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Profiling of key water quality parameters and major *Vibrio* spp. in selected shrimp (*Penaeus monodon*) farms of Gujarat

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Abstract
The present study was conducted to investigate the physicochemical water quality parameters and major *Vibrio* spp. present in the shrimp (*Penaeus monodon*) farms in Gujarat. Water samples were collected from July to December 2024 from 20 *P. monodon* farms across four districts (five sites per district), including Navsari, Surat, Bhavnagar, and Bharuch districts. The water quality parameters, *viz.,* temperature, pH, salinity, dissolved oxygen (DO), total alkalinity, and total hardness, ranged from 28.00±0.577 to 30.67±0.333 ºC, 7.93±0.055 to 8.33±0.058, 13.00±0.577 to 25.33±0.333 ppt, 131.67±1.667 to 200.00±10.00 mg/L, and 2600.00±115.470 to 5083.33±104.08 mg/L, respectively. The majority of water quality parameters were well within the acceptable range for normal shrimp culture. Five major *Vibrio* spp. *viz*., *Vibrio parahaemolyticus* (VP), *V.* cholerae (VC), *V. vulnificus* (VV), *V. alginolyticus* (VA), and *V. harveyi* (VH) were found present at respective sites. The species-based identification from water samples revealed that the highest number of *Vibrio* present was VP (49), followed by VC (43), VA (30), VH (12), and VV (6).

Keywords: Water quality parameters, *Penaeus monodon*, shrimp aquaculture, *Vibrio* species, Gujarat

Introduction

Aquaculture, the farming of aquatic organisms, is considered one of the most sustainable sources of animal protein and the fastest-growing food production sector. Global demand is expected to increase aquaculture output by nearly 50% by 2050 (U.N., 2009). In India, agriculture and fisheries have long played a vital role in ensuring nutritional security, generating employment, earning foreign exchange, and supporting the livelihoods of millions of people. However, meeting the food needs of a growing population is challenged by expanding population pressure, diminishing natural resources such as land and water, and the impacts of climate change. Declining production trends, the indiscriminate use of scarce resources, and unpredictable seasonal patterns further complicate the situation. Shrimp production remains in high global demand, contributing significantly to the economic growth of developing countries while meeting nutritional requirements and creating employment opportunities (Henchion *et al.*, 2017) [23]. Among crustaceans, penaeid shrimps are the most economically important farmed species. The production of *Penaeus monodon* has varied considerably over the past five years, with a notable decline of 22.04% in 2020-21, likely due to pandemic-related disruptions. Production rebounded from 2021-22 onward, with successive growth rates of 46.72%, 55.68%, and 36.06%, reaching 85,752 metric tons in 2023-24. In India, *P. vannamei* production reached approximately 1,097,481 tons, while *P. monodon* production was estimated at 63,041 tons during 2022-2023 (MPEDA, 2023) [33].

Water quality is a critical factor in *Penaeus monodon* aquaculture, as it directly influences shrimp growth, survival, disease resistance, and the overall sustainability and productivity of the farming system. It also shapes the microbial ecology of the culture environment and affects the health of the cultured species. Maintaining optimal parameters such as temperature, pH, dissolved oxygen, salinity, ammonia, nitrite, and alkalinity is essential for sustaining a stable and beneficial microbial community that can suppress pathogenic organisms and support shrimp health. Poor water quality, including elevated ammonia and nitrite concentrations or reduced dissolved oxygen levels, can disrupt microbial balance and promote the proliferation of opportunistic pathogens such as *Vibrio* spp.,

ultimately contributing to disease outbreaks in shrimp farms. Among water quality parameters, temperature and salinity are considered the most influential factors determining the global distribution of *Vibrio* species (Deeb *et al.*, 2018) [17].

The declining land and water resources prompted food producers and researchers to improve cultivation methods to increase yields per unit area. This push led to the widespread use of aquatic inputs, such as feed and chemicals, which ultimately damaged water bodies. Increased intensification and advanced technologies raise the occurrence of diseases and stress on aquatic animals, disrupting the industry (Thornber *et al.*, 2020) [50]. Viral, bacterial, fungal, and parasitic pathogens affect shrimp. Since shrimp lack an acquired immune system and do not respond to vaccinations, shrimp aquaculture faces more challenges than fish farming (Thornber *et al.*, 2020) [50]. Notably, the highest economic loss was caused initially by white spot syndrome virus (WSSV), followed by bacterial diseases, with an estimated loss of over USD 6 billion to the shrimp farming industry (Lightner *et al.*, 2012) [31]. Among bacterial pathogens affecting shrimp, bacteria in the genus *Vibrio* remain the most significant, causing substantial economic damage to aquaculture (Anh *et al.*, 2010; FAO, 2013) [2, 20]. *Vibrio* species are halophilic Gram-negative bacteria with a single flagellum and a curved rod shape, commonly found in aquatic environments. Furthermore, *Vibrio* spp. is prevalent in aquatic environments, including crustaceans, fish, mollusks, and others (Haque *et al.*, 2023) [22]. Of the 30 *Vibrio* species, twelve are considered human pathogens; among these, *V. cholerae, V. parahaemolyticus*, and *V. vulnificus* are the most frequently reported zoonotic pathogens (Bonnin-Jusserand *et al.*, 2019; Rahman *et al.*, 2020a) [8, 44]. Some strains and species of *Vibrio* are infectious and pathogenic, causing the disease "*Vibrio*sis." Among the major bacterial diseases in crustaceans, *Vibrio*sis is the most common and severe in aquaculture, as opportunistic or pathogenic *Vibrio* bacteria can be devastating (Kumar *et al.*, 2017) [29]. Estimated annual economic losses in India during 2018-19 (in USD million) due to *Vibrio*sis included income loss of 39.93, employment loss of 0.31, and overall economic loss of 40.24 (Patil *et al.*, 2021) [40].

