

Research Repository

Microbial contamination and foodborne pathogens in farmed and shucked retail oysters (*Saccostrea cucullata*) in eastern Thailand

Accepted for publication in Food Control

Research Repository link: <https://repository.essex.ac.uk/43939/>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the published version if you wish to cite this paper.

<https://doi.org/10.1016/j.foodcont.2026.112659>

Microbial contamination and foodborne pathogens in farmed and shucked retail oysters (*Saccostrea cucullata*) in eastern Thailand

Narinratana Kongjandtre¹, Supattra Muangham², On-ong Chanprasartsuk³, Uraiwan Intamaso⁴, Penchan Laongmanee⁵, Michael Steinke⁶ and Wansuk Senanan^{1*}

¹ Department of Aquatic Science, Faculty of Science, Burapha University, Thailand

² Department of Microbiology, Faculty of Science, Burapha University, Thailand

³ Department of Food Science, Faculty of Science, Burapha University, Thailand

⁴ Medical Technology, Faculty of Allied Health Sciences, Burapha University, Thailand

⁵ Faculty of Marine Technology, Burapha University, Thailand

⁶ School of Life Science, University of Essex, United Kingdom

* Corresponding author: Department of Aquatic Science, Faculty of Science, Burapha University, Thailand. E-mail address: wansuk@go.buu.ac.th (W. Senanan)

Abstract

This study investigated microbial contamination in oysters from eastern Thailand through two complementary assessments: an environmental survey of farmed oysters in Chon Buri and a post-harvest survey of retail-shucked oyster products collected from markets in Chon Buri and Trat provinces. Phase 1 evaluated environmental conditions and microbial contamination in surrounding waters from five stations in the Bang Pakong River and Chon Buri Bay, with farmed oysters sampled from two stations between October 2021 and March 2022. Phase 2 examined microbial quality in retail oysters collected during the dry and wet seasons in 2024. Samples were categorized as aseptically shucked oysters (ASO), freshly shucked oysters (FSO), and packed shucked oysters (PSO). Environmental conditions varied among stations, particularly salinity (0.2–32.8 psu), dissolved oxygen (3.7–5.6 mg/L), total suspended solids (32.4–75.5 mg/L), and nutrient concentrations. Stations near river discharge and coastal communities showed elevated total coliform concentrations exceeding 1,000 MPN/100 mL. Total bacterial counts (TBC) in farmed oysters ranged from 4.9 to 9.1 log CFU/g. TBC and *Vibrio* spp. concentrations varied significantly with sampling period, season, station, and product type ($P <$

0.05). Retail oysters collected during the wet season showed significantly higher TBC values than those collected during the dry season, while ASO and FSO samples exhibited significantly higher *Vibrio* spp. concentrations than PSO samples ($P < 0.05$). *Salmonella* spp. were detected in 5% (6/120) of retail oyster samples, whereas *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* were not confirmed by PCR. These findings highlight the influence of environmental conditions and post-harvest handling on oyster microbial quality in eastern Thailand.

Keywords: oyster aquaculture, foodborne pathogen, microbial contamination, *Vibrio* spp., *Salmonella*, food safety, coastal water quality, post-harvest handling; sustainable aquaculture

1. Introduction

Oysters are among the major aquatic products consumed in coastal regions worldwide and are highly valued for their distinctive taste and nutritional benefits. They are considered a rich source of high-quality proteins, unsaturated fatty acids, vitamins, and minerals (Yu et al., 2021). However, oyster producers globally face increasing challenges in providing safe and high-quality seafood products for a growing population under changing and progressively deteriorating environmental conditions.

In Thailand, primary oyster production areas include Chon Buri and Phetchaburi in the upper Gulf of Thailand, Surat Thani in the lower Gulf of Thailand, and Phang Nga along the Andaman Sea coast. In 2024, Thailand had 1,163 oyster farms, accounting for 22% of shellfish culture farms and generating THB 425 million in production value (Department of Fisheries, 2024). The small rock oyster (*Saccostrea cucullata*) is the predominant species cultivated in Chon Buri using the hanging-culture method. This species is commonly marketed as shucked oyster meat and is highly popular among domestic consumers (Chalermwat et al., 2003; Szuster et al., 2008).

Oysters are filter-feeding shellfish capable of accumulating a wide range of microorganisms from their surrounding aquatic environments (Chinnadurai et al., 2021). The prevalence and composition of microbial communities in oysters can vary considerably depending on geographic location, seasonal conditions, environmental factors, and post-harvest handling practices. Previous studies have investigated oyster-associated pathogens and consumer health

risks (DePaola et al., 2010; Shuping et al., 2023), while recent work has shown that *Vibrio* levels in farmed oysters vary among farms and seasons (Almuhaideb et al., 2026). However, most studies examine microbial contamination in oyster-farming environments separately from the microbial quality of retail products, particularly in tropical estuarine-coastal systems.

Coliform bacteria, particularly fecal coliforms and *Escherichia coli*, are widely recognized as indicators of fecal contamination in shellfish and their growing waters. Their presence in oysters raises significant food safety concerns (Jeamsripong et al., 2018, 2022). In addition, *Vibrio* species are consistently reported as dominant bacterial groups associated with oysters. Among them, *V. vulnificus* and *V. parahaemolyticus* are important foodborne pathogens capable of causing severe illness in humans (Froelich and Noble, 2016). Cases of *Vibrio*-associated infections linked to the consumption of raw or undercooked oysters are increasing worldwide (Huss, 1994; Ryder et al., 2014; Almuhaideb et al., 2026). Therefore, monitoring bacterial indicators and foodborne pathogens in oysters is important for seafood safety, particularly in systems where environmental exposure, shucking, storage, and retail handling can contribute to microbial variation.

The Bang Pakong River and Chon Buri Bay represent estuarine environments with broad salinity gradients ranging from 0.2 to 32.8 psu. Limited information is available on how environmental conditions are associated with microbial contamination in oyster-farming areas in eastern Thailand and how microbial quality varies among shucked oyster products sold in local markets. In 2024, oyster production in eastern Thailand had an estimated value of THB 60 million (Department of Fisheries, 2024). This regional context provides an opportunity to assess oyster microbial quality from both production-environment and retail-product perspectives.

Therefore, this study investigated microbial contamination in oysters from eastern Thailand through two complementary assessments. Phase 1 focused on microbial contamination in surrounding water samples from five stations and farmed oysters from two stations in the Bang Pakong River and Chon Buri Bay on four occasions between October 2021 and March 2022. The objective of this phase was to examine associations among farming locations, environmental conditions and concentrations of microbial indicators and selected foodborne pathogens in oyster and water samples. Phase 2 evaluated the microbial quality of shucked oyster products in two

major local markets in Chon Buri and Trat provinces, eastern Thailand, from April to July 2024. The objectives of this phase were to compare the levels of bacterial indicators and foodborne pathogens, including *Escherichia coli*, *Salmonella* spp., *Vibrio parahaemolyticus*, *Vibrio cholerae*, and *Vibrio* spp. among aseptically shucked oysters (ASO), freshly shucked oysters obtained directly from shucking houses (FSO), and packed shucked oysters (PSO) during the dry season and wet season.

