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Research Article

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Qualitative and Quantitative Characterization of Gut Microbiota in *Macrobrachium Malcolmsonii* in Relation to Water Quality Parameters of Lower Anicut (Anaikarai) Thanjavur District, Tamil Nadu

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Keywords*: M. malcolmsonii; Gut Microbiota; Planktons; Water Quality Parameters; Anakarai*

Abstract

Background

The gut microbial community is one of the richest and most complex ecosystems on earth. Aquaculture industry of the world is facing serious problems due to microbial diseases of pathogenic microbes. On the other hand, probiotics are healthy gut microbiota and play an important role in host development and immune system protection. *Macrobrachium malcolmsonii* is a second largest fast growing prawn occurs commonly in Indian rivers, draining into the Bay of Bengal. *M. malcolmsonii* is also abundant in the river Cauvery, a major perennial river of southern India. Phytoplaktons and zooplanktons are biological indicators whereas, water quality parameters determine the diversity of gut microbiota.

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Results

Qualitative and Quantitative analysis of gut microbiota were carried out in adult *M. malcolmsonii* of length (25.09±3.25) cm and weight (12.05±0.52) gm, within 36 hours of capture from the wild. The quantification of 10-5 dilution resulted in 86 colonies. Qualitative examination of gut microbiota reaveled the presence of bacteria belonging to the genera *Escherichia, Bacillus, Pseudomonas, Salmonella, Vibrio* and *Staphylococcus*. Bacillariphyceae, Nostoceae, Diatomea, Surirellaceae, Skeletonemataceae, Fragilariaceae, Brachionidae, Bacillaricaea and Volvocaceae families of phytoplanktons and Daphniidae, Ceratiaceae, Parameciidae and Hexamitida families of zooplanktons where the predominating planktonic community in water medium of the host species. Water temperature of 26±1°C, P^H 7.3±0.2, dissolved oxygen 3.72±0.23, alkalinity 15.33±7.69, salinity 0.053±0.001 and chlorides 16.2±1.73 were recorded for the water medium of the host species.

Conclusion

Significant increase in alkalinity is attributed to the gut associated microbiota, with distribution of pathogenic groups.

Background

Crustacean aquaculture is a very important commercial activity in several countries of Asia and the Americas. Aquatic ecosystem is known to support the range of organisms. A bacterial species of the gut can influence the health of the host. Freshwater prawn *(Macrobrachium malcolmsonii)*, the second largest fast growing prawn occurs commonly in Indian rivers, draining into the Bay of Bengal. *M. malcolmsonii* [1] and is an omnivorous bottom dwelling freshwater prawn. Several microbial pathogens are known to cause disease in cultured crustaceans and these have been reviewed by several workers [2]. Feeding habits, immune system, ontogenic characters are the some of the factors affecting the gut microbiota [3]. Gut microbiota is found to cluster with environmental microbiota and environmental influences [4]. Planktonic population observation may be used as a reliable tool for bio-monitoring studies to assess the pollution status of aquatic bodies [5]. Freshwater zooplanktons play an important role in ponds, lakes and reservoirs ecosystem and food chain [6]. Presence of *E. coli* and pathogenic gut microbiota as *Salmonella Sps.* is ascertained to fecal contamination and environmental pollution [7]. An investigation on gut microbiota of *M. malcolmsonii* was performed and to characterize the presence of pathogenic and non-pathogenic microbiotic communities and determine their causative factors by assessing the biological indicators and abiotic components of the medium.

Materials and Methods

Collection and Transportation of Specimen

Adults of *M. malcolmsonii* (12.05±0.52cm in length and 25.09±3.25gm in weight) were used for the study. The samples were collected from the lower anicut of Cauvery, Anakkari, packed with ice to maintain minimal temperature ambience and transported to the laboratory, examined for gut microbiota within 36 hrs.

Enumeration of Bacterial Isolates in Serially Diluted Gut Homogenate

Intestine of the specimen was removed by using sterile scalpel, forceps and sterile knife. 5gm of each dissected gut tissue was homogenized with 100ml of 0.85% of NaCl. The resulting homogenate was serially diluted at 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively with 0.85% saline. From the diluted sample around 1.0ml of the sample were transferred to petriplate along with 20ml of the nutrient agar was and is rotated both clockwise and anticlockwise. After solidification the plates were incubated at 37°C for 24 hrs and the total number of colonies were calculated. The total bacterial densities were enumerated by spread plate method by using sterile instruments.