Materials and Methods

Farm Sites: The samples were collected from 20 *Penaeus monodon* farms from four districts of Gujarat: Navsari, Surat, Bhavnagar, and Bharuch. All the selected ponds for sampling were earthen ponds without polyethylene (PE) lining.

Collection of the Sample: Samples were collected randomly from the selected farm locations of Gujarat. All the samples were collected from different farms after 85-100 Days of Culture (DOC) from July to December 2024 (Table 1) from 20 *Penaeus monodon* farms from four districts (five sites from each district), including Navsari, Surat, Bhavnagar, and Bharuch. This is the prerequisite for accurate results that sample collection must be appropriate with a highly aseptic approach, and should avoid any cross-contamination during sampling. The sample tubes and bags were sterilized properly. The water quality parameters were recorded at the sampling sites. Water samples were collected from three different locations of each site into 50 ml sample tubes (Eppendorf, India). Collected samples were transported in an insulated sampling box (Aristo, India) with gel ice packs immediately after collection to maintain a temperature of 4°C.

Water Quality Parameters: According to Abioye *et al.* (2021) [1], the water collection method was carried out. Water temperature, pH, salinity, and dissolved oxygen (DO) were assessed at all sample collection sites using a thermometer, pH meter, and refractometer. The other water quality parameters were DO, total alkalinity, and water hardness, analysed by using Kits (AQUACheck, India).

Identification of *Vibrio* spp.

After collecting all the samples were transported to the laboratory at the College of Fisheries Science, KU, Veraval. The highly sterilized conditions are used during sample collection and processing. The accessories like glassware, scissors, forceps, sample tubes, etc. were sterilized by autoclaving before starting of process. The platform, hands, and other needful things were also sterilized with 70% alcohol before starting the work. 1 ml of water sample was directly added to the 9 ml of Alkaline Saline Peptone Water (ASPW) through a micropipette. All the prepared samples are then placed into the incubator at 37 ◦C for 24 hours to allow enrichment of bacteria (WHO, 1999) [53]. The enriched samples were taken out from the incubator, and streaking was made on the surface of sterile petri plates (90mm, HiMedia, India) containing Thiosulfate Citrate Bile Salts Sucrose (TCBS) medium with quadrant streaking method under the aseptic conditions of laminar airflow. The plates were incubated in an incubator at 37°C for 24 hours. Then, the preliminary examination of the growth characteristics of the grown colonies was observed (color, size, shape) (Plate 3C). Then, the single colonies were picked and streaked on Tryptic Soy Agar (TSA) or Nutrient agar (Hi-media), and the plates were labeled with basic details (sample number, colony number, colony color, and date). Prepared plates were placed in the refrigerator at 4 °C to preserve the colonies for further processes.

Identification of different *Vibrio* spp.

Gram Staining: It is a common process to distinguish two bacterial groups, i.e., Gram-positive and Gram-negative, based on their cell wall characteristics and the colouring of these cells, red or violet. A thin smear was prepared on a clear, sterile glass slide with a targeted colony. It was allowed to dry and fixed with gentle heat. Flooded with Gram's crystal violet for 1 minute and washed with tap water. The smear was again flooded with Gram's iodine and allowed to remain for 1 min. Then, it was decolorized with Gram's decolorizer until the blue dye no longer flowed from the smear, and again washed with tap water. Counter-stained with 0.5% w/v safranin for 20 seconds and rinsed off with water. The slide was allowed to air dry. Examined under a 100x microscope.

Biochemical Tests: The six biochemical tests were performed as per the procedure described by Anupama (2020) [3]. The characterization of different *Vibrio* spp. was done based on Table 2.

Catalase test

It is performed using 3% hydrogen peroxide. A loopful of colonies from a young culture was placed on a clean,

sterilized glass slide and misted with two to three drops of 3% hydrogen peroxide. The result was interpreted based on visual appearance, with a positive response indicated by gas bubble evolution.

Oxidase (Cytochrome oxidase) test (Kovac's method)
A small portion of the colony was scraped using a loop and applied to previously impregnated filter paper with Kovac's reagent (tetramethyl- p- phenylenediamine). The affirmativeness of the test was considered when a blue color appeared within 30 to 60 seconds.

Indole test
The colony of the targeted sample was suspended in tryptone broth and placed for incubation at 37±0.5 °C for 24±2 hours. 0.2- 0.3 milliliters of Kovac's reagent and placed open in the air, and checked result with the appearance of a red color ring.

