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Variations in Physicochemical Parameters of Water and Abundance of *Vibrios* Associated with Giant Freshwater Prawn (*Macrobrachium rosenbergii*) in Selected Reservoirs of Sri Lanka

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ABSTRACT

Exhaustive scrutiny of *Vibrios*, one of the dominant autochthonous microbes in the Giant Freshwater Prawn, (*Macrobrachium rosenbergii*), can profoundly influence the production of GFP. This study aimed to enumerate *Vibrio* populations isolated from GFP and examine their relationship with the physicochemical properties of seven reservoirs in Sri Lanka. GFP samples were collected, homogenized, and plated using the spread plate technique on Thiosulfate Citrate Bile Salt (TCBS) agar to isolate Vibrio spp. *In-situ* measurements of water parameters temperature, pH, conductivity, dissolved oxygen (DO), and Secchi disk depth were taken from each reservoir. Chlorophyll-a and organic matter contents were analyzed in the laboratory. Kruskal-Wallis pairwise comparisons were made to identify significant variations in Vibrio abundance and water quality parameters across reservoirs. Regression analyses assessed the relationships between *Vibrio* abundance and environmental factors. Results showed significant differences in Vibrio abundance among reservoirs. The highest mean *Vibrio* count was observed in GFP from Muthukandiya Wewa (8.19±0.74 log CFU g^{-1}), significantly exceeding counts from Bandagiriya, Handapanagala, and Urusita reservoirs. Among the water quality variables, only pH in Urusita Wewa showed a significant positive correlation ($r = 0.740$, $p = 0.006$) with *Vibrio* abundance. The study infers that the abundance of *Vibrios* is high in Muthukandiya Wewa. Furthermore, the abundance of *Vibrios* in Urusita Wewa is regulated by reservoir pH. An assiduous and perpetual analysis of *Vibrio* abundance and factors of lasting repercussions on its abundance helps facilitate mitigation measures to control *Vibrios*.

Keywords: Abundance, dry zone reservoirs, physicochemical parameters, relationship, trophic state index

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INTRODUCTION

Being a notable foreign income generator, the Giant Freshwater Prawn (*Macrobrachium rosenbergii*), is considered a prominent aquaculture commodity in culture-based fisheries (CBF), particularly in dry zone reservoirs of Sri Lanka (Jones et al., 2021). A multifarious assemblage of bacteria, including *Vibrio* sp., is being found in association with the GFP, cultured in both aquaculture settings and wild habitats. The genus *Vibrio* is responsible for causing a multitude of disease conditions in GFP, especially the luminescent larval syndrome (Rao et al., 2015; Farook et al., 2019; Hooper et al., 2023). The presence of *Vibrio* alone does not imply disease conditions, but they are a part of the natural microflora of GFP. Studies have found a diverse array of *Vibrio* sp. from diseased and healthy GFP. For instance, *V. cholerae and V. vulnificus* have been isolated from farms and hatcheries in China in diseased GFP (Gao et al., 2019) and diseased zoea larvae of GFP (Li et al., 2019), respectively. Moreover, *V. parahaemolyticus* (Khuntia et al., 2008; Rao et al., 2015; Tiruvayipati and Bhassu, 2016) and *V. alginolyticus* (Jayaprakash et al., 2006a; Jayaprakash et al., 2006b; Krishnika and Ramasamy 2013; Ajadi et al., 2019; Li et al., 2020) have also been isolated from GFP. Also, plenty of *Vibrio* sp., namely *V. nereis, V. mediterranei, V. fluvialis, V. proteolyticus,* V. *splendidus* (Jayaprakash et al., 2006b), *V. harveyi* (Ahmmed et al., 2020; Sumon et al., 2018), *V. vulnificus* (Jayaprakash et al., 2006b; Krishnika and Ramasamy, 2013), *V. mimicus, and V. carchariae* (Oanh et al., 2008) have been found associated with GFP from different regions of the globe.