2. Materials and methods

2.1 Study location and sampling

Phase 1: Microbial contamination in farmed oysters and surrounding water samples

This phase investigated microbial contamination in farmed oysters and surrounding waters from oyster farming areas in Chon Buri Bay. Water sampling was conducted at five stations extending from Khao Sam Muk to the Bang Pakong River mouth between October 2021 and March 2022 (Fig. 1a). The stations were selected to represent gradients in salinity and oyster farming activities.

Stations 1 and 2 were oyster nursery areas where farmers used oyster collectors, locally known as “E-pae,” to promote larval settlement. Juvenile oysters were subsequently reared until reaching an appropriate size before being transferred to Stations 3 and 4 for grow-out to market size. Station 5 was located near the Bang Pakong River mouth, where terrestrial runoff and nutrient inputs enter the coastal environment.

Water and oyster sampling was conducted on four occasions: October and November 2021, representing the transition period between the late rainy season and the northeast monsoon, (hereafter referred to as the transition season), and January and March 2022, representing the dry season.

During each sampling event, water quality parameters were measured at the surface of all five stations and near the bottom at Station 5 using a YSI ProSolo multiparameter instrument (YSI Inc., USA). Parameters included water depth (m), transparency (m), temperature (°C), salinity (psu), conductivity (mS/cm), dissolved oxygen (mg/L), and pH. Duplicate 500-mL seawater

samples were collected from each station and transported to the laboratory in insulated boxes. Water samples were also analyzed for dissolved inorganic nitrogen (DIN), including ammonia, nitrite, and nitrate, total suspended solids (TSS), and chlorophyll-*a* following Strickland and Parsons (1972). Total coliform and *E. coli* concentrations were determined to assess the influence of land-based fecal contamination on oyster farming areas.

Oyster samples were collected only from Stations 3 and 4 using hanging-culture ropes supporting clusters of oysters. In the laboratory, whole oysters were externally rinsed with running tap water to remove adhering sediment and debris before being aseptically shucked for tissue collection. Oyster tissues were subsequently homogenized using a blender sterilized between samples, and the homogenates were stored at -40°C for no longer than 24 h before microbiological analysis. Total bacterial count (TBC), *E. coli*, *Vibrio* spp. and presumptive *V. parahaemolyticus* were determined to evaluate microbial contamination in oyster meat.

Phase 2: Microbial quality of oysters after harvest before reaching consumers

Fresh oyster samples were purchased from oyster distribution centers in the Ang Sila–Khao Sam Muk community ($13^{\circ}18'48.3''\text{N}$, $100^{\circ}54'26.1''\text{E}$), Mueang District, Chon Buri Province, and the Tha Som community ($12^{\circ}16'37.2''\text{N}$, $102^{\circ}18'22.2''\text{E}$), Khao Saming District, Trat Province (Fig. 1b), to evaluate the microbial quality of post-harvest oyster products available in local markets. Sampling was conducted on four occasions during 2024: April and May (dry season), and June and July (wet season). Three types of oyster product were collected. Whole oyster with shells were purchased and aseptically shucked in the laboratory (ASO) to evaluate microbial contamination before commercial shucking. Freshly shucked oysters obtained directly from local shucking houses (FSO) represented products that had been manually shucked and maintained on ice prior to sale. Packed shucked oyster (PSO) were purchased as retail products that had been stored in plastic bags on ice. During transportation to the laboratory, FSO and PSO samples were maintain on ice in Styrofoam boxes, whereas ASO samples were transported at ambient temperature to simulate the handling conditions prior to commercial shucking. At each occasion, five replicate samples were collected per sampling period at the different locations, resulting in a total of 120 samples.

Oyster production in eastern Thailand is influenced by regional differences in reproductive cycles, grow-out periods, and harvesting schedules. Consequently, oyster harvests are not available simultaneously across all production areas throughout the year. To maintain a continuous supply to customers, oyster marketed in Chon Buri Province are often transported from several production areas, including Phetchaburi, Chanthaburi, and Trat provinces. As a result, retail products available in local markets may originate from different farming locations and may have undergone different post-harvest handling and distribution practices before reaching consumers.

In this study, the term “retail oyster products” refers to oysters sold through local markets, seafood stalls, shucking houses, or small-scale vendors rather than standardized supermarket or industrial distribution systems. Consequently, post-harvest handling practices, including rinsing, storage temperature, display conditions, and time since harvest, may vary among vendors and product groups and could influence the microbial quality of oyster products.

2.2 Microbial analysis

Determination of total bacterial count

The total bacterial count (TBC) was enumerated according to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) with slight modifications (Maturin & Peeler, 2001). Briefly, 25 g of each homogenized oyster sample was aseptically transferred into 225 mL of sterile phosphate-buffered saline (PBS). The suspension was serially diluted (10^{-1} , 10^{-2} , 10^{-3} , and further dilutions as appropriate). Aliquots (100 μ L) of each dilution were spread in duplicate onto Plate Count Agar (PCA) (TM MEDIA[®], India) and incubated at 35 °C for 24 h. After incubation, plates containing 25–250 colonies were selected for enumeration, and the results were expressed as colony-forming units per gram (CFU/g).

Determination of total coliforms, fecal coliforms, and *Escherichia coli*

Total coliforms, fecal coliforms, and *E. coli* concentrations were determined according to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) using the most probable number (MPN) method (Feng et al., 2020). Briefly, 25 g of each homogenized

oyster sample was aseptically mixed with 225 mL of sterile PBS. Serial dilutions (10^{-1} to 10^{-3}) were prepared and inoculated in triplicate into Lauryl Tryptose Broth (LST) (TM MEDIA[®], India). The inoculated tubes were incubated at 35 °C for 24 h and examined for turbidity and gas production. A loopful from gas-positive LST tubes was transferred into Brilliant Green Lactose Bile Broth (BGLB) (TM MEDIA[®], India) and EC broth (TM MEDIA[®], India). BGLB tubes were incubated at 35 °C and examined for gas production after 24 and 48 h. Tubes showing turbidity and gas production within 48 h were considered positive for total coliforms. EC broth tubes were incubated at 44.5 °C for 24 h. Gas production and turbidity in EC broth were considered positive for fecal coliforms. Positive tubes were recorded, and fecal coliform concentrations were calculated as MPN/g.

For *E. coli* confirmation, an aliquot from each gas-positive EC broth tube was streaked onto Eosin Methylene Blue (EMB) agar (Difco, Sparks, MD, USA) and incubated at 35 °C for 24 h. Colonies exhibiting a characteristic green metallic sheen were considered presumptive *E. coli*. Suspected colonies were further confirmed using biochemical tests, including indole, methyl red, Voges–Proskauer, and Simmons citrate tests (Feng et al., 2002).