Methyl red test
The bacterium to be tested was inoculated into MR VP broth and incubated at 37°C for 24-48 hours. Over the 48 hours, the mixed-acid-producing organisms produced enough acid to overcome the phosphate buffer and remain acidic. The pH of the medium was tested by adding 5 drops of MR reagent, and the development of a red color was considered positive, while the yellow color was MR-negative.

Voges-Proskauer (VP) test
The suspected culture was incubated for 48±2 hours at 37±0.5 °C after inoculating it into MR-VP broth. 3 ml of Barrett's reagent A and 1 ml of Barrett's reagent B were added to the tubes and shaken for a few seconds. With the cap off, the media were exposed to oxygen and observed for the next 15-30 minutes. The appearance of the red color indicated a positive result.

Citrate utilization test
Simmons citrate agar slant was prepared and gently inoculated with a colony by delicately pressing the tip of the needle to the slant and incubating for 18-24 hours at 37 °C. If the organism can utilize citrate, the medium changes its color from green to blue.

Statistical analysis
The experiment was conducted using a completely randomized sampling. The bacterial identification data were analyzed by using Microsoft Excel. The water quality parameters and analysis of data were subjected to One-Way Analysis of Variance (ANOVA) using SPSS software to determine significant differences among treatment groups. A Duncan's Multiple Range Test (DMRT) was applied for post-hoc comparison of mean values. The level of statistical significance was set at $p<0.05$.

Results

Water Quality Parameters of Navsari District
Sampling from five different locations of Navsari was Samapor (M1), Karadi-1 (M2), Karadi-2 (M3), Matwad-1 (M4), and Matwad- 2 (M5), taken on the 7th of July, 2024. The water quality data are presented in Fig. 1. All the recorded quality parameters were non-significant ($p>0.05$) between the sites of Navsari, and also well within the culturable limit for *P. monodon*. The range of water quality parameters, temperature, pH, salinity, total alkalinity, total hardness, and DO of the sites was 28.70±0.333 to 29.33±0.333 ºC, 8.00±0.058 to 8.32±0.137, 24.33±0.333 to 25.33±0.333 ppt, 166.00±3.055 to 175.67±3.84 mg/L, 4900.00±173.20 to 5060.00±196.72 mg/L, and 5.00±0.058 to 5.10±0.058 mg/L, respectively.

Water Quality Parameters of Surat District
Five different locations from Surat district, Koba-1 (M6), Koba-2 (M7), Koba-3 (M8), Mandroi-1 (M9), and Mandroi-2 (M10) were collected on September 12, 2024. No significant differences ($p<0.05$) were observed in the case of water quality parameters between all sites in Koba, but there were significant differences from Mandroi farms (Fig. 2). The water temperature and pH were observed to range from 28.00±0.577 to 30.67±0.333 °C and from 7.90±0.058 to 8.17±0.033, respectively. Where the farms of Koba showed significantly higher temperatures and pH compared to Mandroi, in contrast, significantly lower dissolved oxygen was observed (4.90±0.058 mg/L) at Koba compared to Mandroi (5.83±0.176 mg/L). The water salinity, alkalinity, and hardness were observed as non-significant ($p>0.05$) in all the farms with a range of 13.00±0.577 to 15.33±0.333 ppt, 189.33±0.667 to 200.00±10.00 mg/L, and 2600.00±115.470 to 3066.67±66.667 mg/L, respectively.

Water Quality Parameters of Bhavnagar District
The samples for water quality parameters were collected from five sites in Bhavnagar district: Kotada-1 (M11), Kotada-2 (M12), Gundala-1 (M13), Gundala-2 (M14), and Jashwantpara-1 (M15) on November 15, 2024, as presented in Fig. 3. The temperature and pH values ranged from 28.33±0.33 to 30.33±0.33 ºC and 7.93±0.055 to 8.33±0.058, respectively. The farms of Kotada showed the lowest temperature (28.33±0.333 ºC) and pH (7.93±0.055) compared to all other farms. Whereas Jashwantpara farms showed significantly higher temperature (30.33±0.333 ºC) and pH (8.33±0.058). The DO values showed an inverse relationship with temperature, as farms of Kotada showed significantly higher DO (5.20±0.058 mg/L) and significantly lower (4.90±0.058 mg/L) DO at Jashwantpara farms. Although levels of alkalinity varied modestly between sites, with a range of 131.67±1.667 to 158.00±4.726 mg/L. The observed range of salinity and hardness was between 18.00±0.577 to 21.67±0.882 ppt, and 3676.67±116.237 to 4400.00±57.735 mg/L, respectively.

