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Water pollution influencing contamination of *Vibrio* bacteria in the coastal aquaculture area of Chanthaburi and Trat Provinces

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ABSTRACT

Since 1982, the Eastern coastal area of Thailand has been developed from the Eastern Seaboard Project (ESP) to the Eastern Economic Corridor (EEC). The marine ecosystem of Chanthaburi and Trat Provinces was polluted by anthropogenic activities such as agriculture, transportation, tourism, fisheries, and urban communities. This study aims to investigate the marine environmental quality and the contamination of the *Vibrio* bacteria (*V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*) in the coastal aquaculture area of Chantaburi and Trat Provinces. Environmental sampling areas were designated at seven stations eastward from Tamai to Klongyai districts (about 150 km long stretch). The physicochemical parameters, including temperature, salinity, conductivity, pH, dissolved oxygen (DO), NH_3, and major anions e.g. NO_2^-, PO_4^{3-} of seawater samples were measured at designated stations. Marine samples, including cockles (*Anadara granosa*), mussels (*Perna viridis*), oysters (*Saccostrea cucullata*) and white shrimp (*Litopenaeus vannamei*) were collected from aquaculture areas located in the estuarine ecosystem with simple random sampling. Our studies revealed that major inorganic substance concentrations followed this consequence order $PO_4^{3-} > NH_3 > NO_2^-$. The physicochemical properties indicated that seawater quality has been varied within the marine quality standard class III for aquaculture. The prevalence of *V. parahaemolyticus* and *V. vulnificus* showed in all bivalve samples were detected at 0.36 to 4.30 MPN/g and below the detection limit (0.3 MPN/g), respectively, whereas *V. cholerae* was not detected. This study also concluded that the periods of environmental sampling did not significantly influence the seawater quality and the level of *Vibrio* contamination. However, the difference of infection rates for *V. parahaemolyticus* and *V. vulnificus* depended on the marine species.

Keywords: Water pollution, *Vibrio* bacteria, Coastal aquaculture area

INTRODUCTION

Vibrio is a Gram-negative bacteria, typically with lipopolysaccharide in the outer membrane, that belongs to the Proteobacteria phylum, Gammaproteobacteria class, the most diverse class of Gram-negative bacteria. The Vibrionaceae family comprises aquatic bacteria that mostly thrive in warm waters and tolerate various salinity levels, including freshwater, brackish, and marine waters [1].

Nowadays, the global aquaculture sector has grown continuously, and it is currently an important contributor to aquatic animal protein for human consumption [2]. The Thai government has to decide how they will control their national aquaculture production. Multiple bivalve species are economically important both natural and farmed populations, while white shrimp are produced mainly for export [3, 4]. Foodborne infections with the genus *Vibrio* are a serious problem in Thailand and a major cause of gastroenteritis, particularly from traditional consumption of raw or undercooked seafood. Most of these patients are affected by *V. parahaemolyticus* and *V. cholerae* and to a lesser extent by *V. vulnificus* [5]. *Vibrios* belong to the microbiota of infectious bivalves, which can concentrate bacteria in their edible tissues and body fluids, including the hemolymph [6]. The aquacultural harvests from the Gulf of Thailand were more contaminated than *Vibrio*, while bivalves and white shrimps showed a high frequency of contamination [7-9].

The marine mollusks showed a great correlation to environmental variability investigation in the coastal areas and river mouth ecosystems [10]. Bivalve molluscs as bioindicator organisms have been used to assess pollution levels in the aquatic environment [11]. The bivalve species, including oysters (*Saccostrea*

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cucullata), mussels (*Perna viridis*), and cockles (*Anadara granosa*) are abundance species and commercial seafood in the Eastern part of Thailand [7, 12]. Whereas the white shrimp (*Litopenaeus vannamei*) is used to focus on the potential *Vibrio* disease, due to *Vibrio* is recognized as a major cause of seafood-borne illness [13, 14].