Determination of *Vibrio* spp. and qualitative analysis of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*

Vibrio spp. were quantitatively analyzed following the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) method with slight modifications (Kaysner et al., 2004). Briefly, 25 g of homogenized oyster sample was mixed with 225 mL of PBS, homogenized, and incubated at 35 °C overnight. Following incubation, serial dilutions (10^{-2} , 10^{-3} , 10^{-4} , and additional dilutions as appropriate) were prepared by transferring 1 mL of the previous dilution into 9 mL of PBS. Aliquots (100 µL) of each dilution were spread-plated in duplicate onto Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar (TM MEDIA[®], India) and incubated at 35 °C for 24 h. Colonies were enumerated and expressed as CFU/g.

Presumptive *Vibrio* species were identified based on colony morphology on TCBS agar. Presumptive *V. cholerae* colonies appeared yellow, flat, and 2–3 mm in diameter with opaque centers. Presumptive *V. vulnificus* colonies appeared colorless, whereas presumptive *V. parahaemolyticus* colonies appeared green-centered on TCBS agar. Putative colonies were

further confirmed using biochemical characterization, including oxidase tests, sodium chloride tolerance, and Triple Sugar Iron (TSI) agar (Difco, Sparks, MD, USA) (Andrews et al., 2023).

Qualitative analysis of *Salmonella* spp.

The presence of *Salmonella* spp. was determined according to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) method (Andrews et al., 2023). Briefly, 25 g of homogenized oyster sample was added to 225 mL of PBS and incubated at 35 °C for 24 h for pre-enrichment. Subsequently, 1 mL of the pre-enriched sample was transferred into 10 mL of selenite cystine (SC) broth (Difco, Sparks, MD, USA), while another 1 mL aliquot was transferred into 10 mL of tetrathionate (TT) broth (Difco, Sparks, MD, USA). The enrichment broths were incubated at 35 °C for 24 h. Following enrichment, cultures were streaked onto Xylose Lysine Deoxycholate (XLD) agar (Difco, Sparks, MD, USA) and incubated at 35 °C for 24 h. Presumptive *Salmonella* colonies appeared as pink colonies with or without black centers. Presumptive isolates were biochemically confirmed using Triple Sugar Iron (TSI) agar, Lysine Iron Agar (LIA), and urea agar (Difco, Sparks, MD, USA).

2.3 Multiplex PCR

DNA was extracted and purified from oyster homogenates (120 oyster samples) using QuickExtract[™] DNA Extraction Solution (LGC Biosearch Technologies, UK) according to the manufacturer's instructions. Multiplex PCR was performed to confirm bacterial identification by amplifying species-specific marker genes. The *uidA* gene was used for the confirmation of *E. coli*, following the modified method described by Godambe et al. (2017), whereas the *invA* gene was amplified for the confirmation of *Salmonella* spp. according Buehler et al. (2019) with minor modifications. The *toxR* gene was used to identify *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* following the method described by Bauer and Rørvik (2007) with modifications. Each PCR reaction was performed in a final volume of 50 µL containing 1× HotStart PCR master mix (Bio Basic Inc., Singapore), 10 µM of each specific primer (*uidA*, *invA*, and *toxR*), and 1 µL of DNA template. PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 1 min. PCR products were separated by electrophoresis on 1.5% agarose gels stained with

Safe-Red™ (SMART SCIENCE CO., LTD.) and visualized under UV illumination. Amplicons were identified based on the expected product size.

Molecular confirmation using multiplex PCR was conducted only for oyster samples collected during Phase 2 (retail market survey). In Phase 1, presumptive *Vibrio* species were identified based solely on selective media and biochemical characterization.

2.4 Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the effects of sampling period, station, season, oyster product type, and their interactions on environmental parameters and microbial concentrations in water and oyster samples collected from farming areas and retail markets. Microbial counts were log₁₀-transformed prior to statistical analysis. Duncan's multiple range test (DMRT) was subsequently used for post-hoc comparisons. All statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). Principal component analysis (PCA) was performed using PRIMER version 7 software (Clarke and Gorley, 2006) to evaluate the relationships among environmental variables.

3. Results

3.1. Spatial and temporal variation in environmental conditions and microbial contamination in water and oyster samples

3.1.1 Environmental variability

Water depth varied among sampling stations in Chon Buri Bay. Stations 1–4 were relatively shallow, with water depths ranging from 2.2 to 4.9 m, whereas Station 5 was the deepest site, with an average depth of 11.2 m. Dissolved oxygen concentrations at Station 5 were significantly lower than those recorded at the other sampling sites ($P < 0.05$). In contrast, most other water quality parameters showed relatively consistent patterns among sampling locations (Table 1).

Total suspended solids (TSS) were significantly highest at Station 5 ($P < 0.05$), whereas chlorophyll-*a* concentrations were highest at Station 4 ($P < 0.05$). The highest ammonium (NH₄⁺)

concentrations were observed at Station 1, while nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were highest at Station 5 (Table 2).

Principal component analysis (PCA) of environmental variables showed that the first two principal components explained 67.1% of the total environmental variability. The first principal component (PC1) accounted for 45.2% of the total variation and was primarily associated with chlorophyll-*a*, and TSS (Fig. 2a–c). The second principal component (PC2) explained 21.9% of the total variation and was mainly associated with ammonium, nitrite, and nitrate concentrations (Fig. 2d–f).

Table 1 Mean ($\pm$ SD) water quality parameters at five sampling stations in Chon Buri Bay during the study period (October 2021–March 2022). Different superscript letters in the column for DO indicate statistical groupings ($P < 0.05$).

Station	n	Depth (m)	Trans. (m)	Temp ($^{\circ}\text{C}$)	Sal range (psu)	Sal (psu)	Cond.(mS)	DO (mg/L)	DO%	pH range
1	4	2.5 ± 0.31	1.0 ± 0.32	28.4 ± 0.40	13.1-31.6	23.9 ± 8.15	40.1 ± 12.33	4.1 ± 0.62^b	58.7 ± 7.45	7.8-7.9
2	4	2.6 ± 0.22	1.6 ± 0.35	29.2 ± 0.38	14.7-32.8	27.5 ± 8.57	46.1 ± 13.06	5.0 ± 0.28^b	73.9 ± 8.19	7.6-7.9
3	4	4.5 ± 0.39	1.1 ± 0.68	28.9 ± 0.48	11.0-31.8	26.0 ± 10.03	43.5 ± 15.59	5.2 ± 0.22^b	78.4 ± 3.87	7.8-8.2
4	4	3.6 ± 0.28	0.7 ± 0.19	29.1 ± 0.38	10.6-31.0	22.8 ± 8.88	38.8 ± 13.93	5.6 ± 1.05^b	79.6 ± 8.52	7.7-7.9
5-Surface	4			29.3 ± 1.03	0.2-28.2	13.9 ± 15.72	24.6 ± 24.95	3.7 ± 0.72^a	53.3 ± 12.03	7.5-8.4
5-Bottom	4	11.2 ± 0.87	0.4 ± 0.18	29.2 ± 0.85	0.2-28.2	16.4 ± 13.43	28.1 ± 22.26	3.9 ± 0.48^a	53.0 ± 10.12	7.1-8.2

Abbreviations: n: number of sampling events; Trans.: transparency; Temp: water temperature; Sal range: minimum-maximum salinity; Sal: salinity; Cond.: conductivity; DO: dissolved oxygen; DO%: percentage of dissolved oxygen.