Water Quality Parameters of Bharuch District
Hansot-1 (M16), Hansot-2 (M17), Hansot-3 (M18), Vansnoli-1 (M19), and Vansnoli-2 (M20) are the five sites in Bharuch district used for the sample collections. All samples were collected on the 6th of December 2024. The water temperature, pH, alkalinity, and DO were observed to be non-significant ($p>0.05$) between all the sites, with a range of 28.33±0.333 to 29.00±0.577 ºC, 8.17±0.033 to 8.30±0.058, 191.00±2.082 to 198.00±6.110 mg/L, and 5.00±0.058 to 5.17±0.120 mg/L, respectively. Salinity and hardness were significantly different ($p<0.05$) between Hansot and Vansnoli farms. The salinity (25.00±0.577 ppt) and hardness (4960.00±113.725 mg/L) were observed significantly higher in Hansot, whereas salinity (21.00±0.577 ppt) and hardness (4246.67±133.832 mg/L) were observed lower in Vansnoli (Fig. 4).

Identification of *Vibrio* spp.

A total of 200 colonies were isolated from water samples and screened by biochemical tests. 176 colonies were observed as Gram-negative, and from that, 143 were identified as possible targeted *Vibrio* spp. The result of the number of biochemically identified *Vibrio* spp. Presented in Table 3 showed the highest prevalence of *V. parahaemolyticus* (34.26%), followed by *V. cholerae* (30.06%), *V. alginolyticus* (21%), and *V. harveyi* (8.39%); the least observed species was *V. vulnificus* (4.19%).

Discussion

The result of species diversity aligned with the research outcome of Shanmugasundaram *et al.* (2015) [48], who also observed the highest available species, *V. parahaemolyticus*. Chowdhury *et al.* (2024) [15] performed the catalase test, oxidase test, indole, MR, VP, and citrate test, and identified three species of *Vibrio*, *viz.*, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*. Jayasinghe *et al.* (2010) [25] conducted a study regarding the identification of *Vibrio* spp. by using the oxidase test. They identified different species, including *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, and *V. harveyi*. Mohammed *et al.* (2024) [32] used biochemical tests for the identification of seven *Vibrio* spp., where they identified *V. vulnificus* as the second, while *V. parahaemolyticus* was the third most diverse species. Babu *et al.* (2021) and Wei *et al.* (2014) [4, 52] used Gram staining and biochemical tests for the identification of *Vibrio* spp. *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, and *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi* respectively. Dhayanath *et al.* (2019) [18] identified *V. parahaemolyticus* and *V. cholerae* highest prevailing (31.14%, respectively), whereas in contrast, the second highest of *V. vulnificus* (19.67%), followed by 9.8% of *V. harveyi*, and 8.2% of *V. alginolyticus* through biochemical characterization from commercial shrimp farms in Maharashtra and Gujarat.

Temperature is an important environmental factor for shrimp culture due to its influence on the metabolism of the crustaceans (Ferreira *et al.*, 2011) [21]. The water temperature for this study was observed in the range of 28.00±0.577 to 30.67±0.333 ºC. A temperature range of 28 to 32 °C is considered normal for shrimp culture (Boyd, 1989; Carbajal *et al.*, 2011; Hirono, 1992) [9, 13, 24]. Changes in rates of temperature rates can stress shrimp, and consequently, high mortality rates can be present in the population (Navarro *et al.*, 1997) [36]. Temperature controls the solubility of gases, chemical reactions, and the toxicity of ammonia; also, the demand for dissolved oxygen increases when the temperature is high (Martinez, 1994) [31]. Parmar *et al.* (2023) [39] observed a temperature range from 20 to 30 °C in their study and revealed no significant difference in shrimp. Ramanathan *et al.* (2005) [45] noted that the ideal temperature for black tiger shrimp culture is between 26 °C and 30 °C. In the study of Barman *et al.* (2015) [7], they remarked that environmental changes caused the maximum temperature to reach 34.00±1.183 °C, but shrimp cultivation was unaffected because the organisms' optimal growth temperature is 35 °C.

During the water quality analysis, overall water pH ranged from 7.93±0.055 to 8.33±0.058 across all the sites, which falls within the optimum range for shrimp culture. pH is considered one of the important water quality parameters for successful shrimp farming. pH affects biological organisms. Most aquatic organisms cannot survive when the pH exceeds 4 (acid death point) or increases over 10 (alkaline death point) for an hour or more. Most aquatic species thrive at pH levels between 6 and 9, while they may withstand a wider range. Shrimp producers should aim for a pH range of 6-9 in their ponds, with an optimum of 7-8.5 (Boyd, 2001) [11]. In the study of Jiang *et al.* (2020) [26], they observed temperature ranged from 29.10±0.03 to 32.50±0.05 °C, and pH ranged from 6.92 0.03 to 8.00 0.20 in their study, which is more or less similar to our findings. Reddy (2000) [46] recommended that a pH range of 7.5 to 8.5 was the best for *P. monodon* culture. Salinity plays an important role in the physiological functions of cultured organisms. Salinity observed from all sampling sites was well within the range (13.00±0.577 to 25.33±0.333 ppt) for the culture of *P. monodon*. All penaeid shrimps can grow in a wide range of salinities (Paulraj and Sanjeevaraj, 1982) [41]. *P. monodon* is a marine (euryhaline) species. Still, naturally, they also tolerate a wide range of salinity levels (5 to 35 ppt) (Tantulo and Fotedar, 2006) [49], with the optimum range of salinity being from 15 to 23 ppt (Boyd, 1992; Paez, 2001) [12, 38]. The high concentration of salinity reduces dissolved oxygen in water (Paez, 2001). Muthu (1980) [34, 38] and Karthikeyan (1994) [28] also stated that 13 to 35 ppt salinity ranges were ideal for *P. monodon* culture. The salinity ranges they observed of two different farm sites, 27.10±0.09 and 1.75±0.03, revealed less occurrence of *V. parahaemolyticus* in low salinity (Jiang *et al.*, 2020). Najwa *et al.* (2024) [26, 35] noted that environmental parameters like pH (6.14-7.64), salinity (17.4-24 ppt), and temperature (30.8-31.5 °C) remained within acceptable limits for shrimp farming. Nevertheless, they observed a persistent high bacterial load of *V. parahaemolyticus* and *V. cholerae* despite these controlled conditions.