Isolation and Qualification of Selected Gut Microbiota

The isolates were inoculated over nutrient agar, skim milk agar, Simmons citrate agar, Mannitol salt agar, Mac Conkey agar, triple sugar iron agar, cetrimide agar and TCBS incubated at 37°C for 24 hours.

Estimation of Water Medium Quality

The water samples were collected from the lower Anicut reservoir. Sampling was done at two different stations of the lower Anicut. Temperature and pH of the water samples were recorded at the site. Random samples of water medium were collected for the study of phytoplanktons, zooplanktons, dissolved oxygen, salinity and alkalinity. Samples for dissolved oxygen were fixed at the site and the water samples were transported in sterile bottles for further analysis in laboratory.

Statistical Analysis

The results of the present work were analyzed statistically by using SPSS statistics version 20 package software [8]. Data were obtained as Mean±S.D of triplicate analysis.

Results

Table- 1 represents the enumerated of bacterial isolates in serially diluted gut homogenate. Table-2 represents the qualitative characterization of microbiota. The results of water quality parameters were represented in table -3. Table-4 represents the distribution of planktonic communities in water medium.

Table1: *Enumeration of bacterial isolates in serially diluted gut homogenate*

Serial dilution	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
No of Colonies	Infinite	231	174	86

Table 2: *Qualitative characterization of gut microbiota*

Bacteria	Characterization
Escherichia coil	+
Bacillius spp.	-
Staphylococcus aureus	-
Salmonella spp.	+
Salmonella spp.	+
Pseudomonas aeruginosa	+
Vibrio spp.	+

+Indicating presence of organisms; - Indicating absence of organisms

Table 3: *Estimation of water quality parameters*

Temperature	26±1.00°C
P^{H}	7.3±0.2 unit scale
Dissolved oxygen	3.72mg/l
Alkalinity	15.33mg/l
Salinity	0.053mg/l
Chloride	16.2mg/l

The results are mean value of triplicate analysis ± standard deviation.

Table 4: *Distribution of planktonic communities in water medium (family level)*

Phytoplanktonic Community	Zooplanktonic Community
Bacillariphyceae	Daphniidae
Nostocaceae	Ceratiaceae
Diatomeae	Parameciidae
Surirellaceae	Hexamitidiae
Skeletonemataceae	
Fragilariaceae	
Brachionidae	
Bacillaricaea	
Volvocaceae	