Nearly 147 species of this ubiquitous bacteria with high genotypic and phenotypic similarities have been identified (Sampaio et al., 2022). They are found to be free-living species and associated with aquatic animals and plankton. As one of the natural microflorae, *Vibrios* populate a diverse array of habitats, including the guts of humans and other organisms, suspended in the water of saline, brackish and marine, sediments of aquatic systems, corals and attached as biofilms to different finfish and shell-fish species, plants and varying objects (rocks). *Vibrios* are an essential functional group in aquatic environments for decomposing organic matter and other complex polymers owing to their ability to utilise various substrates, eventually leading to nutrient recycling of these ecosystems (Thompson et al., 2006; Johnson, 2014; Percival and Williams, 2014; Sampaio et al., 2022).

Vibrios have the fastest growth rates, outcompeting most other bacteria species (Thompson et al., 2006; Johnson, 2014; Percival and Williams, 2014; Sampaio et al., 2022). A multitude of factors, including environmental parameters, pollutants, competition, and predators, have varying impacts on the abundance of bacteria in aquatic ecosystems, affecting their structure and function. *Vibrios* exhibit intense seasonal variations in their abundance since the parameters above vary by season. Furthermore, the global abundance of *Vibrios* has also changed dramatically over recent years due to climate changes, including global warming, glacier melting, and rising sea surface temperatures and sea water levels (Hou et al., 2017; Brumfield et al., 2023; Velez et al., 2023). The most extensively explored parameters are water temperature, pH, salinity, and nutrient content. The most preferred temperature range for the growth of *Vibrios* is from 20^0C to 40^0C (Thompson et al., 2006; Percival and Williams, 2014). According to Bergey's Manual of Systematic Bacteriology, the majority of *Vibrio* sp. grow well at 30^0C to 37^0C; almost all *Vibrios* grow at 20^0C, whereas few *Vibrios* prefer lower temperatures, around 4^0C (Garrity, 2007). Concerning pH, most *Vibrio* grow well in alkaline conditions, whereas most *Vibrios* grow in a pH range from 6.5 to 9. *Vibrios*, commonly known as halophiles, need Na^+ to nurture their growth. Na^+ is an essential growth factor for *Vibrios*. However, the cumulative impact of all growth requirements can ensure the growth of *Vibrios*, even in freshwater conditions. Furthermore, most of the *Vibrio* sp. are facultative anaerobes that can depend on varying substrates (Thompson et al., 2006; Garrity, 2007; Percival and Williams, 2014).

Being an opportunistic pathogen, *Vibrios*, a type of gram-negative, oxidase-producing, rod-shaped bacteria, can significantly impact the production of GFP by causing harmful diseases. Therefore, investigating the abundance of *Vibrios* associated with GFP is crucial for enhancing production by managing their growth and health status. Despite extensive exploration of how certain parameters regulate the abundance of *Vibrios*, the effects of other parameters remain unexplored. Furthermore, such research is considered a grey area in the Sri Lankan context. Therefore, the present study aims to elucidate the impacts of the physicochemical parameters in regulating the abundance of *Vibrios* associated with the GFP captured from selected Sri Lankan reservoirs.

MATERIALS AND METHODS

Collection of Giant Freshwater Prawns

The present study focused on seven reservoirs located in the Hambantota and Monaragala districts of Sri Lanka, where the highest production of Giant Freshwater Prawn (GFP) was recorded among the reservoirs where culture-based fisheries (CBF) is practiced. The former includes Bandagiriya Wewa (BN), Mahagal Wewa (MH), Ridiyagama Wewa (RD), and Weerawila Wewa (WR), while the latter includes Handapanagala Wewa (HP), Muthukandiya Wewa (MT), and Urusita Wewa (US). The GFP samples were collected from fish landing sites of each reservoir immediately after the fishing boats arrived at the sites. Individual prawns (Average total length = 26.53±345 cm, Average body weight = 278.13±45.26 g) were placed into separate, sterilised polythene bags, stored on ice, and transported to the laboratory of the Department of Livestock Production, Faculty of Agricultural Sciences, at Sabaragamuwa University of Sri Lanka.