Coastal water pollution is an increasingly significant environmental effect and public health illness [15]. Untreated wastewater and urban runoff are the principal sources of marine pollution [16]. These pollutants can inhibit the growth, reproduction, and survival of flora and fauna, leading to a decline in biodiversity. Physicochemical parameters are a limiting factor in the marine ecosystem and the most important environmental variable [17]. The various physicochemical measurements carried out in the coastal seawaters reveal the relationships between environmental quality and intensive anthropogenic activities [18, 19]. Hence, the physicochemical properties of seawater, including temperature, salinity, conductivity, pH, dissolved oxygen (DO), ammonia (NH_3), and major anions (NO_2^-, PO_4^{3-}) are fundamental indicators for the assessment of changing the marine environment and reflecting land use development. Numerous indices regarding seawater quality and pollution status were conducted in Thailand, while the operation of these parameters was followed by a manual seawater sampling and analysis of PCD Thailand and international standards [20, 21].

The inner eastern coastal area of Thailand is the most important coastal aquaculture area and is a central source of recreation, as well as travel destinations, conservation areas, and comfort zones. In recent years, Thailand's Special Economic Zone (SEZ) was approved by the Thai government in 2015, covering the Chanthaburi and Trat coastal areas [22]. These marine zones have seen significant spatial development in the past decade. As a result, they have transformed into important shrimp farming areas, tourism hotspots, and urban communities. Several reports indicated that anthropogenic activities as a source of water pollution are fed back into the marine ecosystem [23-26].

Here, we designed an analysis of water pollution influencing contamination and abundance of *Vibrio* bacteria by collecting marine samples along the Chanthaburi and Trat coastal areas from 2014 to 2022. Precisely, we determined the correlation between water quality parameters and the infection of *Vibrio* in commercial marine seafood with the statistical data.

MATERIALS AND METHODS

Environmental sampling station

The eastern coastal areas cover two provinces, namely Chanthaburi and Trat Provinces, which were considered the locations for the marine sampling (Figure 1). The seawater samples were selected to represent environmental quality at designated stations. All study sites were recorded by Global Positioning System (GPS) (SD ± 5 m) as shown in Table 1.

Three bivalve species, including cockles (*Anadara granosa*), mussels (*Perna viridis*), and oysters (*Saccostrea cucullata*), as well as white shrimps (*Litopenaeus vannamei*) were collected from coastal aquaculture areas using simple random sampling. The surface seawater samples were collected at a depth of 100 cm using a vertical sampling procedure for physicochemical analysis.

All species samples were dredged and cleaned with seawater and stored in 4.0 °C iceboxes to the medical laboratory within 24 hours after collection.