Plate 1: *Characterization of Bacillius sps in Skim Milk Agar*

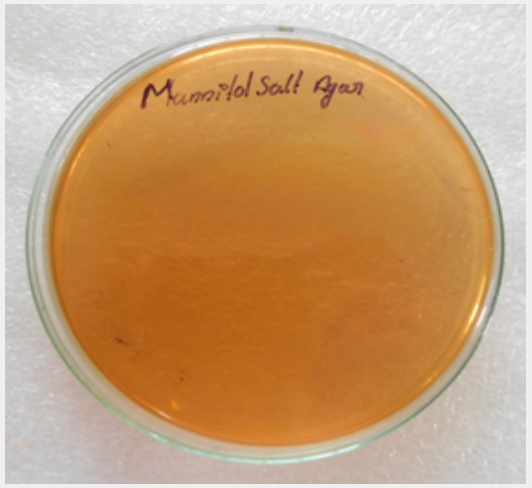

Plate 2: *Characterization of S. aureus in Mannitol agar*

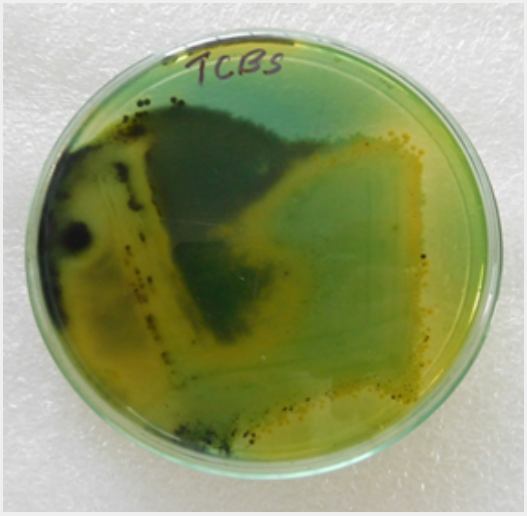

Plate 3: *Characterization of Vibrio sps in TCBS agar*

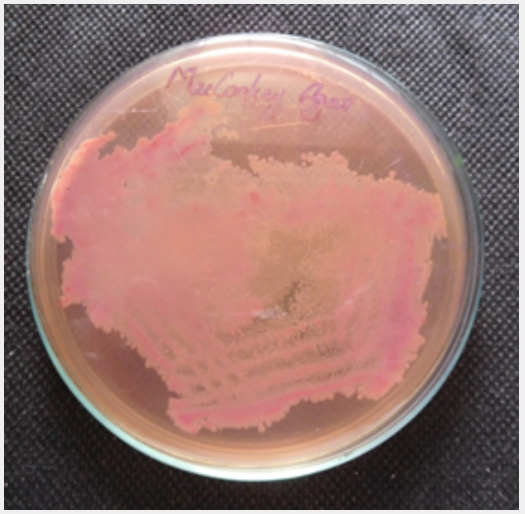

Plate 4: *Characterization of E. coli in MacConkey Agar*

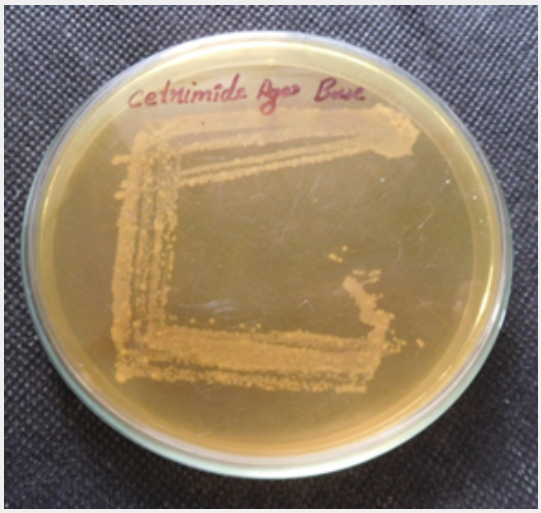

Plate 5: *Characterization of P. aeruginosa in Cetrrimide Agar*

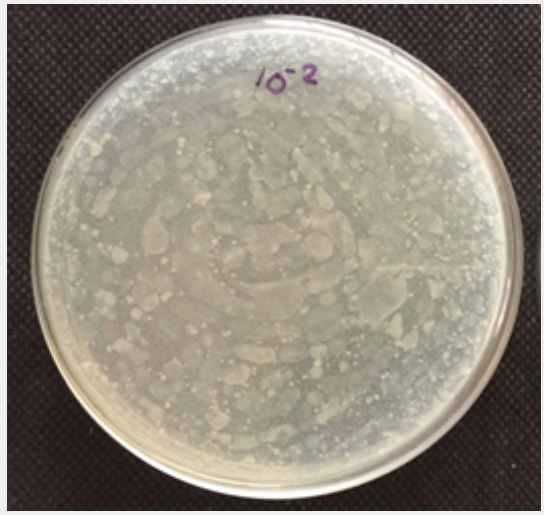

Plate 6: *Enumeration of bacterial isolates in10^{-2} serially diluted gut homogenate*

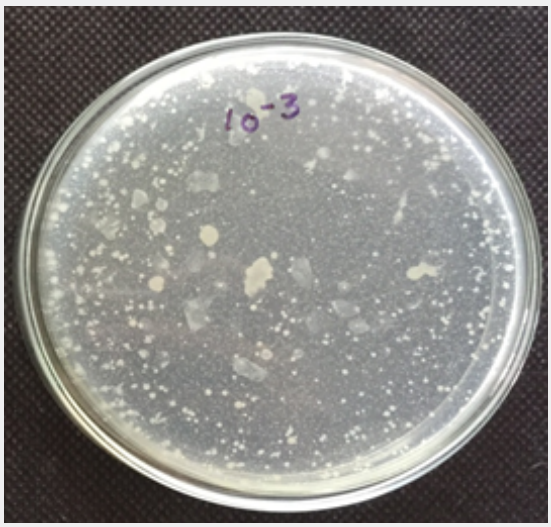

Plate 7: *Enumeration of bacterial isolates in 10^{-3} serially diluted gut homogenate*