Preparation of GFP Samples and *Vibrios* Cultures

Following transportation, the samples were first weighed. Then, the individuals were washed with sterilised distilled water in a volume similar to their weight. After that, these whole individuals were homogenised separately using a Stomacher (BagMixer 400cc, France). Next, the homogenised samples were inoculated in Alkaline Peptone Water (APW) (1 sample:10 APW ratio) and incubated at 30 ^{0}C for 24 hours for enrichment. For the enumeration of *Vibrios*, 500 µL of each prepared and enriched sample was cultured on Thiosulphate Citrate Bile Sugar (TCBS) using the spread plate technique. Then, the inoculated plates were incubated at ambient temperature overnight. After incubation, the colonies grown on TCBS plates were manually counted and expressed as log CFU g^{-1} of Prawn. The colonies were confirmed as *Vibrios* using the following method. After the enrichment, a loopful of each sample was inoculated onto TCBS using the streak plate method and then incubated at ambient temperature overnight. The following day, grown colonies were observed. Single green and yellow colonies were picked separately and inoculated using the streak plate method onto Luria Bertani (LB) agar plates repeatedly to obtain pure colonies. After obtaining pure colonies, they were grown in LB broth and subjected to a battery of biochemical tests for confirmation (Alsina and Blanch, 1994; Noguerola and Blanch, 2008).

Quantifying Physicochemical Parameters

All the physicochemical parameters were recorded from April 2021 to March 2022, concurrently with collecting GFP samples to isolate *Vibrios*. Temperature, pH, conductivity, and dissolved oxygen were measured in situ using a multiparameter water quality meter (Hanna, model HI 98194, USA). Additionally, the Secchi disk depth was measured with a standard black and white Secchi disk with a diameter of 20 cm. Chlorophyll-a was analysed following the method described by Carlson and Simpson (1996). 200 mL of water was collected in brown bottles and filtered through GF/C filters (1.2 µm; 47 mm ø) in the field. The filters were then stored in lightproof, acid-free vials and transported to the laboratory on ice. In the laboratory, the filter papers were macerated in 90% acetone, centrifuged at 2000 rpm for 5 minutes, and the absorption of the extraction was measured at 664 nm and 750 nm before acidification, as well as at 665 nm and 750 nm after acidification using a spectrophotometer (Jenway 6405UV/VIS) (Carlson and Simpson, 1996). Sediment samples were collected at each site using an Ekman grab from the boat. The samples collected in ice-polythene bags were returned to the laboratory to analyse organic matter content. Organic matter content was analysed by dry ashing in a muffle furnace at 550^{0}C for 4 hours (Boyd, 1995).

Data analysis

All statistical analyses were performed

using RStudio 2023.06.0 Build 421. First, a Shapiro-Wilk test was conducted to check the normality of the collected data. Since the data were not normally distributed, the Kruskal-Wallis pairwise comparison test was conducted with Bonferroni adjusted α = 0.002 to determine whether there was a significant difference in the abundance of *Vibrio* sp. isolated from GFP collected from seven study reservoirs. The same test was also conducted to examine the variations in water quality parameters among the reservoirs. Finally, the regression analysis, followed by Pearson correlation coefficient analysis, was performed at α = 0.05 to determine the relationship between the collected water quality parameters and the abundance of *Vibrio* sp. in each reservoir.

RESULTS

The present study reveals the impact of the physicochemical conditions of the water on the growth of *Vibrios* in seven selected reservoirs. Table 1 summarizes the study reservoirs' chlorophyll-a content, conductivity, dissolved oxygen, organic matter content, pH, Secchi disk depth, and temperature. The results of the Kruskal-Wallis pairwise comparison reveal varying differences in water quality parameters among the reservoirs, except for Dissolved Oxygen, pH and Secchi disk depth (Table 2).