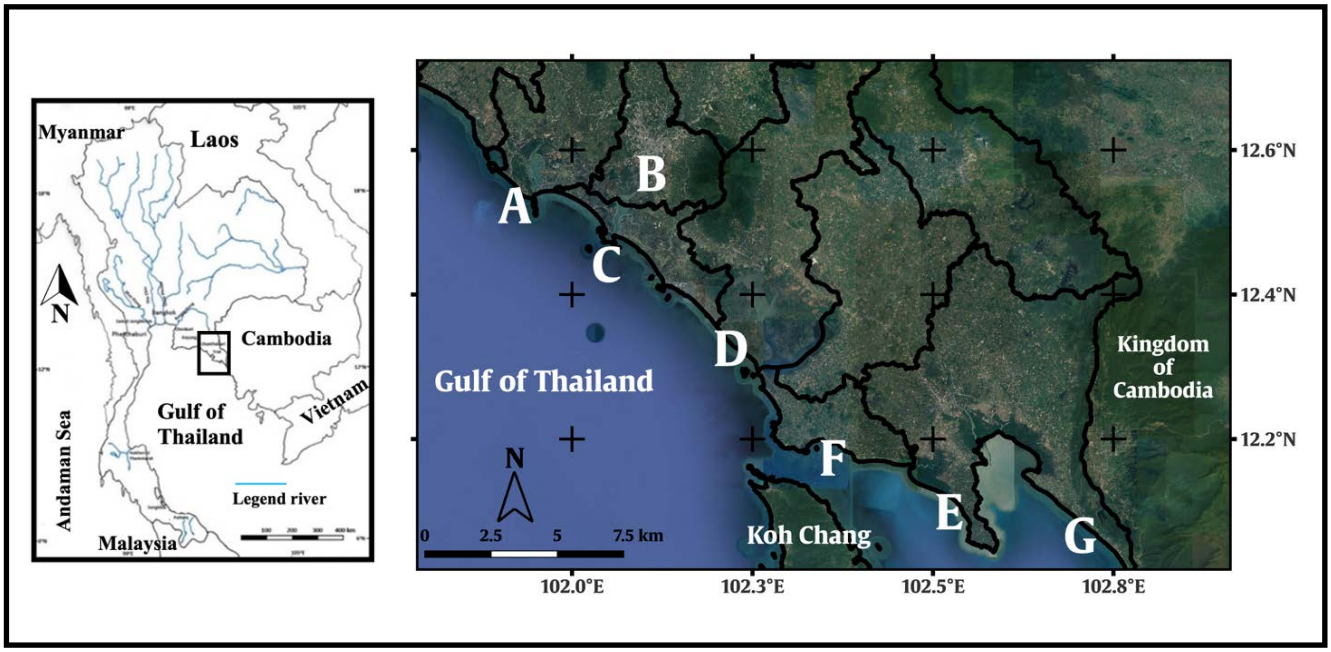

Figure 1 The map of sampling stations along the coastal area of Chanthaburi and Trat Provinces.

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Table 1 Use details of the sampling stations.

Stations	Latitude	Longitude	Description
Chanthaburi coastal areas			
Tha Mai (A)	12.621421°	102.004851°	Tourism landmarks and fisheries
Mueang Chanthaburi (B)	12.606965°	102.104579°	Several anthropogenic activities (Urban community, transportation and oxidation pond treatment)
Laem Sing (C)	12.481633°	102.073803°	Aquaculture in river mouth areas
Khlung (D)	12.454718°	102.221408°	Agriculture, homestay and fisheries
Trat coastal areas			
Mueang Trat (E)	12.243642°	102.515117°	Urban community, disposal site and fisheries
Laem Ngop (F)	12.225371°	102.369298°	Eco-tourism and fisheries
Khlong Yai (G)	12.200052°	102.296142°	Border trade and fisheries

Table 2 The vulnerability assessment of the coastal marine ecosystem of Chanthaburi and Trat Provinces.

Variables	Chanthaburi	Trat
Habitat		
In-shore shallow Seawater	Low	Low to Middle
Sandy beaches	Low	Middle
Rocky beaches	Low	Middle
Inter-tidal mudflats	Middle	Middle to High
Estuaries	Very high	Very high
Seagrass beds	Loss	Loss
Mangroves	Very high	Very high
Selected species		
Cetaceans (3 species)	Very high	Very high
Dugong	Loss	Loss
Migratory shorebirds	Low	Low
Red-backed sea eagle	Low to Middle	Low to Middle

Source: Modification from [34].

Physicochemical measurement

The physicochemical properties were recorded in situ in each station using the electrochemical analyzer (Consort C 932) and salinometer which were calibrated before use. Dissolved Oxygen (DO) is measured by azide modification of the Winkler's titration [20]. Direct nesslerization was performed for NH_3 detection, whereas the colorimetric technique was used to determine nitrite (NO_2^-) and phosphate (PO_4^{3-}) [27, 28].

Bacteriological analysis

Marine samples were prepared by rinsing and scrubbing them under running deionized water to remove debris from the shell, and they were opened using a sterile knife. The edible tissues in an equal amount of phosphate-buffered saline (PBS; pH 7.4) were homogenized in a sterile blender for 90 s [29].