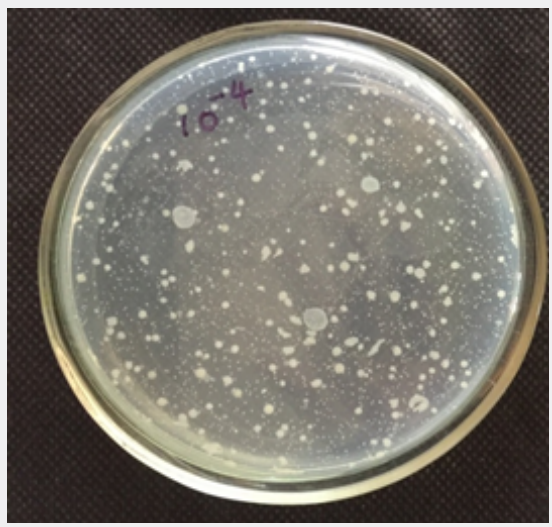

Plate 8: *Enumeration of bacterial isolates in 10^{-4} serially diluted gut homogenate*

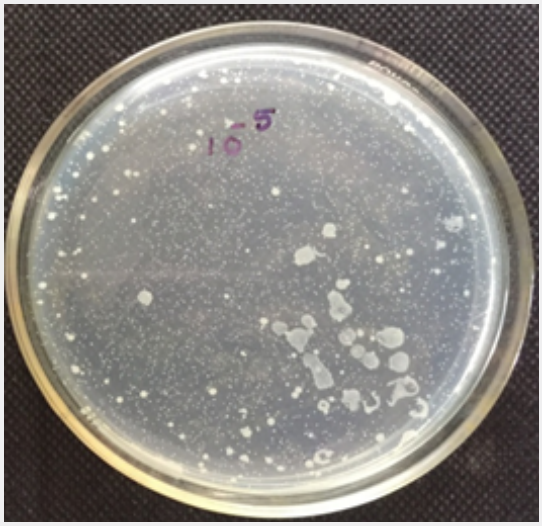

Plate 9: *Enumeration of bacterial isolates in 10^{-5} serially diluted gut homogenate*

