Profile Of Bacterial Composition In Relation To Water Quality Parameters In A Brackish Water Aquaculture System

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Abstracts:
Microorganisms especially bacteria play a vital role in aquaculture system. Naturally occurring bacteria are of both beneficial as well as harmful to the culture organisms. The present study aims at screening the total microbial load and other pathogens to illustrate the common microbes usually present in the aquaculture pond and to understand their role in fulfilling the growth and healthy yield of shrimps. In this context, it is imperative to have regular monitoring of microbial composition in relation to essential water quality parameters for predicting the development of aquaculture industry. This study was carried out in a demonstrate-shrimp aquaculture system of CAS in Marine Biology located at the intertidal region of Vellar estuary. The physico-chemical parameters (Temperature, Salinity, pH, Dissolved Oxygen and Ammonia) were found in the normal range that was suitable for shrimp culture system. The correlation/regression was made between the inter-related parameters in source and pond water to know their influence over others and it was noticed that temperature and salinity ($R^2 = 0.69$; $R^2 = 0.75$) as well as ammonia and pH ($R^2 = 0.286$; $R^2 = 0.601$). ANOVA was also made between the source and pond waters for the parameters examined and it was observed that all the parameters between source and pond waters were found insignificant ($p > 0.05$) except ammonia that showed a significant variation ($p<0.05$) between the source and pond waters. In the present study, the microbial load was noticed with normal range and not shown any microbial diseases in the cultured shrimp. In the source waters, the TPC was found in the range of $9.9x10^{-1}.8x10^{2}$ CFU/ml/10^{-3}, *Vibrio* sp was observed in the range of 72-96 CFU/ml, *Escherichia coli* was found in the range of 102-132 CFU/ml and *Staphylococcus* sp. was noticed in the range of 36-44 CFU/ml. In the pond waters, the TPC was found in the range of $7.4x10^{-1}$-$2.9x10^{2}$

CFU/ml/10^{-3}, *Vibrio* sp was observed in the range of 28-57 CFU/ml, *Escherichia coli* was found in the range of 44-204 CFU/ml and *Staphylococcus* sp. was noticed in the range of 18-76 CFU/ml.

Keywords: Shrimp aquaculture, Physico-chemical parameters, TPC, Pathogens

1.INTRODUCTION

Aquaculture is a substantial force for meeting the demand of food supplementary for the growing population. The unsuitable coastal lands for doing agriculture could be converted for aquaculture farming which plays an equal role in providing nutrient supplementary to the people as that of agriculture farming. Swampy areas or soils with high salt or heavy clay content are found to be suitable for brackish water aqua farming particularly, shrimp culture.

The Asian region accounts for at least 83% of global aquaculture production. Two hundred thousand hectares of brackish water area is available in Tamil Nadu (Santhanakrishnan, 1987) of which only twenty-seven thousand hectares are being used for potential shrimp aquaculture. Microorganisms, especially beneficiary bacteria play a vital role in the aquaculture sector since the health of the culture organisms is determined by the eco-friendly microorganisms that are assisting to improve their immune system. Meanwhile the heterotrophic bacteria decompose the organic matter (excess feed) which results in recycling of nitrogen and phosphorous responsible for influencing the productivity. Both beneficial and harmful microbes (pathogens) are being proliferated naturally but the domination of beneficial microbes should be ascertained for better yield. Microbial diversity is an integral part of aquaculture system and has direct impact on productivity in addition to artificial feed. The dissolved oxygen, ammonia and pH are governed by bacteria and hence the aquaculture system could not be successful without the role of bacterial population (Moriorty, 1997).

Therefore, it is essential to have better management of microbial food web in aquaculture to optimize the production. Apart from the indigenous bacteria in brackish water, provision of shrimp feed and high stocking density leads to high bacterial population stimulating diseases for culture animals. Further, it was reported that the dominant roles in the spoilage of aquaculture system are mainly due to bacteria (Palaniappan, 1982). So, the pond management regarding regularization of bacterial load is to be given priority. In the environment as well as culture ponds Vibrio's and Coliforms are responsible for the disease outbreaks.

