variation in the total bacterial load and *Vibrio* counts in shrimp farming systems throughout culture operation for one crop.

Table 3
Managemental aspects data of Varavade and Chinchkhari farm.

Sr. No.	Managemental aspects	Varavade farm	Chinchkhari farm
1.	Stocked density (Shrimp/m^2)	25	54
2.	Survival (%)	75	70
3.	Feeding frequency	5 times a day	5 times a day
4.	Feeding time (hours)	06:30	06:30
		10:30	10:00
		14:00	13:30
		17:00	16:30
		19:30	19:30
5.	Feeding percentage of body weight		
	DOC 1–7	60-35 %	60-35 %
	DOC 8–14	8 %	8 %
	DOC 15–21	7 %	7 %
	DOC 22–28	6 %	6 %
	DOC 29–35	5 %	5 %
	DOC 36–42	4.5 %	4.5 %
	DOC 43–49	4.2 %	4.2 %
	DOC 50–56	3.4 %	3.4 %
	DOC 57–63	3 %	3 %
	DOC 64–70	2.7 %	2.7 %
	DOC 71–77	2.6 %	2.6 %
	DOC 78–84	2.5 %	2.5 %
	DOC 85–91	2.4 %	2.4 %
	DOC 92–98	2.2 %	2.2 %
	DOC 99–105	2 %	2 %
	DOC 106–112	1.9 %	1.9 %
	DOC 113–120	1.8 %	1.8 %
	DOC 121–127	1.7 %	1.7 %
	DOC 128–134	1.6 %	1.6 %
	DOC 135–141	1.5 %	1.5 %
	DOC 142–148	1.4 %	1.4 %
	DOC 149–155	1.3 %	1.3 %
6.	Total feed utilized (tonnes)	12.5 (6.25tonnes^{-ha})	10.0 (10.0tonnes^{-ha})
7.	Probiotic, lime, chemicals etc.		
	Water Probiotic (Avant ProW)	500 g ha^{-1} before stocking	–
		250 g $ha^{-1\text{-}30days}$ after stocking	250 g $ha^{-1\text{-}30days}$ after stocking
	Soil Probiotic (Spark PS)	5 L $ha^{-1\text{-}15days}$	5 L $ha^{-1\text{-}30days}$
	Mineral (Water)		
	Saldomix	5 kg $ha^{-1\text{-}15days}$	5 kg $ha^{-1\text{-}15days}$
	Lime	10 kg $ha^{-1\text{-}15days}$	10 kg $ha^{-1\text{-}15days}$
	Mineral mate	5 g kg^{-1} of feed	5 g kg^{-1} of feed
	AscoSol-C (Vit C)	5 g kg^{-1} of feed	5 g kg^{-1} of feed
	Xtra-M&G	5 g kg^{-1} of feed	–
	KMnO4	1 kg $ha^{-1\text{-}15days}$	–
	Molasses	10 L ha^{-1}	–
	Potassium Chloride (KCl)	5 kg $ha^{-1\text{-}15days}$	–
8.	Water exchange	After 60 days in every 4 days interval	After 80 days in every 7 days interval

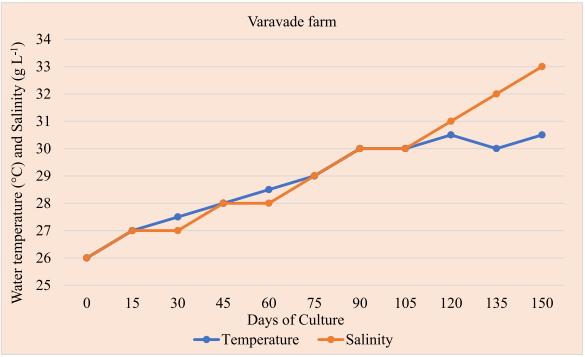

Fig. 9. Water temperature (°C) and Salinity (g L^{-1}) variation in Varavade farm at different days of culture.