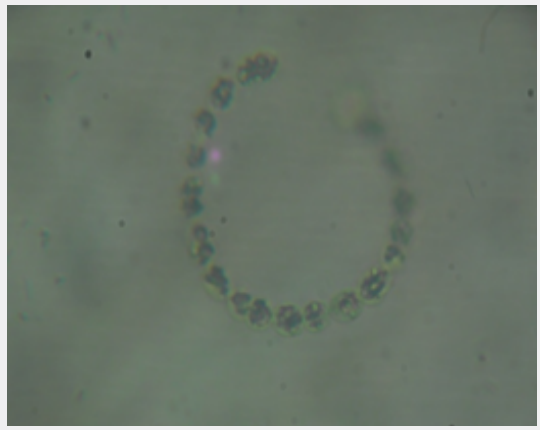

Plate 10: *Nostocaceae*

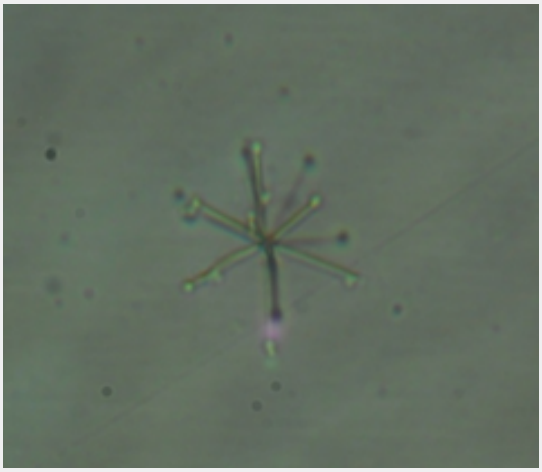

Plate 11: *Fragilariaceae*

Plate 12: *Brachionidae*

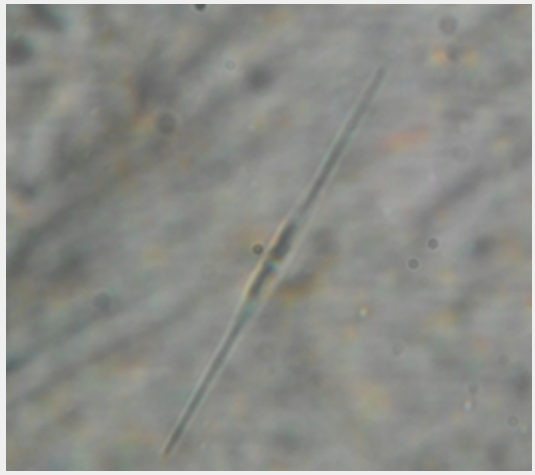

Plate 13: *Bacillariaceae*

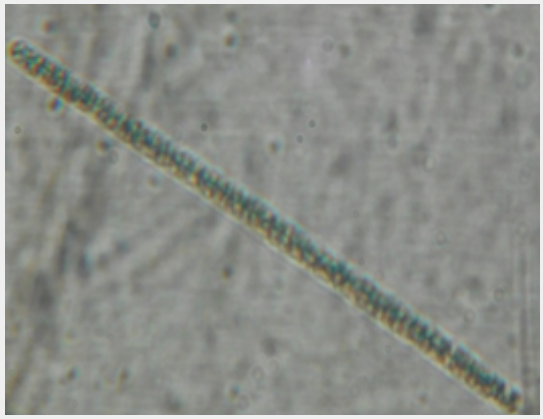

Plate 14: *Skeletonemataceae*

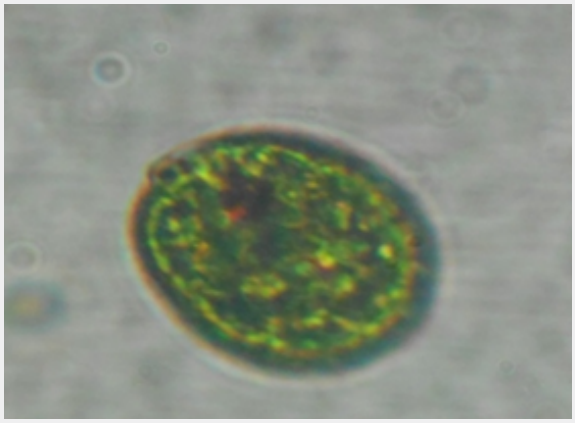

Plate 15: *Volvocace*

Discussion

The intestinal microbiota of animals and humans have attracted much recent attention as it is believed to be a key factor in numerous animal functions including health, growth and disease status [9]. The influence of the gut flora on the host is clearly of great interest in aquaculture, particularly the poor productivity [10]. Marcel *et al.,* (2013) [11] isolated more than one species of bacteria from a single fish, and it is associated with the nature of feed given to the fish, location of sampling sites, nearby human activities and water quality at the fish culture area. Pakingking *et al.,* (2015) [12] reported that the bacterial flora of the fish also reflects the bacterial composition and health status of fish. The significant roles of fish microbiota are to protect the host against pathogenic challenge by production of antagonistic factors, inactivation of pathogenic bacterial toxins or metabolites, stimulation of host immunity and competition with pathogens for attachment sites or nutrients. The pathogenic diseases are usually caused by bacterial species which are facultative pathogenic for both fish and human. In the present study, the species *M. malcolmsonii* were collected and gut analysis is carried out for the isolation and characterization of bacteria. Characterization of gut microbiota in *M. malcolmsonii,* revealed the presence of bacteria belonging to the genera *Escherichia, Bacillus, Pseudomonas, Salmonella, Vibrio* and *Staphylococcus* were identified. Similiarly, a study on bacterial flora is observed in the digestive system of freshwater prawn *M. rosenbergii* showed that the *E. coli* is the prominent bacteria, a reflection on the bacterial flora of the water is suggested by [13]. Vanderzant *et al.,* (1970) [14] reported *S. aureus, Pseudomonas aeruginosa, Enterobacter, Aeromonas* and *Vibrio sps.* as the dominant flora bacteria with E. coli which occupies a less niche in a pond rearing system. *E. coli* is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia*. *E. coli* is often non-pathogenic, although different strains may cause diseases in gastrointestinal, urinary, or central nervous systems in fish reported by Nataro *et al.,* (1998) [15].