Table 2 Mean ($\pm$ SD) concentrations of total suspended solids (TSS), chlorophyll-*a*, ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) at five sampling stations in Chon Buri Bay during the study period (October 2021–March 2022). Different superscript letters within the same column indicate significant differences among stations ($P < 0.05$).

Station	n	TSS (mg/L)	Chl- <i>a</i> ($\mu\text{g/L}$)	NH_4^+ ($\mu\text{g/L}$)	NO_2^- ($\mu\text{g/L}$)	NO_3^- ($\mu\text{g/L}$)
1	12	39.05 ± 13.54^a	4.05 ± 1.86^b	474.53 ± 237.83^c	31.38 ± 16.35^b	159.29 ± 53.36^b
2	12	32.44 ± 6.75^a	2.39 ± 0.93^a	161.31 ± 131.58^{ab}	20.62 ± 15.83^a	74.42 ± 47.87^a
3	12	36.63 ± 11.32^a	5.00 ± 2.77^b	123.49 ± 85.44^a	21.80 ± 18.39^a	85.45 ± 30.71^a
4	12	44.43 ± 13.89^a	9.21 ± 9.06^c	148.34 ± 96.55^{ab}	31.30 ± 23.28^b	95.13 ± 48.00^a
5-Surface	12	58.86 ± 13.02^b	5.15 ± 2.62^b	183.41 ± 154.93^b	102.21 ± 92.88^c	310.40 ± 237.65^c
5-Bottom	12	75.46 ± 23.75^b	4.48 ± 2.34^b	183.80 ± 133.10^b	109.11 ± 90.10^c	286.41 ± 209.14^c

Abbreviations: n: number of replicates; Chl-*a*: Chlorophyll *a*; NH_4^+ : ammonium; NO_2^- : nitrite; NO_3^- : nitrate

3.1.2 Concentrations of total coliforms and *Escherichia coli* in seawater

The mean concentrations ($\pm$ SD) of total coliforms and *E. coli* in seawater across sampling sites and seasons are presented in Fig. 3. Significant effects of station and the interaction between station and season were observed for total coliform concentrations ($P < 0.05$), with the highest concentrations detected at Stations 1 and 5, independent of the season (Fig. 3a). In contrast, *E. coli* concentrations at Station 3 were significantly lower ($P < 0.05$) than those at station 1 (both seasons) and station 5 (transition season only) (Fig. 3b).

3.1.3 Total bacterial counts and concentrations of *Vibrio* spp., *Vibrio parahaemolyticus*, and *Escherichia coli* in oysters

The mean concentrations ($\pm$ SD) of total bacterial counts (TBC), *Vibrio* spp., *V. parahaemolyticus*, and *E. coli* in farmed oyster samples from sampling stations ST3 and ST4 in the defined sampling periods are presented in Fig. 4. Sampling period had significant effect on TBC ($P < 0.001$), primarily due to the significantly higher concentrations observed in March 2022 compared with the other sampling periods (Fig. 4a).

Vibrio spp. concentration were significantly affected by sampling periods ($P < 0.001$), with a significant interaction between sampling period and station ($P < 0.05$) (Fig. 4b). In contrast, sampling period was the only factor significantly affecting presumptive *V. parahaemolyticus* ($P < 0.001$) and *E. coli* ($P < 0.01$) concentrations (Fig. 4c–d). March 2022 exhibited significantly higher concentrations of *Vibrio* spp., presumptive *V. parahaemolyticus*, and *E. coli* than the other sampling periods.

3.2. Comparison of bacterial contamination levels among shucked oyster sample groups across dry and wet seasons in two oyster markets in eastern Thailand

3.2.1 Bacterial contamination in oyster samples

In Phase 2, a total of 120 oyster samples were analyzed, comprising three product groups: laboratory-prepared aseptically shucked oysters (ASO, $n = 40$), freshly shucked oysters obtained

from shucking houses (FSO, n = 40), and packed shucked oysters purchased from retail vendors (PSO, n = 40).

Total bacterial counts (TBC) differed significantly by season ($P < 0.001$), with a significant interaction between season and sampling site ($P < 0.001$) (Fig. 5a-c). Mean TBC values were higher during the wet season, reaching approximately 9.2 log CFU/g, compared with approximately 7.2 log CFU/g during the dry season (Fig. 5a-c).

During the wet season, TBC was consistently high (8.9-9.2 log CFU/g) and showed relatively little variation among oyster product type. In contrast, TBC during the dry season exhibited greater variability, with a wider distribution of both low and high bacterial counts. Several samples with particularly high TBC were also observed during the dry season, suggesting greater temporal variability that may be associated with environmental or site-specific factors.

Differences in TBC between Ang Sila and Tha Som were smaller than the seasonal effects although the significant season $\times$ sampling site interaction indicates that site-related patterns varied between seasons. TBC values were comparable among the three sample groups (ASO, FSO, and PSO) across the sampling periods. Overall, these findings suggest that season was the main factor associated with TBC levels, while sampling site and oyster product type had relatively minor effects on bacterial count variability.

3.2.2 Total coliforms, fecal coliforms, and Escherichia coli in oyster samples

Total coliform concentrations were consistently high, with many samples exceeding 2,400 MPN/g. Fecal coliform concentrations ranged from from 3.00 ± 0.00 to 9.60 ± 10.23 MPN/g (Fig. 5d-f), whereas *E. coli* concentrations were consistently below 3 MPN/g. Fecal coliform concentrations differed significantly among sampling occasions ($P < 0.001$), with the highest concentrations observed in April, although the median values were similar between the dry and wet seasons.

3.2.3 Occurrence of Vibrio species in oyster samples

Season, sampling site, and oyster product type significantly affected *Vibrio* spp. concentrations ($P < 0.001$). During the dry and wet seasons, mean *Vibrio* spp. counts in ASO, FSO, and PSO samples collected from Ang Sila and Tha Som ranged from 4.84 ± 0.99 to 6.61 ± 0.72 log CFU/g (Fig. 5g-i).

Samples collected from Tha Som exhibited significantly higher *Vibrio* spp. concentrations than those collected from Ang Sila ($P < 0.001$). In addition, ASO and FSO samples showed significantly higher *Vibrio* spp. concentrations than PSO samples ($P < 0.001$). Temporal variation in *Vibrio* spp. concentrations was also observed. *Vibrio* spp. concentrations in April were significantly lower than those in July, whereas the highest concentrations were recorded during May and June (Fig. 5g-i).