The chlorophyll-a content of the seven reservoirs varied from 04.87 μgL^{-1} in Handapanagala Wewa to 34.05 μgL^{-1} in Bandagiriya Wewa, where the seven reservoirs had significantly different chlorophyll-a concentrations. Bandagiriya Wewa showed the highest conductivity, which was 571.68±77.49 μScm-1, while Muthukandiya Wewa recorded the lowest conductivity, 204.35±38.91 μScm^{-1}. There were significant conductivity differences ($p < 0.002$) among the reservoirs.The highest and lowest dissolved oxygen levels, 10.73±1.08 mgL-1 and 09.71±0.77 mgL^{-1}, were observed in Ridiyagama Wewa and Handapanagala Wewa, respectively. Notably, none of the reservoirs showed a significant difference ($p > 0.002$) in their recorded dissolved oxygen levels. The organic matter content of the reservoirs varied from 16.08±1.56 mgL^{-1} in Ridiyagama Wewa to 38.00±1.41 mgL^{-1} in Bandagiriya Wewa. Similar to the chlorophyll-a content, there were significant differences ($p < 0.002$) in organic matter content among reservoirs. The pH values of the reservoirs varied from 08.13±0.44 in Weerawila Wewa to 07.47±0.33 in Ridiyagama Wewa. The lowest Secchi disk depth was recorded in Bandagiriya Wewa (0.33±0.03m), and the highest was in Urusita Wewa (0.41±0.07m). Intriguingly, reservoirs had no significant differences in pH and SDD values ($p < 0.002$). The highest temperature was recorded in Urusita Wewa(29.06 ± 0.87°C), while the lowest was in Muthukandiya Wewa (28.23 ± 0.22°C). Urusita Wewa's temperature differed significantly ($p<0.002$) from all other study reservoirs except Mahagal Wewa. The overall averages of the physicochemical parameters of all reservoirs were 12.25±10.02mgL^{-1} of chlorophyll-a, 367.72±141.26 μScm^{-1} of conductivity, 10.17±0.42 mgL^{-1} of DO, 7.86±0.31 of pH, 0.38±0.03m of SDD, and 28.59±0.25^{0}C of temperature (Table 1 and Table 2).

Biochemical tests revealed that all the counted colonies were Vibrios. The mean total number of *Vibrio* species associated with GFP collected from seven reservoirs ranges from 5.61±2.38 log CFUg^{-1} of prawn in Weerawila Wewa to 8.19±0.74 log CFUg^{-1} of prawn in Muthukandiya Wewa. The total number of *Vibrio* species associated with GFP showed varying differences among the reservoirs. The Kruskal-Wallis pairwise comparison test indicates a significant difference between the mean total number of *Vibrios* associated with the GFP between Bandagiriya Wewa and Muthukandiya Wewa, Handapanagala Wewa and Muthukandiya Wewa, and Urusita Wewa and Muthukandiya Wewa (Figure 1). There was a significantly higher mean total number of *Vibrios* in the GFP from Muthukandiya Wewa (8.19±0.74 log CFU g^{-1} of prawn).

The regression analysis examining the mean total number of *Vibrios* associated with the GFP in each reservoir and the collected water quality parameters revealed that none of the parameters significantly correlated with the bacterial abundance, except for the pH in Urusita Wewa. (Table 3). According to the Pearson correlation coefficient, a significant positive correlation ($r = 0.740$, $p = 0.006$) was observed between bacterial abundance and the pH of Urusita Wewa.

Table 1. Mean values (± SD) of physicochemical parameters in the study reservoirs in the dry zone, Sri Lanka.

Reservoir	Temperature (^0C)	pH	Chlorophyll-a Content (μgL^{-1})	Organic Matter Content (mgL^{-1})	Dissolved Oxygen (mgL^{-1})	Conductivity (μScm^{-1})	Secchi Disk Depth (m)
Bandagiriya Wewa	28.57±0.50	07.70±0.31	34.05±1.23	38.00±1.41	10.34±0.83	571.68±77.49	0.33±0.03
Mahagal Wewa	28.60±0.22	08.04±0.28	08.55±0.22	28.83±1.64	10.52±0.93	364.25±51.40	0.35±0.04
Ridiyagama Wewa	28.63±0.81	07.47±0.33	10.95±0.36	16.08±1.56	10.73±1.08	488.08±135.83	0.37±0.06
Weerawila Wewa	28.44±0.36	08.13±0.44	13.31±0.44	26.58±1.78	09.73±0.70	427.17±33.32	0.38±0.05
Handapanagala Wewa	28.62±0.22	07.83±0.21	04.87±1.47	23.00±2.30	09.71±0.77	329.48±36.79	0.40±0.03
Muthukandiya Wewa	28.23±0.22	07.79±0.13	07.66±2.86	30.33±1.83	10.36±0.79	204.35±38.91	0.40±0.01
Urusita Wewa	29.06±0.87	07.70±0.23	06.34±1.87	33.00±2.80	09.80±0.41	189.00±41.16	0.41±0.07

Table 2. Kruskal-Walis pairwise comparison of physicochemical parameters in the study reservoirs in Sri Lanka (BN - Bandagiriya Wewa, MH - Mahagal Wewa, RD - Ridiyagama Wewa, WR – Weerawila Wewa, HP – Handapanagala Wewa, MT – Muthukandiya Wewa, and US – Urusita Wewa).