Alkalinity is related to important factors in shrimp culture as the buffer effect on daily variation of pH in the pond, setting the soluble iron precipitated, and in ecdysis (molting) and growth. In the present study, total alkalinity ranged from 131.67±1.667 to 200.00±10.00 mg/L. Waters with total alkalinities less than 15 or 20 mg/liter usually contain less productivity. A total alkalinity of 20 to 150 mg/L is a suitable level for the productivity of aquaculture. A total alkalinity range of 200 to 250 mg/L is also acceptable (Boyd & Pillai, 1985). In the study of Parmar *et al.* (2023) [39], they recorded a 122.60-359.72 mg/L range of total alkalinity. According to Chakravarty et al (2016) [14], in shrimp culture ponds at Narsapurapupeta, Kajuluru, and Kaikavolu villages of East Godavari district, Andhra Pradesh, total alkalinity values ranged between 120 and 500 mg/L, showing higher values than the optimum ranges. Bajaniya *et al.* (2019) [5] observed that alkalinity ranged between 140 and 300 mg/L in shrimp farms in Gujarat, and mentioned that it is within the desirable limit in most of the ponds.

The total hardness was observed with a range of 2600.00±115.470 to 5083.33±104.08 mg/L in the present study. Parmar *et al.* (2023) [39] reported a total hardness range between 2987.00 - 8464.32 mg/L. Bajaniya et al (2019) [5] and Saksena *et al.* (2006) [47] recorded hardness levels of 1990 to 12100 mg/L and 265 and 5945 mg/L, which were also beyond the expected limit. Similarly, Darwin *et al.* (2017) [16] also observed that the hardness of the water fluctuated from 2640 to 6722 mg/L.

The dissolved oxygen was recorded with a range of 4.90±0.058 to 5.83±0.176 mg/L while collecting samples from different locations. Putri *et al.* (2020) [43] also detailed

that the optimal range of 3.8-7.1 mg/L is for shrimp farming. Junda (2018) [27] suggested that dissolved oxygen concentrations between 2.6 and 6.1 mg/L have shown good growth and survival in land-based, extensive shrimp farms. In the study of Bajaniya *et al.* (2019) [5], they recorded 3.5 to 5.3 mg/L from different shrimp farm locations of Gujarat.

Shrimp ponds are more stressful habitats than estuaries or other confined water bodies (Direkbusarakom *et al.*, 1998) [19]. This is mostly owing to high organic matter levels and dissolved oxygen oscillations, which influence the makeup of natural bacterial populations. Under normal settings, temperature increases will result in a larger diversity of *Vibrio* species (Barbieri et al, 1999; Pfeffer *et al.*, 2003) [6, 42]. When high temperatures and salinity conditions prevail, certain species, such as *V. parahaemolyticus*, will dominate (OIE, 2018; Williams and LaRock, 1985) [37, 54].

Changes in water quality can harm the growth and survival of farm-raised shrimp by causing stress and hindering the breakdown of organic matter at the pond's bottom. The study emphasized that even within permissible ranges, variations in temperature, pH, and especially salinity significantly influence bacterial proliferation. Therefore, careful regulation of water quality parameters, particularly maintaining stable salinity and optimal pH, is essential in reducing the bacterial load and managing disease risks in shrimp farming operations (Jiang *et al.*, 2020) [26].

Conclusion

The recorded water quality parameters across all sampling sites largely remained within the optimal range required for healthy shrimp growth, indicating stable environmental conditions during the culture phase. Despite this, the detection of five pathogenic *Vibrio* spp. underscores the persistent risk of bacterial prevalence in shrimp aquaculture systems. Among the isolates, *V. cholerae* was the most dominant, followed by *V. parahaemolyticus*, *V. alginolyticus, V. harveyi*, and *V. vulnificus*. The presence of these species highlights the need for continuous monitoring and early detection strategies to prevent potential disease outbreaks. Overall, the findings emphasize the importance of integrating routine water quality assessment with microbial surveillance to ensure sustainable shrimp farming practices in the region.