The observation of *Vibrio* contamination was prepared following methods outlined in the Bacteriological Analytical Manual (BAM) for food sampling/preparation [30]. *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* were isolated from marine species by cultured in alkaline peptone water (APW) and thiosulfate citrate bile sucrose (TCBS), which was modified from BAM [31]. Each APW culture sample was sub-cultured on TCBS agar and incubated at 35°C for 18 - 24 hours to observe fermentative colonies. *Vibrio* densities in contaminated samples were calculated using the scored MPN table, and each dilution was measured in triplicate [32].

Data analysis and quality control

A prevalence of *Vibrio* for each of the four species was tested for significant differences by analysis of Chi-square; $p < 0.05$ was accepted for significant value. The influence of time on study periods and the physicochemical parameters of seawaters were analyzed using one-way analysis of variance (ANOVA) with $p < 0.05$ was classified as statistically significant. Post hoc comparisons, LSD (Least Significant Difference) were applied to report differences among the sampling periods; $p < 0.05$ was accepted as a significant value. The statistical inferences for hypothesis

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tests were confirmed with a 5% significance level. All statistical analyses were performed using SPSS 18.0 software (Serial No.5083337). The quality control procedures were implemented through rigorous standardization by the PCD (Pollution Control Department), in accordance with seawater analysis guidelines.

RESULTS AND DISCUSSION

Land use of coastal area of Chanthaburi and Trat Provinces

The eastern coastal region of Thailand has been an important part of the country's economic development. The real gross regional product (GRP) of the eastern region increased significantly from around 500,000 THB in 1994 to 1.7 million THB in 2018 [33]. The terrestrial area of Chanthaburi and Trat Provinces is the most agricultural landscape. It covers more than 60 percent of the total land use. The agricultural areas are a mixture of many kinds of tropical fruits known for their tasteful flavor. The coastal areas of Chanthaburi and Trat Provinces are affected by four major river basins, including the Wang-Ta-Nord, Chanthaburi, Welu, and Trat river basins, as shown in Table 2. Land use activities from the river basins during heavy rainfall may also significantly affect the marine coastal area and the dynamic of *Vibrio* spp. abundance [35].

Physicochemical parameters

The physicochemical parameters are used to determine environmental quality along the coastal areas of Chanthaburi and Trat Provinces. Table 3 reported the annual average of physicochemical properties from 2014, 2018 and 2022. The variation in seawater quality was elucidated with a Box plot diagram based on the minimum, maximum, first, median, and third quartile (Figure 2). The seawater quality monitoring in study sites could be categorized as a marine water quality standard class III. Additionally, the investigation of seawater samples revealed that the environmental quality of study areas was unpolluted.

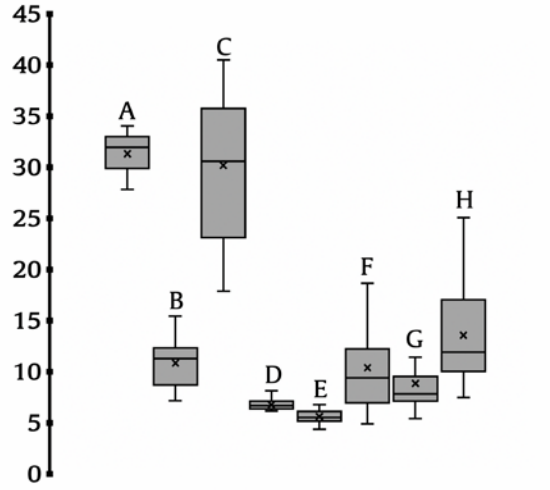

Figure 2 Box plot of physicochemical parameters of seawater samples (A: Temperature; B: Salinity; C: Conductivity; D: pH; E: Dissolved Oxygen (DO); F: Ammonia-Nitrogen; G: Nitrite-Nitrogen and H: Phosphate-Phosphorus).