Variable (α)		Pairwise Comparison (β)						
		BN	MH	RD	WR	HP	MT	US
Temperature	BN	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	MH	-	-	<0.001	<0.001	0.001	ns	ns
	RD	-	-	-	<0.001	<0.001	ns	<0.001
	WR	-	-	-	-	<0.001	<0.001	<0.001
	HP	-	-	-	-	-	ns	ns
	MT	-	-	-	-	-	-	ns
	US	-	-	-	-	-	-	-
pH	BN	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	MH	-	-	<0.001	ns	<0.001	ns	ns
	RD	-	-	-	<0.001	<0.001	<0.001	<0.001
	WR	-	-	-	-	ns	0.001	<0.001
	HP	-	-	-	-	-	<0.001	<0.001
	MT	-	-	-	-	-	-	ns
	US	-	-	-	-	-	-	-
Chlorophyll-a Content	BN	-	-	ns	ns	<0.001	<0.001	<0.001
	MH	-	-	ns	<0.001	ns	<0.001	<0.001
	RD	-	-	-	ns	ns	<0.001	<0.001
	WR	-	-	-	-	-	<0.001	<0.001
	HP	-	-	-	-	-	<0.001	<0.001
	MT	-	-	-	-	-	-	ns
	US	-	-	-	-	-	-	-
Organic Matter Content	BN	-	ns	ns	ns	ns	ns	ns
	MH	-	-	ns	ns	ns	ns	ns
	RD	-	-	-	ns	ns	ns	ns
	WR	-	-	-	-	ns	ns	ns
	HP	-	-	-	-	-	ns	ns
	MT	-	-	-	-	-	-	ns
	US	-	-	-	-	-	-	-

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		BN	MH	RD	WR	HP	MT	US
Conductivity	BN	-	-	<ns	ns	<0.001	<0.001	<0.001
	MH	-	-	ns	<0.001	ns	<0.001	<0.001
	RD	-	-	-	ns	ns	<0.001	<0.001
	WR	-	-	-	-	-	<0.001	<0.001
	HP	-	-	-	-	-	<0.001	<0.001
	MT	-	-	-	-	-	-	ns
	US	-	-	-	-	-	-	-
Secchi Disk Depth	BN	-	ns	ns	ns	ns	ns	ns
	MH	-	-	ns	ns	ns	ns	ns
	RD	-	-	-	ns	ns	ns	ns
	WR	-	-	-	-	ns	ns	ns
	HP	-	-	-	-	-	ns	ns
	MT	-	-	-	-	-	-	ns
	US	-	-	-	-	-	-	-
Dissolved Oxygen	BN	-	ns	ns	ns	ns	ns	ns
	MH	-	-	ns	ns	ns	ns	ns
	RD	-	-	-	ns	ns	ns	ns
	WR	-	-	-	-	ns	ns	ns
	HP	-	-	-	-	-	ns	ns
	MT	-	-	-	-	-	-	ns
	US	-	-	-	-	-	-	-

ns= not significant at 0.002* *. α and β are samples 1 and 2, respectively. p values are mentioned for the significant comparisons. Significant values were adjusted by Bonferroni correction for multiple tests.