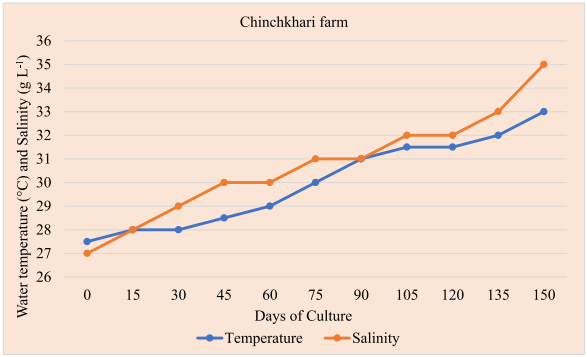

Fig. 10. Water temperature (°C) and Salinity (g L^{-1}) variation in Chinchkhari farm at different days of culture.

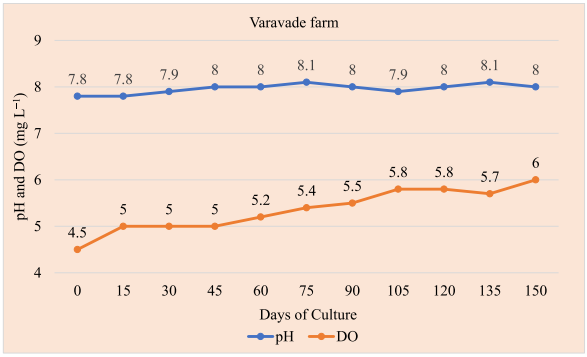

Fig. 11. pH and dissolved oxygen (mg L^{-1}) variation in Varavade farm at different days of culture.

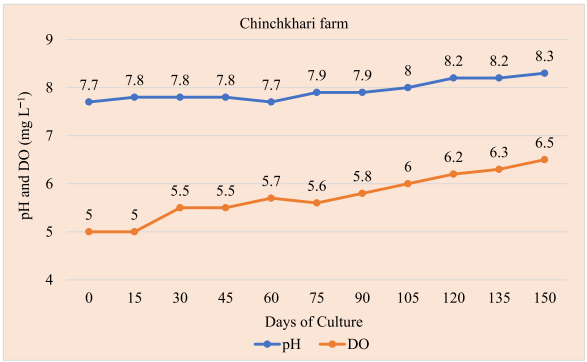

Fig. 12. pH and dissolved oxygen (mg L^{-1}) variation in Chinchkhari farm at different days of culture.

4.1. Quantitative assessment of TPC in waters

The range of total bacterial load obtained in the present study was higher than the values reported as 5.0×10^2 to 8.8×10^3 CFU mL^{-1} by Foneska [24] in Sri Lanka and 1.8×10^3 to 4.47×10^3 CFU mL^{-1} by Sharmila et al. [25], and also higher than as reported by Janakiram et al. and Anand et al. [26,27]. The values obtained in the present study were also higher than those recorded (10^5 CFU mL^{-1} of pond water) in Southeast Asian countries [28] and slightly higher with 3.4×10^7 CFU mL^{-1} [29] than the results seen in present studies. George [30] observed that the total heterotrophic count (THC) of pond water in Farm I ranged from 2.50×10^2 to 3.25×10^4 CFU mL^{-1}, and in Farm II was also quite similar, where the range was 1.60×10^4 to 3.30×10^4 CFU mL^{-1} in the water samples. Tompo [31] observed a wide range of the number of heterotrophic bacteria in semi-intensive pond water such as 10^3–10^9 CFU mL^{-1} during the crop period. It is clear that with the advancement of culture systems and intensification, the bacterial trend increased with the culture period, and cultured animals became more susceptible to various diseases. In Indonesia recorded an increase in the bacterial count with the increase in rearing duration and peaked at day 60 [29], but in the present study, the microbial community of

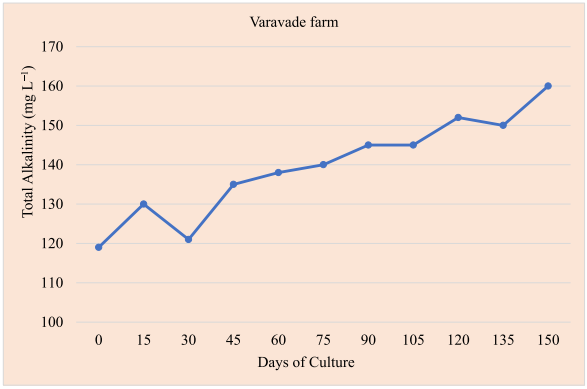

Fig. 13. Total alkalinity (mg L^{-1}) variation in Varavade farm at different days of culture.