The brackish water aquaculture system that was selected for the present study is a demonstration pond of CAS in Marine Biology, located at the bank of Vellar estuary and opposite to Marine Biological station. The estuarine water is found to suitable for shrimp culture and as result many private aqua farms are located at both sides of Vellar estuary. In this estuary, environmental variables are also ideal which are found to be the added strength to improve the aquaculture system in this region. The temperature, salinity, pH, dissolved oxygen, rain fall and nutrients of this environment are the factors which mainly influence the production and successful growth of plankton. The changes in the physico-chemical and biological parameters of this estuary are being regulated by regular tidal action (R. Chandran, K. Ramamoorthi, 1984). In southeast coast of India, particularly Cauvery River basin at near shore is developed with number of aquaculture farms due to swampy nature since these areas are not suitable for agricultural cultivation. Coastal Aquaculture Authority, India has launched several guidelines and special schemes for promoting the fish and shrimp farming along the coastal belt. In the Cauvery basin, Coleroon and Vellar estuaries are well taken for shrimp farming and flourishing since 1990s onwards. Our center's demonstration shrimp farm was selected for the present study which is located at the bank of Vellar estuary because of their ecological importance. The aquatic microorganisms are the immediate responders to these pollutants/contaminants so, their diversity study is considered as good bio-indicators of organic pollution arise out of sewage and or aquaculture wastes. The poor maintenance of aquaculture system would make favorable conditions for harmful

microorganisms, especially pathogenic bacteria to multiply and cause detrimental diseases to the culture animals and also to human beings via consumption. The poor-quality water, if discharged into the natural water that will contaminate the nearby ecosystem and affect the other animals also and hence, this study takes its importance on the ecological point of view and for the development of aquaculture system effectively in this coastal belt without collapsing the aquaculture industry and natural ecosystem. Further this study helps to acquire knowledge on the best bacterial indicators of aquaculture system and make use of bacterial index and contamination factor analysis to predict the range of risk due to microbes to the culture organisms.

2. Materials and Methods

2.1. Study area:

The study was conducted in the demonstration brackish water shrimp culture facility of CAS in Marine Biology located at the intertidal region of Vellar estuary, Parangipettai (Lat; 11^{o} 29'N and Long; 79^{o} 46'E). Parangipettai is a small coastal town, about 30 km south of Cuddalore, situated on the east coast of Tamil Nadu comprising riverine, estuarine, backwater, mangrove and neritic biotopes. The Vellar estuary is the downstream basin of Vellar river that has its source in the Servarayan hills at Salem district, Tamil Nadu and after running through areas of red sand, leached sand, laterized black soil and loamy red soil, forms an estuarine ecosystem here before emptying into the Bay of Bengal and is moderately restricted having average width of 100 meters and average depth of 2-3 meters. This estuary relates to the Coleroon estuary by the backwater network channels forming Vellar-Coleroon estuarine complex that supports the mangrove vegetation (Pitchav aram Mangroves).

2.2. Sample collection:

2.3. Water sample:

Water samples were collected at every week from the source (Estuary) and shrimp culture pond (Demonstration Pond) during the entire culture period (28th July-21st November 2022) for the assessment of the crucial physico-chemical parameters and microbial quality. The sterilized glass containers were used to collect the water samples aseptically during the early hours. Immediately after collection, the water samples were brought to the laboratory for necessary analysis.

2.4. Analysis of physico-chemical parameters in water:

The physico-chemical parameters such as water temperature and pH were measured *in situ* by calibrated instruments of mercury thermometer, pH pen (pH EP-3). The salinity was determined by titration method of using $AgNO_3$ and potassium dichromate and for the assessment of dissolved oxygen, the water samples were collected in DO bottles of 125ml capacities without turbulence and thus collected samples were fixed for DO by adding manganese sulphate followed by alkaline iodide. Winkler's titration method was followed for the assessment of DO (Strickland and Parsons, 1972). Ammonia was assessed by following the method of (Hansen et al.1999)

2.5. Microbiological Analysis:

The Total Plate Count (TPC) and pathogens were evaluated by following the method described in the APHA (2012) manual.

2.6. Serial dilution:

Serial dilution involves the process of taking a sample and diluting it through a series of standard volumes of sterile diluents, which can either, be distilled water or 0.9% saline. Then, a small, measured volume of each dilution is used to make a series of pour or spread plate containing Nutrient Agar. Serial dilution (10^{-1},10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) of water samples was prepared using sterile 50% estuary water and 0.1ml of diluted sample was spread on a petri plates.