Table 3 Seawater quality along the coastal areas of Chanthaburi and Trat Provinces, Thailand of 2014, 2018 and 2022.

Physicochemical parameters	Year 2014	Year 2018	Year 2022	Criterion*
Temperature (°C)	29 - 33	30 - 34	28 - 34	a
	31.14 ± 1.57	32.14 ± 1.35	30.86 ± 2.19	
Salinity (g/L)	8 - 13	9 - 14	9 - 16	b
	10.29 ± 1.70	11.71 ± 1.98	12.71 ± 2.50	
Conductivity (μS/cm)	18.39 - 32.45	23.54 - 40.26	19.55 - 40.11	-
	24.83 ± 5.14	33.90 ± 5.44	32.10 ± 6.96	
pH	7.04 - 8.11	7.09 - 8.95	7.36 - 8.09	7.0 - 8.5
	7.43 ± 0.37	7.66 ± 0.65	7.75 ± 0.34	
Dissolved Oxygen (mg/L)	5.32 - 7.09	6.08 -7.65	6.05 - 7.11	≥ 4
	6.24 ± 0.65	6.72 ± 0.55	6.55 ± 0.44	
Ammonia-Nitrogen (μg/L)	5.83 - 10.18	6.78 -20.12	9.17 - 13.47	≤ 70
	8.05 ± 1.68	11.34 ± 4.28	11.29 ± 1.48	
Nitrite-Nitrogen (μg/L)	7.15 - 18.21	6.32 - 12.13	8.09 - 11.37	55[c]
	10.67 ± 4.44	8.87 ± 1.89	9.39 ± 1.23	
Phosphate-Phosphorus (μg/L)	10.46 - 25.32	9.64 - 20.23	8.31 - 19.56	≤ 45
	15.61 ± 5.17	13.09 ± 3.90	13.91 ± 4.39	

*The announcement of the National Environment Board No.27 (B.E. 2549) regarding the specification of the standard of seawater

a - Naturally but changing by no more than 1 °C

b - Changing no more than 10 percent of the lowest value

c - AMEQC (ASEAN Marine Environmental Quality Criteria)

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The statistical comparison did not reveal a significant difference between the seawater quality and the sampling periods ($p > 0.05$), except for conductivity. Multiple comparisons (LSD) revealed a significant difference in conductivity values across the study periods period ($p < 0.05$). The conductivity variation in seawater is affected by the presence of inorganic dissolved solids such as chloride, nitrate, phosphate, and other anions. Mostly, essential nutrients enter the marine environment through urban rinsing, irrigation drainage, and agricultural runoff, while the high nutrient levels in seawater may lead to phytoplankton abundance and eutrophication [36]. In this study, the sampling sites revealed the degree of variation in terrestrial water pollution, which is frequently observed in estuarine coastal areas due to locally accumulated contaminants from domestic wastewater, fertilizer usage in orchards, and natural watershed erosion. A septic tank with an anaerobic filter was recommended for practice in solving water pollution problems in urban areas [37]. Conversely, nanomaterials, biosolids, and electrobiological techniques have efficiently removed nutrients from agricultural wastewater [38, 39].

Contamination of Vibrio bacteria

The contamination of *Vibrio* spp. was investigated in the marine samples collected along the coastal aquaculture area of Chanthaburi and Trat Provinces, which produced seafood products for Thailand's domestic consumption and also exported. The results showed the infection of *V. parahaemolyticus* in all marine species, but *V. cholerae* was not detected, except in three bivalve species that were contaminated with *V. vulnificus* (Table 4; Figure 3). However, abundance of *Vibrio* species was below the seafood safety standard for fishery products, indicating no potential risks for human consumption. The main contaminated source of *Vibrio* bacteria is the discharge from untreated wastewater, urban sewage and infectious leachate, especially during extremely heavy rainfall, which are very threatening to the downstream ecosystem [19].