Salmonella is a gram negative, rod shaped, pathogenic bacteria of water bodies. *Salmonella* belongs to *Enterobacteriaceae* family. *Salmonella enteritidis* is the main culprit in fishes. The major part of the Salmonella sps. are aquatic environment species; however, fish and fishery products have been renowned as a carrier of food-borne pathogens were reported by *Salmonella* survival in water depends on biological (macro and micro invertebrate) and physical factors (e.g. temperature). Salmonella is associated with organic loading of the aquatic environment and poor water quality [16]. *Vibrio* species are part of the natural microbiota of wild and cultured prawns and become the pathogens for the natural defense mechanisms of the prawn [17]. The high prevalence of *Vibrio*, particularly in the gut, reinforce the opinion while members of this genus are pathogenic, they are not primary pathogens which exist in and around prawns in the environment as part of their normal microbiota [18]. However, Somework on disease syndromes of penaied shrimp have been caused by *vibrio* species which behave more like true pathogens than opportunistic invaders [19]. Thus, the *Vibriosis* is controlled by vigorous water management and sanitation to prevent the entry in the culture water and to reduce stress on the prawns [20].

Bacillius species has been isolated from the gut of marine fish and applied as probiotics. It is a non-pathogenic bacterial species. The term probiotic is currently used to name ingested microorganisms associated which is benefits for humans and animals [21]. Many *Bacillius* strains isolated from marine fish could inhibit potential pathogens [10]. *Pseudomonas spp.* population in the gut of healthy adult freshwater prawn, were observed by [22]. *Pseudomonas* is often encountered in sea water, sediments, phytoplankton and zooplankton [23].

Pseudomonas shows the highest production in the amylase activity in rocky crab *(Plagusia dentipes)* were reported by [24]. *Staphylococcus aureus* is known as the enterotoxin producing agent and a microorganism which is poisonous [25].

Water quality is an important aspect in aquaculture system. Non-optimum water physico chemical parameters (dissolved oxygen, pH, salinity, ammonia, temperature etc.) and poor management practices (overfeeding, inadequate nutrition, overcrowding etc.) can cause stress to the cultured fish and thus make them more susceptible to disease outbreaks [26]. The parameters like temperature, pH, dissolved oxygen, alkalinity, salinity and chlorides were estimated from the water sample.