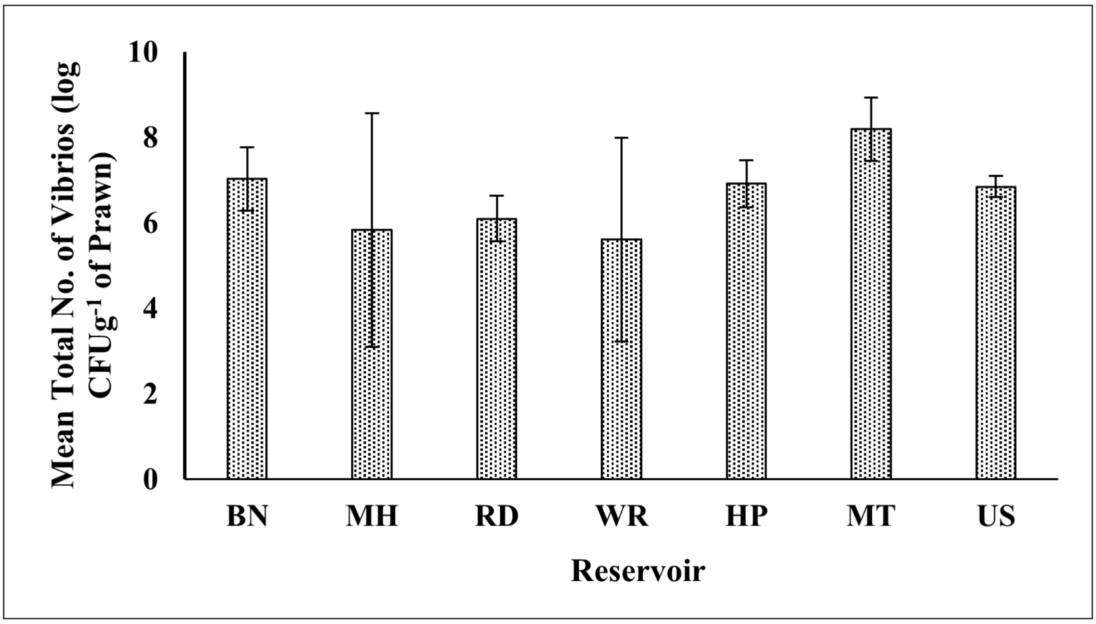

Figure 1. The *total Vibrio* count associated with *Macrobrachium rosenbergii* (Mean ± SD) collected from seven reservoirs in Sri Lanka (BN - Bandagiriya Wewa, MH - Mahagal Wewa, RD - Ridiyagama Wewa, WR – Weerawila Wewa, HP – Handapanagala Wewa, MT – Muthukandiya Wewa, and US – Urusita Wewa).

Table 3. Kruskal-Walis pairwise comparison test of mean total *Vibrio* count associated with *Macrobrachium rosenbergii* from the selected seven reservoirs in Sri Lanka.

	Pairwise Comparison (β)						
Variable (α)	**BN**	**MH**	**RD**	**WR**	**HP**	**MT**	**US**
BN	-	ns	ns	ns	ns	0.01	ns
MH	-	-	ns	ns	ns	ns	ns
RD	-	-	-	ns	ns	ns	ns
WR	-	-	-	-	ns	ns	ns
HP	-	-	-	-	-	0.03	ns
MT	-	-	-	-	-	-	0.01
US	-	-	-	-	-	-	-

ns= not significant at 0.002 **. α and β are samples 1 and 2, respectively. P-values are mentioned for the significant comparisons. Significance values were adjusted using the Bonferroni correction for multiple tests.

DISCUSSION

Vibrios, which are opportunistic yet virulent pathogens found in both wild and aquaculture species, as well as in humans, exhibit diverse dynamics in their abundance in relation to physicochemical parameters. Examining these diverse relationships in detail is crucial, as fluctuations in physicochemical parameters can serve as ideal indicators of Vibrio outbreaks in aquatic ecosystems (Johnson, 2014; Brumfield et al., 2023). Furthermore, recent climate changes, such as global warming, glacier melting, and increased algal blooms and eutrophication, have altered the abundance and distribution of *Vibrios* across varying ecosystems around the globe due to these changes in physicochemical parameters (Velez et al., 2023).