3.2.4 Occurrence of Salmonella spp. in oyster samples

The occurrence of *Salmonella* spp. was detected in 6 out of 120 samples, corresponding to an overall prevalence of 5%. Among the positive samples, five were obtained from Ang Sila, Chon Buri Province, whereas one positive sample was detected in Tha Som, Trat Province. In terms of seasonal distribution, three positive samples were detected during the dry season and three during the wet season.

3.2.5 Confirmation of Escherichia coli, Salmonella spp., Vibrio cholerae, Vibrio parahaemolyticus, and Vibrio vulnificus using uidA, invA, and toxR marker genes

Multiplex PCR detected the presence of *E. coli* and *Salmonella* spp., whereas *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* were not detected. Isolates positive for the *uidA* were confirmed as *E. coli*, whereas isolates positive for the *invA* gene were confirmed as *Salmonella* spp. Among the 120 oyster samples analyzed, *E. coli* was the most frequently confirmed species, with a prevalence of 13.3% (16/120), followed by *Salmonella* spp. at 5.0% (6/120).

4. Discussion

The results indicate that microbial contamination in oyster farming areas of eastern Thailand is shaped by both environmental variability and the biological accumulation capacity of oysters to

accumulate microorganisms through filter-feeding. Although the environmental and retail surveys were conducted during different sampling periods, the two datasets provide complementary insights into microbial contamination in oyster farming environments and retail oyster products. The environmental survey helps explain spatial and temporal variability in microbial exposure conditions at farming sites, whereas the retail survey reflects microbial quality after harvest, handling, and distribution. Therefore, the findings should not be interpreted as direct farm-to-retail tracking, but as evidence that oyster safety is influenced by both environmental conditions and post-harvest factors.

4.1. Environmental variability and farming conditions

Station 5, located near the Bang Pakong River mouth, was characterized by lower salinity and dissolved oxygen concentrations, but higher total suspended solids (TSS), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations compared with the other sampling stations. In contrast, the highest ammonium (NH_4^+) concentration was observed at Station 1, which was situated within a drainage canal. Higher chlorophyll-*a* concentration at Station 4 may indicate enhanced phytoplankton productivity associated with oyster grow-out activities. Overall, water quality at Stations 1–4 was generally consistent with the Thai Coastal Seawater Quality Standards for Aquaculture (Class 3), particularly because dissolved oxygen concentrations remained above 4 mg/L.

The environmental variability observed in this study likely reflects the influence of freshwater runoff and estuarine circulation in the Bang Pakong River and Chon Buri Bay. Runoff from the watershed may increase suspended particles and nutrient inputs near the river mouth, resulting in elevated TSS concentrations, particularly at Station 5. Similar observations have been reported in estuarine systems where suspended solids decrease with increasing distance from river discharge areas due to dilution of sediments and water-exchange processes (Bordalo et al., 2016). Our findings are also consistent with those reported by Srisunont and Srisunont (2022), who investigated cockle culture areas in Klong Khone and Bang Tabun in the upper Gulf of Thailand and found that dissolved oxygen concentrations were positively associated with salinity, but negatively associated with temperature and dissolved inorganic nitrogen compounds, including NH_4^+ , NO_2^- , and NO_3^- .

Total coliform concentrations permitted in coastal aquaculture waters in several Asian countries, including Indonesia, Japan, Thailand, and Vietnam, are generally limited to 1,000 MPN/100 mL (Rafiq et al., 2025). In the present study, total coliform and *Escherichia coli* concentrations at Stations 3 and 4 remained relatively low and were consistent with the standard during the transition season and dry season. In contrast, Stations 1, 2, and 5 exceeded the recommended limits, likely due to their proximity to land-based activities and freshwater discharge sources. Similarly, studies conducted in Panay and Ivisan, Philippines, reported that total coliform, fecal coliform, and *E. coli* concentrations in water samples exceeded permissible limits (Nuñal et al., 2023). These findings suggest that fecal contamination in nearshore oyster farming areas may be influenced by inadequate sanitation management and terrestrial runoff, particularly during periods of increased freshwater discharge during the wet season (Jeamsripong et al., 2018; 2022; Nuñal et al., 2023). Greater distance from shore and enhanced water exchange may promote dilution processes, thereby reducing nutrient and fecal-indicator concentrations in offshore farming areas (Bordalo et al., 2016). In addition, previous studies have demonstrated that *E. coli* concentrations in oysters are strongly influenced by environmental conditions, showing a negative association with dissolved oxygen and a positive association with precipitation (Jeamsripong et al., 2018).

The concentrations of indicator microorganisms observed in oysters collected from Chon Buri Bay suggest substantial microbial accumulation within farmed oysters. Total bacterial counts (TBC) ranged from 4.9 to 9.1 log CFU/g, with peak concentrations recorded in March 2022 at Stations 3 and 4. These values exceeded the Japanese microbiological guideline for raw oysters intended for consumption ($<5.0 \times 10^4$ CFU/g or approximately 4.7 log CFU/g). Elevated bacterial loads in tropical oyster farming environments may be associated with increased water temperatures, high organic matter availability, and the filter-feeding behavior of oysters, which facilitates microbial accumulation from surrounding waters. Despite the elevated TBC values, *E. coli* concentrations remained relatively low, with the highest concentration observed during the dry season in March 2022 reaching only 8.4 MPN/100 g, which remained within the acceptable limit of <230 MPN/100 g according to the Thai Agricultural Standard (TAS 7022-2012).

Sampling period and the interaction between sampling period and station significantly affected *Vibrio* spp. concentrations, where concentrations reached peaks of approximately 5.5-6.1 log

CFU/g. Presumptive *V. parahaemolyticus* concentrations based on culture and biochemical characterization were highest in March 2022, reaching 150 and 210 MPN/g at Stations 3 and 4, respectively. These concentrations were substantially lower than those previously reported from oyster farming areas in Phang Nga Bay (Andaman Sea, Thailand), where average concentrations reached 8.5×10^7 MPN/g. Seasonal variation in presumptive *V. parahaemolyticus* has also been reported in oysters from Thailand, with the highest concentrations occurring during the rainy and winter seasons (Jeamsripong et al., 2018). Differences in salinity, temperature, nutrient availability, and hydrodynamic conditions among farming areas may contribute to regional variability in *Vibrio* abundance. In addition, the elevated *Vibrio* concentrations observed during March may be associated with increasing seawater temperature and nutrient enrichment during the late dry season, which may promote bacterial proliferation in estuarine oyster farming environments.