Fishes are poikilotherms and the body temperature depends on their external environment. Ibrahim *et al.,* (2004) investigated the effect of temperature on the in vitro adhesive ability of potential fish probiotics [27]. Ismaila *et al.,* (2016) performed a comparative study on water quality parameters (temperature, dissolved oxygen, ph, ammonia) between Terengganu river and Pedu lake were reported a significant influence of high water temperature associated with higher number of bacterial isolates from eyes, kidney and brain of cage cultured red hybrid tilapia fish [28]. Temperature is an important physical factor, which influence the other hydrological parameters. In this study, the maximum water temperature (26±1°C) was recorded, where *E. coli* can grow and divide in a wide range of temperatures (20-40°C). The minimum temperature reported for the growth of *Salmonella sps.* is 6.2°C [29]. The climatic warming is one of the reported factor for pathogenic association in water medium [30]. In this study pH (7.3±0.2) was recorded. *Escherichia coli, staphylococci,* and *Salmonella sps.* will grow optimally at pH 7. Similarly, the value of pH 7.6 was recorded by Nambirajan *et al.,* (2012) at lower Anicut Thanjavur District Tamilnadu [29]. Dissolved oxygen is an important chemical factor for respiration which would get influenced by aquatic organisms. The dissolved oxygen of water sample was measured. In this study, the value of dissolved oxygen (3.72±0.23) was recorded from the water sample. *Vibrio* and *Pseudomonas* species correlates with the concentration of dissolved oxygen from the water sample. However, Dissolved oxygen is not found to be a significant factor affecting the distribution of bacterial strains in cage cultured red hybrid tilapia, Oreochromis niloticus and O. mossambicus as reported by [28]. Salinity is the total concentration of dissolved ions in the freshwater. In this study, the value of salinity is (0.053±0.001) was recorded from the water sample, which is compared with normal freshwater value. Alkalinity of water is a major of its capacity to neutralize acids. In this study, the value of total alkalinity is estimated (15.33±7.69) were recorded from the water sample. Chlorides is the indicators of contamination with animal and human waste. The estimation of chloride is estimated by using Mohr's titration method. In this study, the value of chloride is estimated (16.2±1.73) were recorded from the water sample. In this study, the presence of alkalinity is much significant for the growth of bacteria. Physico chemical parameters of the water may influence the density of bacterial population [31]. This study revealed that the physico chemical parameters which influence the presence of various bacteria from the freshwater prawn *M. malcolmsonii.* However, the reports of chlorides, salinity, alkalinity on gut associated microbiota in aquatic species is limited. Similarly, all measured water quality parameters showed their importance in influencing the occurrence of bacteria. Water quality have been identified and discussed as important factors in influencing the presence of non-pathogenic and pathogenic bacteria.

Major sources of water-borne pathogens includes: domestic animals, wildlife, and humans [32]. *Escherichia coli,* a Thermophilic coliform found in all mammal faeces. *E. coli* survives in drinking water for between 4 and 12 weeks, depending on environmental conditions (temperature, microbiota, etc.). Under the conditions in distribution systems, *E. coli* will be much more long-lived [33]. Pathogenic gut microbiota as *Salmonella Sps.* is ascertained to fecal contamination and environmental pollution [7]. The principal habitat of *Salmonella* is the intestinal tract of humans and animals [33]. *Salmonella* are constantly found in environmental samples, because they are excreted by humans, pets, farm animals, and wild life. Municipal sewage, agriculture pollution, and storm water runoff are the main sources of these pathogens in natural waters [1]. Salmonellae do not seem to multiply significantly in the natural environment, but they can survive several weeks in water and in soil if conditions of temperature, humidity, and pH are favorable [33]. However, there is no published data on environmental pollution of the water medium due to industrial contaminants. Inspite, agricultural practices are one of the major livelihood of the people of Anakarai, defining the human source or agricultural source of contamination [29].

Despite the vastly different forms of interactions, symbiotic and pathogenic bacteria have in common that they are adapted to a particular environmental niche represented by the host organism or compartment thereof. Influence of biotic and abiotic fctors on symbiotic association has been reported for *rhizobium sps.* [36]. Dehler *et al.,* (2017) investigated on association between gut microbiota and environmental microbiota stated a difference in microbiotic community of host gut and its environment. Many bacterial species found in the intestine were not detected at in the environmental water samples, suggesting a symbiotic mode of association with the host, which is not possible in free living state on water medium [4,37]. Phytoplankton and zooplankton is called as biological indicators. Barivona *et al.,* (2017) reported that plankton's diversity in fresh waters is very important because most of planktons can be used as environmental indicators [38]. Species that respond predictably to environmental conditions were used as bioindicators for particular variables of aquatic ecosystems, the dynamics of which are related to environmental changes [39,40].

Conclusion

Gut microbiota analysis of *M. malcolmsonii* revealed the presence of *Vibrio, Salmonella, E. coli,* which finds agricultural and fecal contamination as the major environmental sources at Kollidam. However, presence of Bacillus species is attributed to be beneficial for *M. malcolmsonii* as a potent probiotic species enhancing immune tolerance to these pathogenic groups. High alkalinity of the water medium favours survival of the pathogenic as well as non- pathogenic groups.

Abbreviations

TCBS: Thiosulphate Citrate Bile Salts Sucrose
SPSS: Statistical Package for Social Science

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