The effect of chlorophyll-a content, temperature, conductivity, pH, dissolved oxygen, organic matter content, and Secchi disk depth was determined as they are the main influential physicochemical parameters that regulate the abundance of *Vibrios* (Brumfield et al., 2023; Velez et al., 2023). Chlorophyll-a content, a substitute for phytoplankton biomass in aquatic ecosystems, is also an ideal predictor of the trophic status of reservoirs. Due to its relative ease and low cost, measuring chlorophyll-a content is preferred as a forecast for phytoplankton biomass and primary productivity of water bodies. (Carneiro et al., 2014: Pallavi et al., 2024; Sanuja et al., 2024). The recorded chlorophyll-a concentrations of the present study are corroborated by Sanuja et al. (2024), who document similar chlorophyll-a concentrations in all seven reservoirs studied, except for Mahagal Wewa. According to Pallavi et al. (2024), surface water temperature, nutrient availability, water clarity, and light penetration are the primary drivers of chlorophyll-a content in water bodies. Furthermore, the present study's findings align with those of Brezonik et al. (2019), who suggested an inverse relationship between chlorophyll-a content and the Secchi depth of water bodies. Despite the well-known fact that temperature and chlorophyll-a are positively correlated, no such positive correlation between temperature and chlorophyll-a was observed in the present study. This finding is supported by Pallavi et al. (2024), in which the authors recorded no or little association between temperature and chlorophyll-a.

There can be substantial variations in the physicochemical parameters of water systems, even within a country, due to climate conditions, altitude, geography and soil, annual rainfall, anthropogenic activities and land usage around the water bodies (Somasundaram et al., 2024; Shamsudduha et al., 2025). According to the results, the reservoirs of the Hambanthota district did not show any significant differences in temperature, pH, conductivity, SDD, and DO among themselves. Similarly, pH, chlorophyll-a, SDD, and DO did not differ significantly among the reservoirs of the Monaragala district. However, the reservoirs of the two districts showed notable variations in temperature, conductivity, chlorophyll-a, and organic matter content. These observed variations may be since the reservoirs are located in two separate districts, Hambanthota and Monaragala, which are classified under two different climate zones, with the former belonging to the dry zone and the latter belonging to the intermediate zone (Somasundaram et al., 2024). The Trophic State

Index (TSI), a key indicator of nutrient content and other physicochemical characteristics, provides insight into the productivity of lakes, reservoirs, and other water bodies. There is a bidirectional dependency between physicochemical parameters and the TSI of the reservoirs (Carlson, 1996). Among the studied reservoirs, Bandagiriya Wewa, Mahagal Wewa, Muthukandiya Wewa, Ridiyagama Wewa, and Weerawila Wewa were classified as eutrophic reservoirs, while Handapanagala Wewa and Urusita Wewa were classified as mesotrophic (Sanuja et al., 2024). Therefore, the observed variations in the physicochemical parameters of these reservoirs may be due to deviations in TSI.

The average values of physicochemical parameters in all seven reservoirs challenge the recorded values of chlorophyll-a content, conductivity, DO, pH, and temperature of reservoirs in dry zone areas from 2010 to 2012 by Sudharma et al. (2013). These average values of study parameters are slightly higher than those recorded in 2012. A significant impact of global climate change could explain these varying physicochemical parameters over the years (Deepananda et al., 2012; Brumfield et al., 2023).

Quantification of bacteria, including *Vibrios*, is pivotal in almost every field, despite the bacteria are pathogenic, beneficial, or neutral. Among the multitude of approaches used to determine bacterial abundance, such as relative abundance, Most Probable Number (MPN), quantitative reverse transcription polymerase chain reaction (qRT-PCR), MPN-PCR, Enzyme-Linked Immunosorbent Assays (ELISA), and Next Generation Sequencing (NGS) (Kallastu et al., 2023), the plate count method used in the present study is the most extensively employed.

The abundance of *Vibrios* varied among the seven reservoirs, and the GFP captured from Muthukandiya Wewa had the highest number of *Vibrios*. The findings contradict those of Jayasinghe et al. (2022), which reported the highest *Vibrio* abundance in the Bandagiriya reservoirs. A multitude of temporal and spatial factors govern the abundance of *Vibrios* in water bodies. One possible reason for this varying distribution is that various aspects, such as transmission, dissemination, and proliferation related to the abundance of Vibrio-like pathogens, are governed by environmental variables (Brumfield et al., 2023).