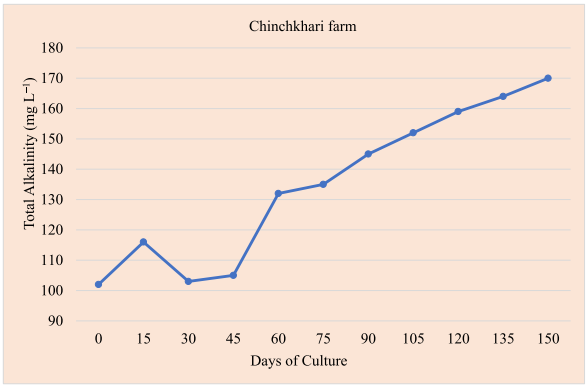

Fig. 14. Total alkalinity (mg L^{-1}) variation in Chinchkhari farm at different days of culture.

Varavade farm peaked at day 135–150 of the harvesting period and in Chinchkhari farm, it peaked at day 135. Later, a decrease in the bacterial community was observed, probably attributed to the managemental measures and a decrease in shrimp density due to its partial harvesting.

Management measures undertaken on the farms, such as water exchange, treatment of ponds using lime and occasional probiotic application, could have contributed to such variations in the bacterial load in the pond water of Varavade farm. Water exchange leads to dilution of the nutrients and consequent reduction in the bacterial load in pond water. This could, therefore, be used as a method to regulate the total heterotrophic count, which might increase uncontrollably due to the increase in nutrients generated due to the wastage of excess feed and faecal matter in the culture system. Higher values of TPC observed in the Chinchkhari farm during the entire period of study might, therefore, be an indication of the existing differences in the management practices that were followed in these two shrimp farms.

4.2. Quantitative assessment of TPC in the sediments

The TPC of sediment samples (CFU g^{-1}) was found to be consistently higher than the bacterial load in the pond water (CFU mL^{-1}) in both farms. A similar observation was recorded by Allan et al. [32] in model shrimp farming ponds and in modified extensive shrimp culture ponds by Janakiram et al. [26]. However, in the studies of Allan et al. and Janakiram et al. [26,32], the bacterial load was relatively on the lower side than that of the present work might be due to less biomass of the modified extensive culture systems. In the studies of George [30], in Farm I, the THC varied from 3.60×10^4 to 1.83×10^6 CFU g^{-1} and in Farm II, it ranged between 1.48×10^5 to 1.3×10^6 CFU g^{-1} in the pond sediments. In the present study, the TPC of pond water in the Varavade farm ranged from 4.35 to 6.32 log10 CFU mL^{-1} and in the sediments, the TPC varied from, 4.99 to 7.25 log10 CFU g^{-1}. The TPC count in the Chinchkhari farm was also quite higher, ranging between 5.22 and 8.17 log10 CFU mL^{-1} in the water samples while it was, 5.87 to 8.45 log10 CFU g^{-1} in the case of sediments. The TPC of pond sediments did show a statistically significant difference in both of the farms, though an increasing trend of the count was noted from the beginning to the end of the culture. In the shrimp ponds of China, Song et al. [33] recorded counts of 1.26×10^6 to 3.72×10^6 CFU g^{-1} in Southeast Asian countries. A total viable count in the range of 4.00×10^5 to 2.228×10^8 CFU g^{-1} was recorded by Peranginangin et al. [34], while Sharmila et al. [25] reported a value $>10^6$ CFU g^{-1} in semi-intensive system. Balakrishnan [35] also observed that the counts of THC were higher in shrimp farm sediments than in shrimp farm water. Tompo [31]

observed that the number of heterotrophic bacteria in the sediments was widely ranged between 10^6–10^{12} CFU g^{-1}. In comparison with the studies of Tompo [31], the present study showed a relatively low count of the microbial community during the culture period, which was between 10^4–10^7 CFU g^{-1} count (TPC) in the sediments of Varavade farm and 10^5–10^8 CFU g^{-1}in the sediments of Chinchkhari farm. According to Janakiram et al. [26], depending on a wide range of biotic, abiotic and anthropogenic factors prevailing in the locality, bacterial loads could vary in different ponds. The authors reported higher bacterial loads in semi-intensive ponds compared to modified extensive ponds due to high stocking densities associated with high fertilizer and feed inputs.