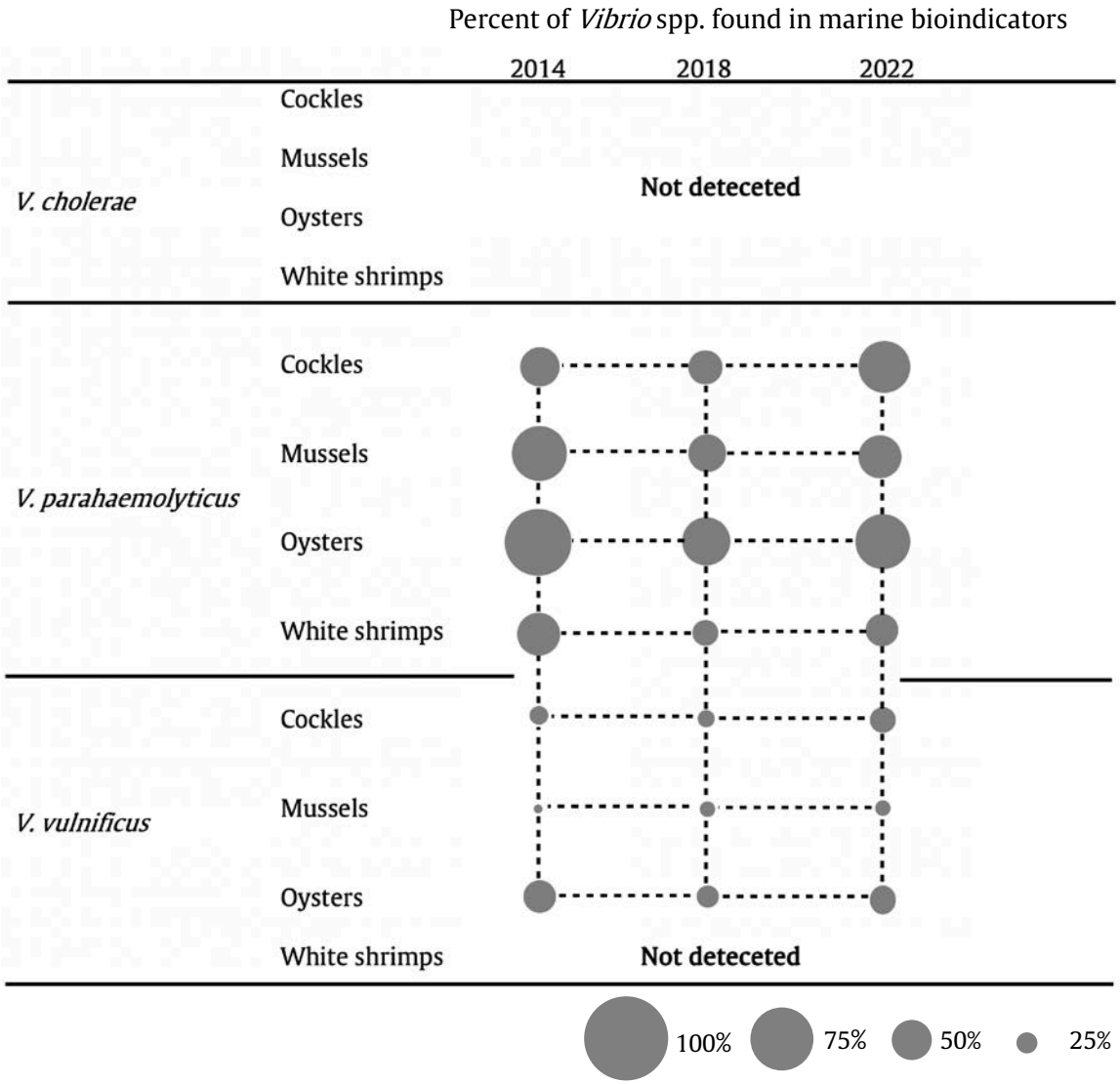

Figure 3 Distribution of *Vibrio* contamination in marine samples along the coastal areas of Chanthaburi and Trat Provinces from 2014 to 2022.