2.7. Total Plate Count:

About 0.1ml from the appropriate desired dilution series was poured onto the center of the surface of agar plates and spread the sample evenly over the surface of the agar using L-shaped sterilized glass rod. The plates after inoculation were incubated in the incubator in an inverted position at 28±2^{o}C for 18-24hrs. Duplicate samples were prepared for each dilution.

After incubation, the plates were taken for the determination of total number of colony forming unit (CFU) by calculating the average of each set of duplicates and expressed as CFU/ml of the homogenate and the representatives of the colonies were again sub-cultured for the identification of individual bacteria.

2.8. Morphological characteristics of the isolates:

To identify the bacterial species based on the morphological characteristic features, different selective media such as MacConkey Agar (Coliforms), Eosin Methylene Blue Agar (*Escherichia Coli*), Manitol Salt Agar (*Staphylococcus* sp), XLD agar (*Salmonella* sp*)* TCBS agar (*Vibrio* sp) were used and the pathogens were confirmed.

2.9. Gram staining:

Gram staining is a procedure used to characterize bacteria as Gram-positive or Gram-negative, based on the chemical and physical properties of their cell walls (Bergey et al., 1994). Gram staining was carried out for all the isolates.

A thin smear of the isolate was made with saline in the center of the clean glass slide. The smear was air-dried by gently passing over the flame at low temperature. The smear was flooded with crystal violet for one minute and then washed off gently under tap water. The smear was then de stained by allowing 95% alcohol to flow over it and washed gently under tap water, and counterstained with safranin for 2 minutes. Safranin was washed off with tap water and the slide was dried. The stained smear was observed under the microscope, having 10X and 100 X objective lens. Gram-positive bacteria were confirmed if the primary stain of crystal violet solution is retained and not with safranin, causing the appearance of violet/purple under microscope whereas the bacterial cell that lost the primary stain and retain the secondary stain is confirmed as Gram-negative and the appearance would be red when viewed under microscope.

2.10. Biochemical identification of the isolates:

The isolates were subjected to the biochemical tests such as Indole production test, Methyl red test, Vogues Proskauer test, Citrate utilization test, Catalase test, Gelatinase test, Urease test, Hydrogen sulphide test, Oxidase test, Coagulase test, Nitrate reduction test, Carbohydrate test, Starch hydrolysis test and Glucose utilization test to identify the bacterial species.

2.11. Indole production test:

The prepared peptone broth was sterilized and after cooling, it was inoculated with the culture. The test culture was incubated at room temperature for 24 h. After incubation, 0.2 ml of Kovac's reagent was slowly added to amyl alcohol using a stirrer and then hydrochloric acid was added. The culture was mixed well and left for 5 minutes. The positive result was confirmed after examining for a cherry red colored ring appears at the top of the surface the medium tube.

2.12. Methyl red test:

The prepared MR-VP broth with glucose was sterilized and after cooling, it was inoculated with the culture. The test culture was incubated at 37°C for 48 hrs. After incubation, 5-6 drops of Methyl Red solution containing methyl red and ethanol was added. The culture was mixed well and left for 5 minutes. The positive result was confirmed after examining for a bright red as result of glucose fermentation.

2.13. Voges- Proskauer test:

Pure bacterial culture was inoculated in sterile MRVP broth and incubated at 35-37^{o} C for 24-48 hours. Following the incubation, 6 drops of 5% alpha naphthol followed by 40% of potassium hydroxide were added to the culture broth and shaken vigorously. The positive result was confirmed after examining for the pink color during 30 minutes period.

2.14. Citrate utilization test:

A loopful of bacterial culture was streaked on Simmons citrate agar slant and incubated at 35-37^{o}C for 24-48 hours. The positive result was confirmed after examining for the green to deep blue color at the surface of the test tube.

2.15. Catalase test:

A loopful of bacterial culture was smeared on a sterile glass slide and 2-3 drops of hydrogen peroxide (3 ml of Hydrogen peroxide and 97

ml of Distilled water) were placed on the smeared region. The positive result was confirmed after examining for the formation of immediate bubbles on the smear.