Since a varying abundance of *Vibrios* was observed among the reservoirs, further analysis was conducted to correlate the measured physicochemical parameters of each reservoir with the abundance of *Vibrio* sp. Previous studies have thoroughly explored *Vibrios*' dependency on the physicochemical parameters of water, but not specifically on dry zone reservoirs in Sri Lanka. According to Wong et al. (2024), the abundance of *Vibrios* in water is primarily correlated with either positive or negative relationships to chlorophyll-a, Total Suspended Solids (TSS), Dissolved Organic Nitrogen (DON), and Dissolved Organic Carbon (DOC). Additionally, Chen et al. (2020) reported an increasing number of *Vibrios* in eutrophic reservoirs, leading to elevated nutrient levels and increased phytoplankton and zooplankton populations. On the contrary, the escalating TSI and nutrients facilitate the rapid spread of diseases caused by a diverse array of *Vibrios*.

The present study's findings rebut most of the previous findings made by several authors, who found significant relationships between water quality parameters and the abundance of *Vibrios*. The growth of various *Vibrio* species, including *V. cholerae, V. vulnificus*, *V. parahaemolyticus, V. splendidus*, and *V. alginolyticus*, was regulated solely by temperature and pH (Randa et al., 2004; Wang and Gu, 2005; Eiler et al., 2007; Brumfield et al., 2023; Velez et al., 2023), salinity (Armada et al., 2003; Randa et al., 2004; Brumfield et al., 2023), and nutrient content of water (Armada et al., 2003; Eiler et al., 2007) or the cumulative effect of above parameters. In line with the present findings, Brumfield et al. (2023) have not found any significant impact of Dissolved Oxygen, conductivity and Total Dissolved solids on the growth of *V. parahaemolyticus* and *V. vulnificus*. Wong et al. (2024) have found no effect of temperature, DO, and salinity on the abundance of *Vibrios*. Moreover, Chlorophyll-a content and salinity did not regulate the seasonal abundance and variation of *V. vulnificus* (Randa et al., 2004). One of the leading possible causes for the present finding in which the water quality parameters do not significantly contribute to the abundance of *Vibrios* may be the pronounced genetic diversity, which enables them to grow at a broader range of environmental parameters by administering a multitudinous of survival mechanisms viz biofilm formation, attachment, nutrition acquisition, immune evasion, and other mechanisms (Johnson, 2014). However, the present study found that pH has a significant positive effect on the abundance of *Vibrios*. pH has been identified as one of the main stress factors to *Vibrios* in their natural environments due to algal blooms, eutrophication, pollution and changes in CO_2 concentrations. Generally, the optimum growth of *Vibrios* occurs within the pH range of 5 to 9, in which they thrive under more alkaline pH conditions. Intriguingly, some *Vibrios* have developed various mechanisms, such as genetic mechanisms related to cadBA genes responsible for acid tolerance, making it possible for them to survive in acidic conditions. *Vibrios* are adapted to survive in a broad spectrum of pH levels, from high alkaline to high acidic conditions, owing to these mechanisms (Rhee et al., 2002; Liu et al., 2024; Pattano and Mittraparp-arthorn,2025). Therefore, authors suggest future studies to explore how the abundance of *Vibrios* associated with GFP is influenced by the pH of the reservoirs.

CONCLUSION

The present findings indicate that GFP collected from Muthukandiya Wewa supports a significantly higher number of *Vibrios* than other reservoirs. Furthermore, except for dissolved oxygen, Secchi disk depth, and pH all other observed physicochemical parameters (temperature, conductivity, chlorophyll-a content, and organic matter content) varied significantly among reservoirs to different degrees. The pH of Urusita Wewa demonstrated a strong positive correlation with the abundance of *Vibrio* sp. The study concludes that further research is necessary to clarify the relationship between the abundance of Vibrio species and the physicochemical environment of the reservoir by selecting a larger number of reservoirs from geographically diverse locations.

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CONFLICT OF INTEREST

The authors affirm that no conflict of interest could possibly affect the reported work in this paper.

AUTHOR CONTRIBUTIONS

Conceptualization: E. G. K. Y. C. B., H. A. D. R., U. A. D. J., K. H. M. A. D.; methodology: E. G. K. Y. C. B., H. A. D. R.; formal analysis: E. G. K. Y. C. B., H. A. D. R.; Data curation: E. G. K. Y. Y. C. B., H. A. D. R., K. H. M. A. D.; writing- Original draft preparation: E. G. K. Y. C. B., writing- review and editing: H. A. D. R., U. A. D. J., K. H. M. A. D.

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