4.2. Seasonal effects on microbial contamination

The present study demonstrated clear seasonal variation in microbial contamination of oyster products marketed in eastern Thailand. Total bacterial counts (TBC) in all oyster product types exceeded the microbiological quality criteria established by the Department of Medical Sciences, the U.S. Food and Drug Administration, and Food Standards Australia New Zealand, which generally recommend maximum limits ranging from approximately 5.0 to 6.2 log CFU/g for raw oyster products intended for human consumption (Department of Medical Sciences, 2017; U.S. Food and Drug Administration, 2009; Food Standards Australia New Zealand, 2011). Significantly higher TBC values were observed during the wet season compared with the dry season, suggesting that environmental conditions associated with increased rainfall, humidity, and organic matter input may enhance bacterial proliferation in oyster products.

In the wet season, TBC values were consistently high, as reflected by the relatively narrow distribution pattern observed in the boxplots. In contrast, TBC values during the dry season exhibited greater variability and a wider range, indicating more heterogeneous bacterial contamination patterns. Similar seasonal trends have been reported in tropical coastal environments, where elevated seawater temperatures and rainfall-associated runoff promote microbial growth and survival (Choudhary et al., 2023). Increased freshwater discharge during

the wet season may also transport organic matter, nutrients, and terrestrial microorganisms into oyster harvesting areas, thereby enhancing bacterial accumulation in filter-feeding shellfish.

Fecal coliform concentrations in oyster samples exceeded the recommended microbiological limits for raw oyster consumption established by the Department of Medical Sciences (2017), although *Escherichia coli* concentrations remained within acceptable limits. According to Thai regulations, oysters may be categorized as restricted for raw consumption when fecal coliform concentrations exceed the specified standard, even if *E. coli* concentrations remain below the regulatory threshold (<3 MPN/g). Internationally, microbiological criteria for shellfish safety are commonly based on *E. coli* concentrations. The Codex Alimentarius recommends *E. coli* concentrations below 700 MPN/100 g of shellfish meat, whereas the European Commission (2007) classifies shellfish harvesting areas as Class A when *E. coli* concentrations are below 230 MPN/100 g or fecal coliform concentrations are below 300 MPN/100 g, allowing direct human consumption without further processing. In contrast, Class B shellfish areas permit *E. coli* concentrations up to 4,600 MPN/100 g and require depuration, relaying, or heat treatment before consumption.

Compared with previous studies conducted in Thailand, microbial contamination levels observed in the present study were comparatively lower. Jeamsripong et al. (2018) reported substantially higher concentrations of total coliforms (9,310 MPN/g), fecal coliforms (6,430 MPN/g), and *E. coli* (4,580 MPN/g) in oyster samples. Similarly, Kanchanamala et al. (2024) reported total coliform concentrations ranging from 267 to 16,600 MPN/g, fecal coliform concentrations ranging from 667 to 9,330 MPN/g, and *E. coli* concentrations ranging from 1 to 8,100 MPN/g in oysters collected from coastal aquaculture areas.

Interestingly, mean fecal coliform concentrations were higher during the dry season than during the wet season, although median values between seasons were relatively similar. This discrepancy suggests that several extreme values may have contributed to the higher dry-season mean concentrations. Spatial variation was also observed, with oyster samples collected from Tha Som generally exhibiting slightly higher fecal coliform concentrations than those from Ang Sila. These differences may reflect variations in local environmental conditions, sanitation

management, hydrological characteristics, and proximity to sources of domestic wastewater discharge.

In addition to environmental factors, post-harvest handling and supply chain conditions may also influence microbial contamination levels in oyster products. Previous studies have demonstrated that oysters stored under ambient conditions exhibit increasing most probable number (MPN) counts over time, whereas oysters stored on ice show reduced bacterial levels. Furthermore, prolonged transportation and delayed distribution to retail markets have been associated with significantly increased MPN counts in oysters (Nuñal et al., 2023). These findings suggest that inadequate temperature control during storage and transportation may contribute to elevated bacterial contamination in retail oyster products.

Previous studies have demonstrated that fecal indicator bacteria in oyster farming areas are strongly influenced by rainfall, salinity, freshwater discharge, and human activities (Mignani et al., 2013). Coliform densities are often positively associated with rainfall and negatively associated with salinity, particularly in estuarine systems affected by untreated sewage discharge or inadequate sanitation infrastructure. Increased tourism and coastal urbanization may further contribute to microbial contamination in oyster harvesting areas. Collectively, these findings suggest that oyster cultivation areas in eastern Thailand remain vulnerable to fecal contamination and may pose potential food safety risks if oysters are consumed raw or undercooked. Therefore, improved sanitation management, routine microbial monitoring, depuration processes (Chinnadurai et al., 2021), cold-chain management (Love et al., 2020), and adequate cooking practices remain important measures for reducing bacterial contamination and enhancing seafood safety for consumers.

4.3. Differences among shucked oyster sampling groups

This study demonstrated that total bacterial counts (TBC) in all oyster sample groups, including aseptically shucked oysters (ASO), freshly shucked oysters (FSO), and packed-shucked oysters (PSO), exceeded the microbiological quality criteria established by the Department of Medical Sciences and the U.S. Food and Drug Administration ($<5 \log \text{CFU/g}$), as well as the standards established by Australia and New Zealand ($5.7\text{--}6.2 \log \text{CFU/g}$) (U.S. Food and Drug

Administration, 2009; Food Standards Australia New Zealand, 2011). These findings indicate substantial bacterial contamination in retail oyster products marketed in eastern Thailand.

Previous studies have generally reported lower bacterial loads in oyster products than those observed in the present study. Intarakul et al. (2011) reported total bacterial counts of 3.38 ± 0.52 log CFU/g for aseptically shucked oysters, 7.11 ± 1.55 log CFU/g for freshly shucked oysters, and 6.67 ± 1.52 log CFU/g for packed-shucked oysters collected from retail shops in Chon Buri Province, Thailand. Similarly, Ukwo et al. (2024) reported bacterial contamination levels ranging from 3.25 ± 0.04 to 3.84 ± 0.04 log CFU/g in the mangrove oyster (*Crassostrea gasar*), while Kim et al. (2024) reported aerobic plate counts of 1.86 log CFU/g in freshly harvested oysters and 3.95 log CFU/g in shucked oyster products. These values were considerably lower than those observed in the present study.

The elevated bacterial loads observed in oysters from Ang Sila, Chon Buri Province, and Tha Som, Trat Province, may be associated with environmental and anthropogenic factors influencing oyster farming areas in eastern Thailand. These coastal regions are located near local communities, industrial activities, and riverine discharge systems, including the Bang Pakong and Welu Rivers. Freshwater runoff and nutrient enrichment may promote plankton blooms and increase suspended organic matter, thereby creating favorable conditions for microbial proliferation. Due to their filter-feeding behavior, oysters can accumulate suspended particles, phytoplankton, and pathogenic microorganisms from surrounding waters (Kanchanamala et al., 2024). Similar contamination events have previously been associated with heavy rainfall, sewage discharge, stormwater runoff, and other coastal pollution sources near oyster harvesting areas (Shuping et al., 2023). These findings raise concerns regarding public health risks, particularly because oysters are frequently consumed raw or only partially cooked.