4.3. Quantitative assessment of vibrios in waters

Dalmin et al. and Gomez-Gil et al. [36,37] recorded that in the water/sediment samples and intestinal bacteria of cultured shrimp of farms, the main genera were *Vibrio*, *Pseudomonas*, *Aeromonas* and *Corynebacterium*. Bacteria belonging to the *Vibrio* spp. seemed not only to be common in the shrimp farming environment, but also to be considered a serious pathogen causing massive mortality in shrimp hatcheries and grow-out ponds worldwide [38–40]. Bacterial isolated more than 50 % of gram-negative bacteria belonging to 14 genera from the samples collected from Tuticorin shrimp farms [25]. Otta et al. [13] compared the prevalence of *Vibrio* in shrimp farms on the east and west coasts of India. Bacteriological characterization of water from 12 farms on the west coast of India indicated that *Vibrio* spp. constituted between 56 % and 86 % of the microbiota of individual farms. It was also noticed, moreover, similar species composition for the samples from the east coast of India. Among *Vibrio* species, *Vibrio alginolyticus* accounted for 5.2–23.4 % of the microbiota in various farms on the east coast of India. In the present study, *Vibrio* spp. were found to be ranging between 15.97 and 63.14 % of the total bacterial load in all the water samples of Varavade farm. The TVC of water from the Varavade farm varied from 4.01 to 5.63 log10 CFU mL^{-1}, and showed a statistically significant difference in the *Vibrio* load among DOC 0 to DOC 90 and DOC 120 to DOC 135 of the samples. In Chinchkhari farm, the TVC ranged from 4.75 to 6.89 log10 CFU mL^{-1} in water samples. Heenatigala and Fernando [41] studied the occurrence of bacterial species causing vibriosis in Sri Lankan shrimp pond culture systems and observed that the TVC ranged from 0 to 5×10^3 CFU mL^{-1} in pond waters. Widiyanto et al. [42] assessed the *Vibrio* and heterotrophic microbiota of *Penaeus vannamei*. The results showed that *Vibrio* populations fluctuated greatly, with the population ranging between 1.4–29.0 $\times$ 10^2 CFU mL^{-1}. Gopal et al. [43] recorded that the relative abundance of *Vibrio* species was higher on the west coast farms (about 10^4 CFU mL^{-1} water) compared to the east coast farms (about 10^2 CFU mL^{-1} water). Alagappan et al. [44] also studied the total vibrio counts in the pond water of two ponds, and it was found to be between 1.8×10^2 and 2.6×10^2 CFU mL^{-1}. Thakur et al. [45] reported four types of *Vibrios*, i.e., *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum,* and *V. vulnificus* in the total CFU values ranged from 1.8×10^1 to 7.8×10^4 CFU mL^{-1} for shrimp pond waters. In Indonesia, it was found that vibrio count peaked at day 60 at a concentration of 1.4×10^4 CFU mL^{-1} in the pond water samples [29]. Alagappan et al. [44] also noticed counts in the pond water of two ponds between 1.8×10^2 and 2.6×10^2 CFU mL^{-1}. However, compared to these studies [29,41–45], the vibrio count in the present study was on the higher side, ranging from 10^4–10^6 CFU mL^{-1} of water samples for both the farms along the Ratnagiri coast. This higher count might be due to the difference in the interval of water exchange, aeration and organic matter on the bottom of the pond that triggers the growth of the bacteria.

4.4. Quantitative assessment of vibrios in sediments

The TVC of sediment samples collected from different ponds of Varavade farm ranged from 4.64 to 6.56 log10 CFU g^{-1}, while in Chinchkhari farm, the values ranged from 5.16 to 6.70 log10 CFU g^{-1}. The presence of *vibrios* ranging from 3.0×10^3 to 1×10^6 CFU g^{-1} at 80 DOC was reported in sediments of different farms in Indonesia [46]. Ganesh et al. [47] reported the values of *Vibrio cholerae* as 1.9×10^7 CFU g^{-1} at DOC 25 and 2.3×10^7 CFU g^{-1} at DOC 150 and for *Vibrio parahaemolyticus* as 1.3×10^7 CFU g^{-1} at DOC 25 and 5.5×10^7 CFU g^{-1} at DOC 150 in the sediment samples in the brackishwater aquaculture ponds from Tamilnadu. In the sediment samples, a relatively low vibrio count (10^4–10^6) was seen in both farms compared to the study of Moriarty [46].