2.16. Gelatinase test:
The test culture was inoculated into a Nutrient gelatin medium by piercing with a straight inoculation needle. The tubes were then inoculated at 37ºC for 24 hours and the tubes were refrigerated for 15 min. The positive result was confirmed after examining for the rapid liquefaction of gelatin appearance.

2.17. Urease test:
A loopful of bacterial culture was streaked on urea slant and incubated at 35-37ºC for 24-48 hrs. A bright pink color appearance would be the confirmation for a positive result.

2.18. Hydrogen sulphide test:
A loopful of bacterial culture was streaked on a slant and stabbed on the bud of SIM agar and incubated at 35-37°C for 24 hours. The tube that is stabbed with the inoculums turns black indicating the positive results.

2.19. Oxidase test:
A loopful of bacterial culture was smeared with 1-2 drops of water on the surface of the oxidase disc. If the disc turns into dark purple color, it indicates the positive result.

2.20. Coagulase test:
A loopful of bacterial culture was inoculated in sterile Muller Hinton broth and incubated at 35-37º C for 24-48 hours before performing the test. After incubation, 2-3 drops of blood plasma were added to the culture broth. If the clot is noticed, it indicates the positive result.

2.21. Nitrate reduction test:
The test culture was inoculated into sterile nitrate broth and incubated at 37º C for 24-48 hrs. After incubation, five drops of sulfanilic acid solution of reagent 'A' and five drops of an α-naphthylamine solution of reagent 'B' were added into the tube containing the culture and then the tube was thoroughly shaken to mix the reagents with the medium. The appearance of red color within a few minutes indicates positive result.

2.22. Carbohydrate test:
A loopful of bacterial culture was streaked on Triple Sugar Iron slant and incubated at 35-37°C for 24-48 hours. The appearance of the red color shows non-fermentation of the culture which is alkaline. The appearance of yellow color shows fermentation of the culture and forms bubbles that are acidic and would be taken as positive result.

2.23. Starch hydrolysis test:
A loopful of bacteria was streaked on starch agar plates and incubation was done at 35-37ºC at 24-48 hrs. If a clear zone is formed, it indicates that the inoculums hydrolysis the starch and that could be taken as positive.

2.24. Glucose utilization test:
A loopful of culture was transferred into a glucose test tube and rotated for proper mixing and then it was incubated at 35-37ºC for 24 hours. The pale greenish yellow and then turns into definite pale red would be taken as positive result.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical parameters:

It is well known fact that the maintenance of physico-chemical parameters is utmost important in the aquaculture system since these parameters determine the health of the water and its quality. The growth and health of culture animals exclusively depends upon the quality of water. In addition to stocking density and excretion of culture animals, the supply of artificial feed would alter these parameters drastically and if there is any variation in their levels, it favors for disease outbreak. So, monitoring their levels and resolving the variation if any is significant to ensure the health of organisms. With this view, the water quality parameters were assessed on weekly basis to understand the levels of these essential parameters maintained in the aquaculture system (Table 1). The correlation/regression was made between the inter-related parameters to know their influence over others (Table 2) and ANOVA was also made between the source and pond waters for the parameters examined and it was observed that all the parameters between source and pond waters were found insignificant ($p > 0.05$) except ammonia that showed a significant variation

($p<0.05$) between the source and pond waters (Table 3).

3.2. Surface water temperature:

The surface water temperature was found in the range of 26.1-31.3ºC with an average level of 29.2ºC in the source whereas in the pond, it was found in the range of 27.4-32ºC with an average level of 30.3ºC. A significant correlation was noticed between temperature and salinity in source and pond waters (R^2 = 0.69; R^2 = 0.75). Water temperature did not show a significant variation ($P>0.05$) between source and pond, but the values observed with slightly higher in pond water when compared to source. These levels were seemed to be ideal for shrimp culture farms since the candidate species is common to tropical seas.

3.3. Salinity:

As far as salinity is concerned, in source water, the values were found to be in the range of 21.6-32.9 psu with an average value of 28.39 psu and in pond water the range value was found within 22.8-34.8 psu with an average value of 29.93 psu. As in the case of temperature, no noticeable variation ($P>0.05$) was found between the source and pond waters but in pond water, the levels were observed slightly higher than source water. It can be attributed that the temperature and salinity in pond water would slightly increase naturally since the water is locked inside a confined area which favored for elevation of these parameters.