Among the different sample groups, ASO exhibited the highest fecal coliform concentrations, whereas FSO showed the lowest concentrations. This pattern should be interpreted in relation to the sampling design and local retail context. ASO was a laboratory-prepared reference group obtained from whole-shell oysters shucked aseptically under controlled conditions and provided a reference for bacterial loads before market-level processing. The whole-shell oysters used for ASO were commonly stored under ambient conditions and exposed to heat and moisture during

the daytime. In contrast, FSO and PSO samples were shucked, rinsed, and generally stored on ice prior to sale to consumers. The lower fecal coliform concentrations observed in FSO and PSO may suggest that post-harvest preparation steps, including rinsing and storage on ice, help reduce some fecal indicator bacteria. However, because processing steps were not tested independently, the effect of rinsing alone cannot be determined from the present study. These post-harvest conditions may influence fecal coliform concentrations and other microbial loads in oyster products (Nuñal et al., 2023).

4.4. Occurrence of foodborne pathogens

***Vibrio* spp.**

ASO and FSO samples exhibited significantly higher *Vibrio* spp. counts than PSO samples ($P < 0.001$), whereas PSO samples showed lower median values and narrower distributions, indicating comparatively lower and less variable *Vibrio* contamination under the conditions examined. *Vibrio* spp. counts were generally higher during the wet season than during the dry season, with consistently elevated median values, suggesting increased bacterial abundance during periods of higher rainfall and moisture availability. In contrast, the dry season showed greater variability in *Vibrio* concentrations among samples, indicating that *Vibrio* contamination during this period may have been influenced by localized environmental or post-harvest handling factors.

Despite the relatively high *Vibrio* spp. counts, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* were not confirmed by biochemical characterization and multiplex PCR. These findings were consistent with the microbiological criteria established by the European Commission (2007) and the Department of Medical Sciences (2017), which require the absence of pathogenic *Vibrio* species in oyster products. The high abundance of presumptive *Vibrio* spp. observed in this study may therefore reflect the presence of other naturally occurring marine *Vibrio* species, such as *V. alginolyticus* and other non-pathogenic or opportunistic species that commonly inhabit coastal and estuarine environments. In contrast, previous studies have reported the occurrence of pathogenic *Vibrio* species in retail oysters from Mexico (Villicaña et al., 2019), Thailand (Changchai and Saunjit, 2014), and South China (Chen et al., 2010).

Seasonal environmental variability is known to strongly influence *Vibrio* abundance and community composition in oysters and estuarine environments. Previous studies have demonstrated that increased rainfall, elevated temperature, salinity fluctuations, nutrient enrichment, and dissolved oxygen variability can promote *Vibrio* proliferation (Froelich and Noble, 2016; Chen et al., 2020; Simma et al., 2025). The higher *Vibrio* concentrations observed at the Tha Som site may therefore reflect differences in environmental conditions, nutrient availability, hydrodynamic characteristics, and anthropogenic influences between sampling locations. Because *Vibrio* species are naturally associated with marine and estuarine ecosystems, oysters can readily accumulate these bacteria through filter feeding (Jeamsripong et al., 2018). In addition, inadequate refrigeration during retail display and transportation may further increase *Vibrio* abundance in oyster products, particularly under tropical ambient temperatures (Chen et al., 2010). It should also be noted that the multiplex PCR assay used in this study targeted the species-specific *toxR* gene for bacterial identification and was designed to confirm species identity rather than distinguish between virulent and avirulent strains. Therefore, the absence of PCR-confirmed pathogenic *Vibrio* species should be interpreted with the scope of the molecular targets used in this study.

***Salmonella* spp.**

Salmonella spp. were detected in 6 of the 120 oyster samples, representing a prevalence of 5%. Five positive samples were from Chon Buri Province, whereas one was from Trat Province. Positive samples were equally distributed between the dry and wet seasons. The detection of *Salmonella* spp. in retail oysters did not meet the microbiological criteria, established by the Department of Medical Sciences (2017), requiring the absence of this pathogen and this indicates a potential food-safety concern for raw oyster consumption.

Comparable findings have been reported in other shellfish-producing regions. Mudadu et al. (2022) detected *Salmonella* spp. in bivalve mollusks collected from wholesale and retail markets in Sardinia, Italy, while Nuñal et al. (2023) reported the presence of *Salmonella* in mussel and oyster farming areas in the Philippines. In Thailand, Jeamsripong et al. (2018) reported a *Salmonella* contamination rate of 30.6% in oysters collected from Phang Nga Bay (Andaman Sea), with the majority of positive samples occurring during the wet season. *Salmonella*

contamination in shellfish is commonly associated with fecal contamination originating from sewage discharge, untreated wastewater, and surrounding human activities (Pakingking et al., 2022). Following harvest, inadequate temperature control and prolonged storage conditions may further facilitate bacterial proliferation in oyster products. These findings highlight the importance of continuous microbial surveillance, sanitation management, and appropriate post-harvest handling practices to reduce foodborne pathogen risks associated with raw oyster consumption.

4.5 Implications for oyster farming and local markets

Globally, increasing consumption of raw or minimally cooked seafood, particularly oysters, has been associated with foodborne disease outbreaks caused by bacterial pathogens such as *Vibrio* spp. and *Salmonella* spp. Among these, *V. parahaemolyticus* and *V. vulnificus* are recognized as important causes of seafood-associated vibriosis in humans (Jones, 2014; Campbell et al., 2022). Because oysters are filter-feeding organisms, they can efficiently accumulate microorganisms, including fecal indicator bacteria and foodborne pathogens, from surrounding waters.

The findings of the present study suggest that microbial contamination in oysters may be influenced by multiple factors across the production and distribution chain, including environmental conditions, proximity of farming areas to human activities, sanitation management, and post-harvest handling practices. Oyster farming areas located near coastal communities, river mouths, and wastewater discharge sources may be particularly vulnerable to microbial contamination (Jeamsripong et al., 2018). In addition, inadequate temperature control during transportation, retail display, and storage may further promote bacterial proliferation in oyster products (Nuñal et al., 2023).

Environmental conditions such as rainfall, salinity fluctuations, nutrient enrichment, and elevated seawater temperatures may also contribute to increased microbial abundance in oyster farming environments. These factors are particularly important in tropical coastal regions, where warm environmental conditions can favor the persistence and growth of pathogenic bacteria. Consequently, the consumption of raw oysters may pose considerable food safety risks,

especially for immunocompromised individuals and other vulnerable consumer groups (DePaola et al., 2010; Froelich and Noble, 2016).