An increase in the organic matter in the pond bottom deteriorates the water quality and increases the turbidity of the pond water. Simultaneously, the feed requirement of the shrimp ponds increases with the progression in days of culture. However, there is also a proportionate increase in uneaten feed and faecal matter at the pond bottoms that may lead to an increase in the bacterial load. All of these byproducts contribute to the increase in ammoniacal wastes that are responsible for the increasing ammonia and other toxic gases in pond water. The toxic gases might be indirectly contributing to the increase in stress on the culture organisms. Vibrio bacteria become opportunistic pathogens for the hosts once the environment becomes favourable for their growth, such as poor water quality, high stocking density, low dissolved oxygen (DO), unutilized feed, high water temperature, low water exchange, high sludge at the bottom, and inhibition of the animals' natural defence mechanisms [48,49].

Leaño et al. [50] quantified and characterized the bacterial biota in the hepatopancreas of pond cultured *Penaeus monodon* juveniles and found that *Vibrio* species (90.12 %) dominated the system. Srinivas and Venkatrayulu [51] observed *Vibrio* infection of 5.2–27.6 % in *P. vannamei* farms of Andhra Pradesh for four cycles of crop production. Vaseeharan and Ramasamy [14] recorded that Monodon Baculovirus (MBV) infected post larvae (PL) and their environment had higher *Vibrio*-like bacteria than uninfected PL and further added that the Artemia nauplii could be the possible source of *Vibrio* in hatcheries. Felix [52], Abraham and Palaniappan [53] reported that disease-affected shrimps were dominated by *Vibrio harvey*i. In the present study, *Vibrio* spp. is also dominated and contained 15.97–63.14 % of the total bacterial load of the Varavade farm. Chinchakhari farm observed a maximum of 64.81 % *Vibrio* spp. of the total bacterial load in the shrimp pond water. Similarly, bacterial count was dominated by *Vibrio* spp. in sediments, showing 12.50–68.42 % of the total bacterial load of the Varavade farm, and up to a maximum of 18.34 % for Chinchakhari farm of the total bacterial count of the pond.

Appropriate shrimp farm husbandry is one of the most essential aspects of sustainable farming practices. The variations in bacterial count observed in both farms are because of their differential managemental aspects. The increased bacteria count in Chinchkhari farm may be due to less water exchange at regular intervals. Less water exchange may accumulate the organic matter at the bottom and deteriorate the water quality thus increasing the bacterial load. Variations might be attributed to the use of probiotics and, lime at variable doses on both farms.

5. Conclusion

Intensification of shrimp culture practices has led to an acceleration in the number and frequency of incidence of diseases. Diseases as a consequence, have become a major hurdle in successful and sustainable shrimp farming. The emergence of microbial diseases in the shrimp farming system has caused severe financial losses to the shrimp farming communities globally. Therefore, it is very important to diagnose different types of diseases so that an effective solution can be brought about the diseases. The present study was carried out to find out the bacterial pathogens associated with the shrimp, *Penaeus vannamei* culture system along the Ratnagiri coast. In this study, it was found that higher stocking density led to an increase in bacterial load. The bacterial load showed a high concentration of vibrio bacteria out of the total bacteria present in both farms. The bacterial load was observed to increase with the progress of the culture period in both farms. Using different probiotics, disinfectants, and water exchange after certain intervals helped in the decrease of the bacterial community. The study suggests strict adherence to the managemental aspects such as water and soil quality management, feeding management, etc.

CRediT authorship contribution statement

Bhavesh Choudhary: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Anil S. Pawase:** Conceptualization, Formal analysis, Investigation, Resources, Supervision, Validation, Writing – review & editing. **Gajanan S. Ghode:** Formal analysis, Investigation, Resources. **Raju M. Tibile:** Formal analysis, Investigation, Resources. **Varsha R. Bhatkar:** Formal analysis, Resources. **Divyashree Choudhary:** Software, Visualization, Writing – review & editing, Conceptualization, Formal analysis. **Utkarsh Choudhary:** Visualization, Software, Writing – review & editing, Conceptualization, Formal analysis.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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