3.4. pH:

pH indicates the acid-base balance in the water and it is vital parameter for better growth and survival of culture animals. In source water, the values were found to be in the range of 7.7-8.4 with an average value of 8.13 and in pond water the range value was found within 7.7-8.8 with an average value of 8.12. In contrary to records of temperature and salinity, the average pH level in pond water was noticed with slightly lower when compared to source water. No significant variation (ANOVA) was found ($P>0.05$) between the source and pond waters. The report of Ekubo and Abowei (2011) indicates that at lower pH (4-6.5), the growth of culture organisms got retarded due to stress factor. As noticed in the present study, Pooja and Anita (2013) have suggested that the ideal pH for aquaculture system might be maintained at 7-8.5. The studies made by Mauri and Añón (2006) had a good agreement with the present study as the levels were found with desirable conditions.

3.5. Dissolved Oxygen:

The dissolved oxygen was recorded in the range of 2.9-4.65mg/l in source water with an average level of 3.92mg/l while the levels were recorded in the range of 2.7-4.5mg/l with an average level of 3.76mg/l in pond water. Wu et al. (2009) have described that dissolved oxygen is easily saturated with reference to higher temperature. The rapid decrease of dissolved oxygen in the pond would be accelerated because of the density of culture organisms and organic matters. But despite these factors, the normal level of DO was found maintained in the pond could be due to the operation of artificial aerators. Bhatnagar et al. (2004) suggested that 1-3mg/l of DO has sub lethal effect on growth and feed utilization. No significant correlation was noticed between temperature and dissolved oxygen in source and pond waters (R^2 = 0.082; R^2 = 0.041) and it might be due to keeping the pond water aerated through artificial aerators. No significant variation (ANOVA) was found ($P>0.05$) between the source and pond waters. Though dissolved oxygen level showed a slight shortfall in the pond waters but it was maintained in the acceptable range due to the above said reason. It was confirmed since there was no evidence on poor feed intake, reduced growth rate, mortality, behavioral and physiological changes of culture organisms.

3.6. Ammonia:

The ammonia level was recorded in the range of 0.01-0.16µmol/l in source water with an average level of 0.07µmol/l while the levels were recorded in the range of 0.03-0.52µmol/l with an average level of 0.23µmol/l in pond water. Ammonia is by-product that is liberated due to decomposition of excess feed, fecal matters and other organic matters by heterotrophic bacteria. Temperature and oxidation / reduction process accelerate the ammonia in the aquatic ecosystem. Un-ionized ammonia is highly toxic than ionized ammonia. According to EIFAC (1970), 0.6 to

2ppm of un-ionized ammonia is lethal to culture animals. Mauri and Añón (2006) recommended that the level of ammonia in shrimp culture farms should be less than 0.01ppm. In the present study, the levels of ammonia were found to be well below the threshold limit as the values were recorded in microgram/liter and not in milligram/liter. A significant correlation was noticed between ammonia and pH in source and pond waters ($R^2 = 0.286$; $R^2 = 0.601$) and it might be due to the excretion of culture organisms and a significant variation for ammonia between source and pond waters was observed (ANOVA- $P<0.05$)

3.7. Microbial component:

The water samples collected from both pond and source were screened for bacteria and identified based on morphological, cultural, biochemical, and physiological characteristics according to Bergey's Manual of Determinative Bacteriology (Buchanan et al., 1974). The Colony Forming Unit was calculated for TPC and other bacteria by enumerating in the respective selective media. In the source waters, the TPC was found in the range of $9.9x10^{-1}1.8x10^{2}$ CFU/ml/10^{-3}, *Vibrio* sp was observed in the range of 72-96 CFU/ml, *Escherichia coli* was found in the range of 102-132 CFU/ml and *Staphylococcus* sp. was noticed in the range of 36-44 CFU/ml. In the pond waters, the TPC was found in the range of $7.4x10^{-1}$-$2.9x10^{2}$ CFU/ml/10^{-3}, *Vibrio* sp was observed in the range of 28-57 CFU/ml, *Escherichia coli* was found in the range of 44-204 CFU/ml and *Staphylococcus* sp. was noticed in the range of 18-76 CFU/ml (Table 4).