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Table 4 Contamination of *Vibrio* spp. (MPN/g) in 25 g samples of marine species along the coastal aquaculture areas of Chanthaburi and Trat Provinces (7 stations) from 2014 to 2022 (n = 63).

Species	*V. cholerae*		*V. parahaemolyticus*		*V. vulnificus*	
	Prevalence (percent)	MPN/g	Prevalence (percent)	MPN/g	Prevalence (percent)	MPN/g
Cockles	-	n.d.	55.56	0.36 - 1.25	25.40	< 0.3
Mussels	-	n.d.	63.49	0.74 - 2.80	15.87	< 0.3
Oysters	-	n.d.	73.02	0.36 – 4.30	36.51	< 0.3
White shrimps	-	n.d.	47.62	< 0.3 - 3.0	-	n.d.
Safety levels*		n.d.		< 30/g (MPN)		< 30/g (MPN)

n.d. - not detected

*FDA and EPA safety levels of fishery products in regulation and guidance.

Table 5 Growth characteristics of *V. parahaemolyticus* in the marine environment

Variables	Optimum	Range
Temperature (°C)	37	5 - 43
pH	7.8 - 8.6	4.8 - 11
NaCl (%)	1.5 - 3.0	0.5 - 10
Water Activity (WA)	0.981	0.940 - 0.996
Atmosphere (O_2 consumption)	Aerobic	Aerobic-Anaerobic

Source: Modification from International Commission for Microbiological Specifications for Foods

The study found a high abundance of *V. parahaemolyticus* in all marine samples. The chi-square test showed a significant difference in the prevalence of *V. parahaemolyticus* in the marine species ($p < 0.05$), and the sampling periods were not a significant difference ($p > 0.05$). *V. parahaemolyticus* is commonly found in coastal areas and estuarine ecosystems, and it has been isolated in various marine animals [40]. *V. parahaemolyticus* is a mildly halophilic, mesophilic microorganism, and its general growth characteristics are shown in Table 5. Hence, the physicochemical variation can play an important role in the significant contamination of *V. parahaemolyticus* in marine habitats [41-44].

The contamination of *V. vulnificus* was detected in all samples of bivalves at below detection limit (0.3 MPN/g) but was not found in the white shrimp samples during the study periods (Table 4). The statistical analysis revealed a notable disparity in the infection levels of *V. vulnificus* in bivalve samples, with a significance level of $p < 0.05$. A microbiological survey concerned the levels of pathogenic *V. vulnificus* in marine bivalves across various global locations. The reporting indicated infection rates of 17.2% in oysters and 8.0% in mussels [45, 46]. The prevalence rate of *V. vulnificus* contamination is commonly observed in shellfish maturity within coastal areas, but in this study, *V. vulnificus* was not detected in the white shrimp samples. The confirmation of *V. vulnificus* appeared to have the same disease symptoms as naturally infected shrimp. However, the circulating hemocytes of *Litopenaeus vannamei* play a crucial role in self-purification and activate several *Vibrio* pathogens [47]. Furthermore, physicochemical parameters, such as salinity and temperature, influence the occurrence of *V. vulnificus*, while the natural depuration pond was prepared to decrease *Vibrio* contamination [48, 49].

CONCLUSIONS

The present study concludes that physicochemical parameters were within the seawater permissible standard at all sampling stations in the coastal aquaculture area of Chanthaburi and Trat Provinces. The major land use concerns that have polluted the marine coastal area from anthropogenic activities such as recreational renovation, aquaculture, and infrastructure improvement. *V. parahaemolyticus* is an abundant species in the aquaculture area and is commonly isolated from edible bivalves and white shrimp tissues. However, *V. parahaemolyticus* densities also included the permissible limit. The depuration processes were recommended to decontaminate the cultivated fishery products.

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Ethics approvals

Ethical approval was exempted from this study as it operated within the local fishing communities' methods.

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