Table 1: Sample collection: Date of sampling, sampling sites, and type of samples

Sample No.	District	Location	Sample type	Sample Collection Date
M1	Navsari	Samapor	Water	07-07-2024
M2		Karadi-1	Water	07-07-2024
M3		Karadi-2	Water	07-07-2024
M4		Matwad-1	Water	07-07-2024
M5		Matwad-2	Water	07-07-2024
M6	Surat	Koba-5	Water	12-09-2024
M7		Koba-6	Water	12-09-2024
M8		Koba-7	Water	12-09-2024
M9		Mandroi-1	Water	12-09-2024
M10		Mandroi-2	Water	12-09-2024
M11	Bhavnagar	Kotada-1	Water	15-11-2024
M12		Kotada-2	Water	15-11-2024
M13		Gundala-1	Water	15-11-2024
M14		Gundala-2	Water	15-11-2024
M15		Jashwantpara-1	Water	15-11-2024
M16	Bharuch	Hansot-1	Water	06-12-2024
M17		Hansot-2	Water	06-12-2024
M18		Hansot-3	Water	06-12-2024
M19		Vansnoli-1	Water	06-12-2024
M20		Vansnoli-2	Water	06-12-2024

Table 2: Characteristics of different *Vibrio* spp. for TCBS, Gram staining, and biochemical tests

Colony morphology	*Vibrio* spp.				
	V. parahaemolyticus	***V. cholerae***	***V. alginolyticus***	***V. vulnificus***	***V. harveyi***
	Green	**Yellow**	**Yellow**	**Green**	**Green/Yellow**
Gram staining	-Curved-rod (comma)	-Curved-rod (comma)	-Curved-rod (comma)	-Curved-rod (comma)	-Curved-rod (comma)
Catalase	+	+	v	+	+
Oxidase	+	+	+	+	+
Indole	+	+	+	v	+
MR	+	-	+	+	+
VP	-	v	+	-	-
Citrate	-	v	+	-	v

+(positive), -(negative), v(variable)

Table 3: Number of biochemically identified isolates

***Vibrio* spp.**	**Number of biochemically identified isolates**
V. parahaemolyticus	49
V. cholerae	43
V. alginolyticus	30
V. harveyi	12
V. vulnificus	6
Total *Vibrio spp.*	143