In Indole production test, a cherry red colour was obtained in the tube which indicated the positive result for the test. Methyl red test was found with bright red colour which showed the glucose fermentation activity. Pink colour appearance in the Vogues-Proskauer test was found and that was confirmed for the positive result. Catalase test was noticed with the brisk effervescence when Hydrogen Peroxide solution was placed on the smeared culture on the glass slide and that was confirmed as positive. Similarly, oxidase test also performed on the glass slide that was found turned into blue indicating positive result. The tube with inoculums turned into black showed the positive result of hydrogen sulphide production test. In Nitrate reduction test, the inoculums developed with red colour indicating the positive result. In Citrate utilization test, the colour changed into blue colour due to the enzyme secretion. In casein presence test, formation of a clear zone around the organism was noticed and that was confirmed for the positive result. Likewise, in gelatin test, rapid liquefaction of gelatin was observed and that was confirmed as positive result. The bacteria were identified up to genus level as *Vibrio* sp., *E. coli, and Staphylococcus* sp. following the biochemical tests (Table 5). The genus Salmonella was not identified as the biochemical test showed negative results.

The pathogenic studies in the aquaculture system are of paramount important to yield healthy aquaculture products as well as profitable way. The present investigation, an attempt to reveal the presence of pathogenic bacteria and the results showed that *Vibrio* sp., *E. coli, and Staphylococcus* sp were observed as long-term inhabitants in the Vellar estuary. The present study focuses the need for assessing the microbial component of the aquatic system and aquaculture habitat to produce healthy cultured organisms (Balakrish Nair, Martin Abraham et al., 1980). The bacterial loads might have changed due to the organic matter deposited at the pond bottom (Shamila et al., 1996). In the present study, differential microbial component had been found as indicated by the previous studies that aquaculture system influences the microbial load as it is deposited with unfed feed, excretion and other sources for organic matters.

In the shrimp culture ecosystem, most of the bacteria play a negative role as they compete with shrimps for food and oxygen, causing stress and disease (Moriarty, 1997). Generally, gram-negative bacteria were found to be the dominant forms in the shrimp culture ponds (Sung et al., 2003) as noticed in the present investigation. Among the THB, *Vibrio cholera and Vibrio parahamolyticus* play a vital role in the shrimp culture ecosystem (Ruangpan and Kitao, 1991) as they damage water quality that

are responsible for diseases and mortality of the shrimp since they are dominant pathogens in the coastal pond ecosystem (Aiyamperumal, 1992; Ruangpan, et al., 1994). In this study, the *Vibrio* sp was not identified up to species level and the higher numbers of this species might be due to *V. Parahemolyticus* which is common in this estuarine water. The presence of *E. Coli* is assumed to have come from fecal matters and sewage discharges in nearby human inhabitations and *Staphylococcus* sp also found its way from human interventions to the estuaries and aquaculture systems.

4. CONCLUSION

Bacteria have both beneficial and harmful properties that need to be assessed in a shrimp culture system to ascertain the health of cultured animals. The present study aims at screening the total microbial load and other pathogens in relation to the quality of physico-chemical properties to understand the pond maintenance for yielding healthy and best survival rate of shrimps. The present study was carried out in a demonstrate-shrimp aquaculture system of CAS in Marine Biology located at the bank of Vellar estuary. The quality of physico-chemical parameters (Temperature, Salinity, pH, Dissolved Oxygen and Ammonia) determines the microbial quality of aquatic ecosystem. This study confirmed the normal range that was suitable for shrimp culture system showed good maintenance of culture pond. The TPC, *Vibrio* sp, *Escherichia coli* and *Staphylococcus* sp. were observed with positive results whereas *Salmonella* sp. was noticed with negative results. In the present study, the microbial load was noticed with normal range and not shown any microbial diseases in the cultured shrimps indicating best management practices followed in our demonstrative shrimp culture system.