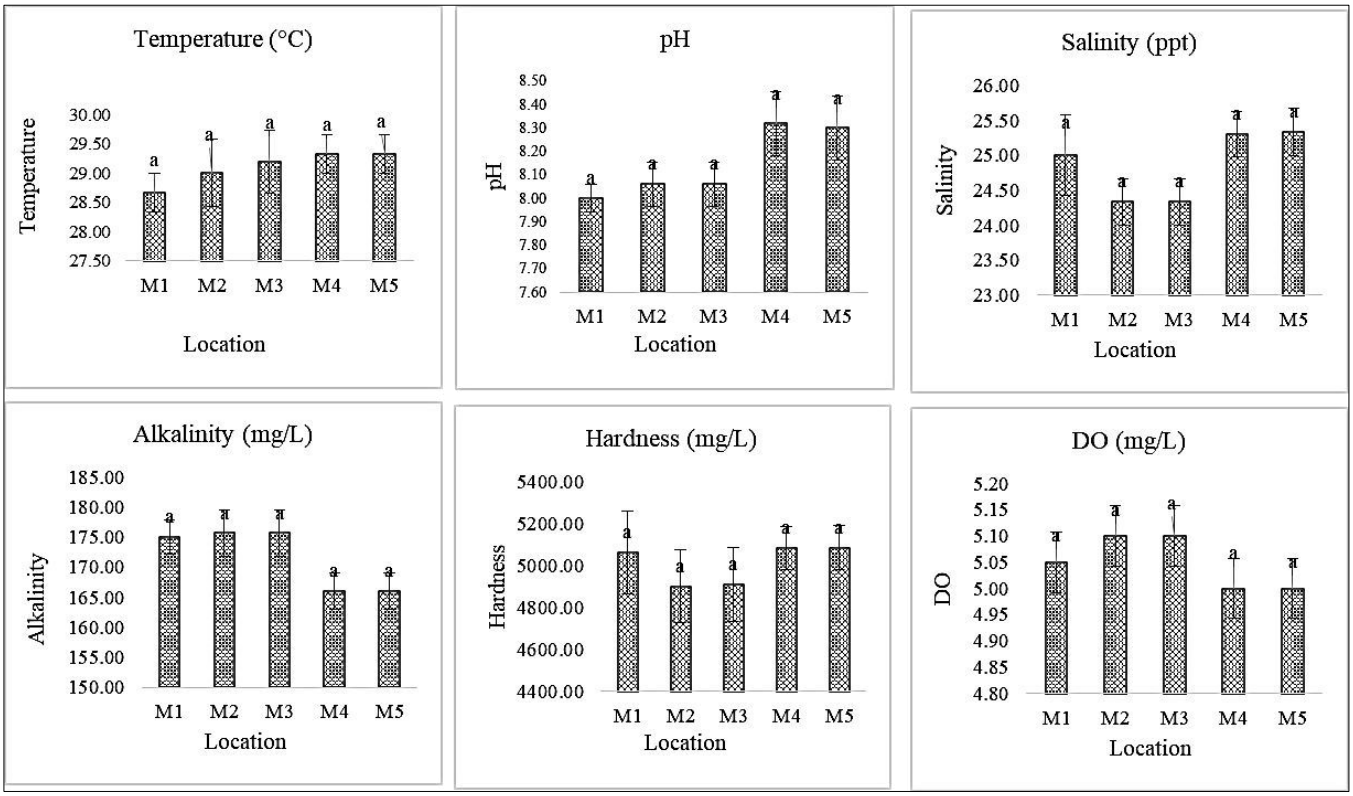

Fig 1: Water quality parameters of Navsari district

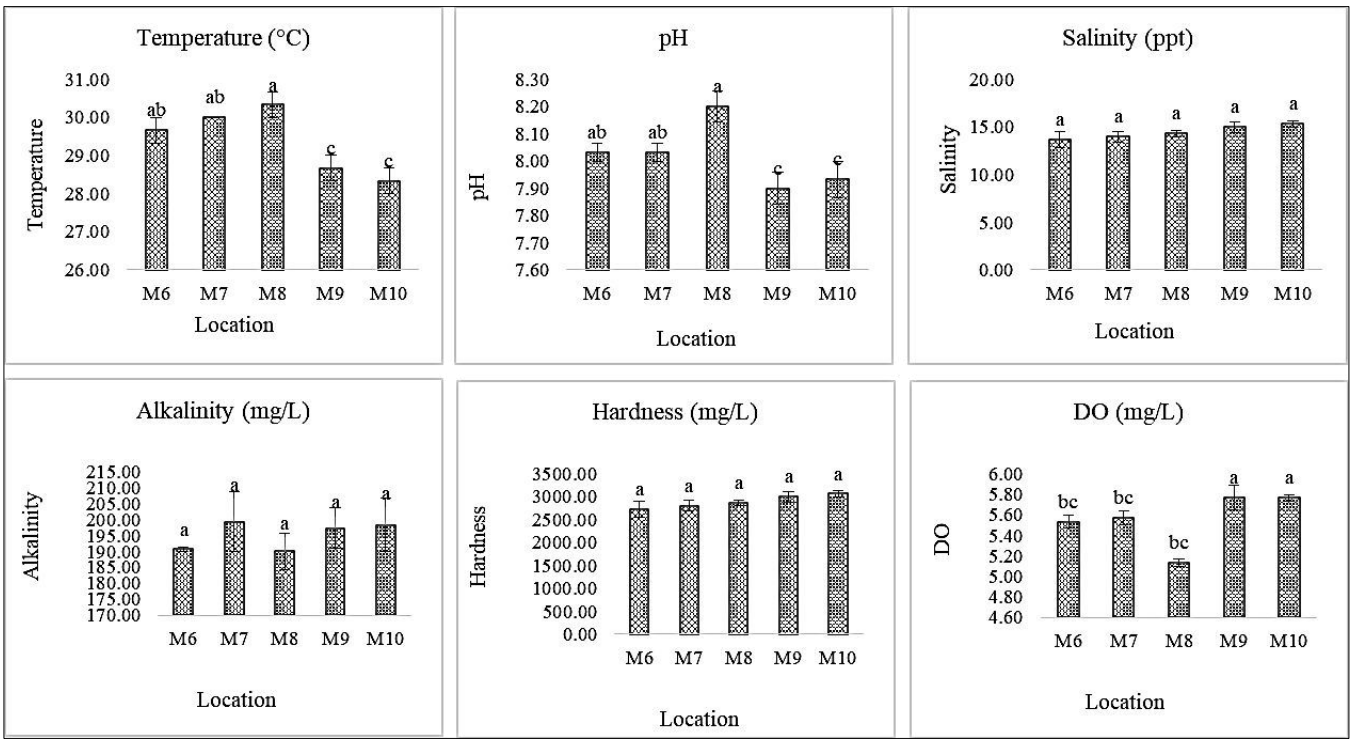

Fig 2: Water quality parameters of Surat district

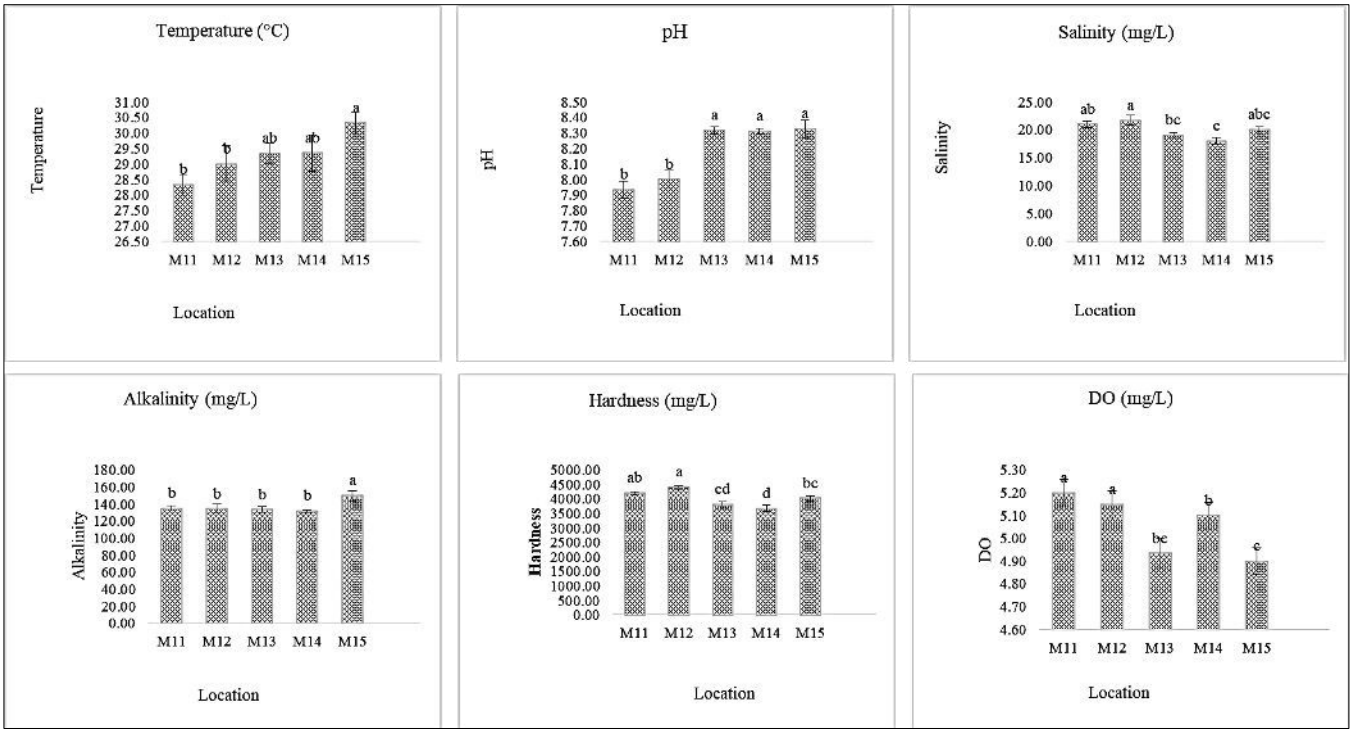