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S.No	DAYS OF CULTURE	DATE OF SAMPLING	TEMPERATURE °C		pH		SALINITY (psu)		DISSOLVED OXYGEN(mg/l)		AMMONIA (NH4) µMol/l	
			source	Pond	source	Pond	source	Pond	source	Pond	source	Pond
1	0	28.07.22	31.30	31.30	8.30	8.80	31.00	34.30	4.40	4.40	0.07	0.06
2	7	04.08.22	30.80	30.90	8.20	8.80	31.10	33.25	4.65	4.50	0.16	0.16
3	15	12.08.22	30.40	31.00	8.40	8.50	30.00	34.10	4.25	4.25	0.08	0.08
4	25	22.08.22	30.90	31.90	8.20	8.40	31.00	32.50	3.60	3.75	0.09	0.08
5	35	01.09.22	31.00	31.20	8.30	8.20	30.20	31.30	4.10	4.00	0.02	0.07
6	44	10.09.22	30.80	31.80	7.70	7.80	32.90	33.10	3.65	3.60	0.04	0.03
7	50	16.09.22	31.00	32.00	8.20	8.00	31.50	34.80	3.76	3.65	0.08	0.05
8	58	24.09.22	29.90	31.10	8.30	7.90	30.90	32.30	4.30	4.20	0.08	0.25
9	70	06.10.22	26.40	30.60	8.20	7.90	28.80	31.85	2.90	2.70	0.12	0.25
10	81	17.10.22	27.80	30.20	8.10	8.00	26.80	28.00	3.40	3.35	0.03	0.52
11	89	25.10.22	26.10	27.40	8.00	7.80	22.20	22.80	4.00	3.50	0.01	0.44
12	97	02.11.22	28.00	27.80	8.10	7.80	21.60	22.80	3.80	3.10	0.06	0.41
13	110	15.11.22	27.00	29.00	7.90	7.70	25.00	23.10	4.10	4.00	0.07	0.47
14	116	21.11.22	28.50	29.20	8.10	7.80	26.80	27.00	4.20	4.00	0.04	0.32
	MAXIMUM VALUE		31.30	32.00	8.40	8.80	32.90	34.80	4.65	4.50	0.16	0.52
	MINIMUM VALUE		26.10	27.40	7.70	7.70	21.60	22.80	2.90	2.70	0.01	0.03
	AVERAGE VALUE		29.21	30.30	8.13	8.12	28.39	29.93	3.92	3.76	0.07	0.23

Table 1. Essential water quality parameters in source and culture pond waters at the demonstration aquaculture system

S.No	Location	Temp. Vs Salinity Regression	Temp. Vs DO Regression	pH Vs Ammonia Regression
1	Source	R^2 =0.695	R^2 =0.082	R^2 =0.286
2	Pond	R^2 =0.750	R^2 =0.041	R^2 =0.601

Table 2. Regression between the related parameters

S.No	Parameters	F value	Degree of Fraction	P value	Variance
1	Temperature	2.956	24.55	0.098	P>0.05
2	pH	0.046	25.05	0.83	P>0.05
3	Salinity	1.005	24.74	0.32	P>0.05
4	Dissolved Oxygen (DO)	0.680	25.66	0.41	P>0.05
5	Ammonia	10.97	14.34	0.004	P<0.05

Table 3. Analysis of variance (ANOVA) of different parameters between source and pond waters

S. No	Days of culture	Date of sampling	TPC CFU/ml Dilution Factor 10^{-3}		*Vibrio* sp CFU/ml		*E Coli* CFU/ml		*Staphylococcus* sp. CFU/ml		Survival rate
			Source	Pond	Source	Pond	Source	Pond	Source	Pond	
1	0	28.07.22	9.9×10^{-1}	7.4×10^{-1}	80	28	102	44	36	18	-
2	25	22.08.22	1.5×10^{-2}	1.2×10^{2}	72	54	132	96	38	22	92%
3	58	24.09.22	1.8×10^{2}	2.4×10^{2}	84	47	110	152	40	30	88%
4	110	15.11.22	1.2×10^{2}	2.9×10^{2}	96	57	116	204	44	76	84%

Table 4. Results of different bacterial counts in the water samples

S.No	No. of samplings	Gram staining	Indole	MR	VP	Citrate	Catalase	Gelatinase	Urease	Hydrogen sulphide	Oxidase	Coagulase	Nitrate	Carbohydrate	Starch	Glucose	ORGANISM
1	4	-ve	+	+	-	+	+	-	-	-	+	+	+	+	-	+	*Vibrio* sp
2	4	-ve	+	+	-	-	+	-	-	-	-	-	+	+	-	+	*E. coli*
3	4	+ve	-	+	-	+	-	+	-	-	+	-	+	-	-	+	*Staphylococcus sp*

Table 5. Cumulative results of different biochemical tests for confirmation of bacteria