Fig 3: Water quality parameters of Bhavnagar district

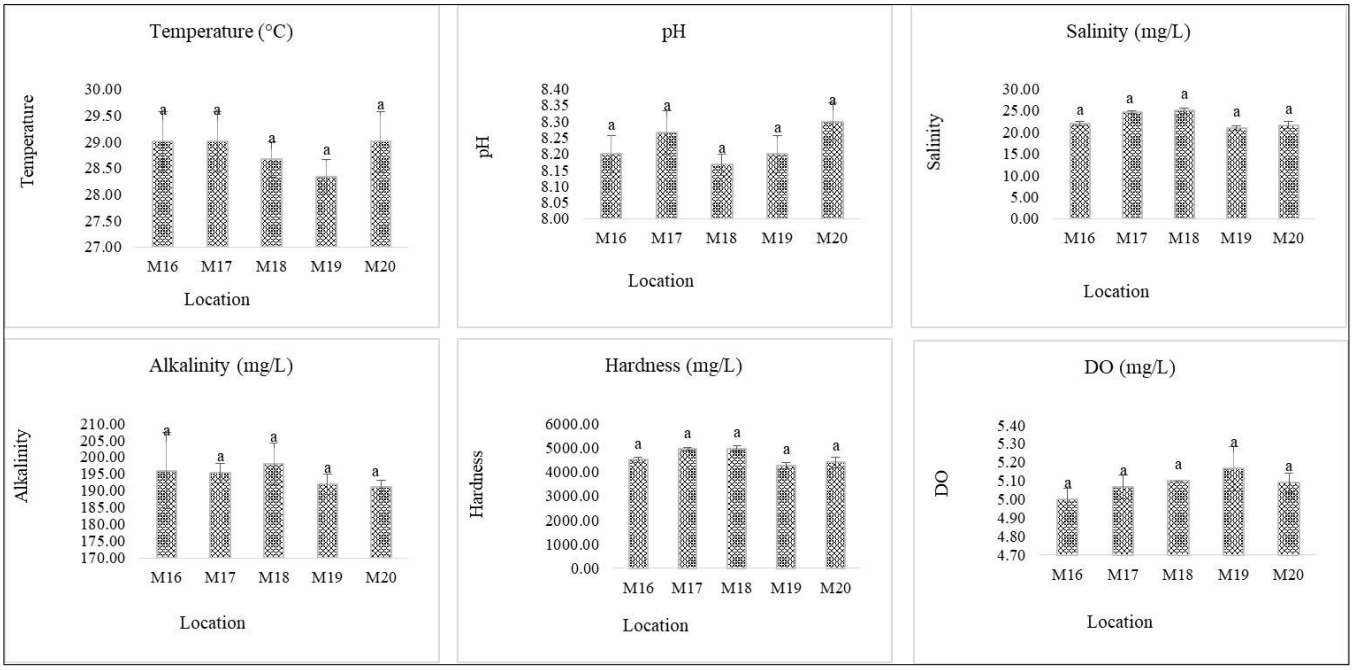

Fig 4: Water quality parameters of Bharuch district

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