Collectively, these findings highlight the importance of integrated seafood safety management throughout the oyster supply chain. Routine microbial surveillance, improved sanitation management in coastal communities, effective cold-chain maintenance, and appropriate post-harvest handling practices are essential for reducing microbial contamination in oyster products. In tropical settings, maintaining a continuous cold chain from harvest to retail is particularly important to minimize bacterial proliferation. Practical measures such as temperature monitoring and logging during transportation and retail display, regular training of vendors and processors in hygienic handling practices, and strengthened regulatory oversight of seafood markets could substantially improve compliance with food safety standards. In addition, depuration systems and adequate cooking practices should be encouraged, particularly when elevated microbial concentrations are detected, in order to enhance consumer safety and reduce the risk of seafood-associated foodborne illness.

4.6 Limitations and future perspectives

This study has several limitations that should be considered when interpreting the findings. First, the environmental survey (2021-2022) and the retail survey (2024) were conducted during different sampling periods. Consequently, direct microbial traceability between specific oyster farming areas and retail products could not be established. Instead, the two phases should be interpreted as complementary assessments of microbial contamination occurring at different stages of the oyster production and distribution chain.

Second, oysters marketed in Chon Buri may originate from multiple production areas, including Phetchaburi, Chanthaburi, and Trat provinces. Therefore, the microbial quality observed in retail oyster products likely reflects the combined influence of farming environments, transportation, storage, and retail handling practices rather than conditions at a single production site.

Third, molecular confirmation was performed only for oyster samples collected during the retail survey (Phase 2). Consequently, presumptive *Vibrio* spp. and presumptive *V. parahaemolyticus* detected during the environmental survey (Phase 1) were identified using selective media and

biochemical characterization only and should therefore be interpreted with caution. Although multiplex PCR targeting the *uidA*, *invA*, and *toxR* genes improved species confirmation compared with conventional culture-based identification alone (Godambe et al., 2017), the assay targeted species-specific marker genes and did not evaluate virulence-associated genes or antimicrobial resistance profiles.

Microbial contamination of oysters may originate from both pre-harvest and post-harvest sources. As filter-feeding organisms, oysters can bioaccumulate microorganisms and other contaminants from surrounding waters during cultivation (Richards et al., 2010), while bacterial concentrations may further increase during transportation, storage and retail display if temperature control and sanitation practices are inadequate (DePaola et al., 2010). Although this study focused on bacterial indicators and selected foodborne bacterial pathogens, important viral pathogens associated with bivalve shellfish, such as norovirus and hepatitis A virus, were not investigated.

Future studies should combine information on bacterial and viral abundance with longer-term environmental monitoring to better characterize seasonal and regional variations in microbial hazards. Additional molecular analyses of virulence-associated genes, antimicrobial resistance, and microbial community composition would provide a more comprehensive understanding of oyster-associated microbial risks. Furthermore, evaluating the effectiveness of depuration systems and post-harvest cold-chain management under tropical conditions would provide valuable information for improving oyster safety and supporting sustainable aquaculture and seafood safety in Thailand.

5. Conclusions

This study provides one of the first integrated assessments of microbial contamination in oysters across farming environments and retail products in eastern Thailand. Environmental conditions, particularly freshwater influence and seasonal variability, affected microbial contamination in farming areas, while post-harvest handling and product type further influenced microbial quality at the retail level. Total bacterial counts exceeded recommended microbiological criteria in many oyster samples, and *Salmonella* spp. were detected in 5% of retail oysters. Although pathogenic

Vibrio species were not confirmed by multiplex PCR, high overall *Vibrio* counts indicated the presence of naturally occurring marine *Vibrio* populations that warrant continued monitoring.

These findings emphasize that oyster safety depends on effective management throughout the production and distribution chain. Continuous environmental monitoring, improved sanitation in oyster-growing areas, maintenance of the cold chain during transportation and in retail, routine microbial monitoring, and appropriate post-harvest interventions, including depuration where appropriate, are essential for reducing foodborne risks. Collectively, this study provides baseline information to support evidence-based seafood safety management and sustainable oyster aquaculture in Thailand.

Credit authorship contribution statement

Narinratana Kongjandtre: Funding acquisition, Writing – original draft, Investigation, Data curation. **Supattra Muangham:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **On-ong Chanprasartsuk:** Methodology, Conceptualization, Writing – review & editing. **Uraiwan Intamaso:** Methodology, Conceptualization, Writing – review & editing. **Penchan Laongmanee:** Methodology, Conceptualization, Writing – review & editing. **Michael Steinke:** Funding acquisition. Writing – review & editing. **Wansuk Senanan:** Supervision, Funding acquisition, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was financially supported by (i) Burapha University (BUU), (ii) Thailand Science Research and Innovation (TSRI), (iii) National Science Research and Innovation Fund (NSRF) (Fundamental Fund: Grant no.95/2567), and (iv) Institutional Links grant ID 623743957 under the University of Essex, and the Office of the Permanent Secretary, Ministry of Higher Education, Science, Research, and Innovation (OPS) to Burapha University and delivered by the British Council.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT to assist with grammar checking and improving the logical flow of the manuscript. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the content of the published article.

References

- Almuhaideb, E., Bowers, J., Johnson, T., Richards, G.P., & Parveen, S. (2026). *Vibrios* in farmed oysters in relation to season and different farms across the Chesapeake and Maryland Coastal Bays, USA. *Food Control* 187, 112079. <https://doi.org/10.1016/j.foodcont.2026.112079>
- Andrews, W.H., Wang, H., Jacobson, A., Ge, B., Zhang, G., & Hammack, T. (2023). BAM Chapter 5: *Salmonella*. In *Bacteriological analytical manual*. U.S. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella>
- Bauer, A., & Rørvik, L. M. (2007). A novel multiplex PCR for the identification of *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*. *Letters in Applied Microbiology* 45(4), 371-375. <https://doi.org/10.1111/j.1472-765X.2007.02195.x>
- Bordalo, A. A., Chalermwat, K., & Teixeira, C. (2016). Nutrient variability and its influence on nitrogen processes in a highly turbid tropical estuary (Bangpakong, Gulf of Thailand). *Journal of Environmental Sciences* 45, 131-142. <https://doi.org/10.1016/j.jes.2016.01.011>
- Buehler, A. J., Wiedmann, M., Kassaify, Z., & Cheng, R. A. (2019). Evaluation of *invA* diversity among *Salmonella* species suggests why some commercially available rapid detection kits may fail to detect multiple *Salmonella* subspecies and species. *Journal of food protection* 82 (4), 710-717. <https://doi.org/10.4315/0362-028X.JFP-18-525>
- Campbell, V. M., Chouljenko, A., & Hall, S. G. (2022). Depuration of live oysters to reduce *Vibrio parahaemolyticus* and *Vibrio vulnificus*: A review of ecology and processing parameters. *Comprehensive Reviews in Food Science and Food Safety* 21(4), 3480-3506. <https://doi.org/10.1111/1541-4337.12969>

- Chalermwat, K., Szuster, B. W., & Flaherty, M. (2003). Shellfish aquaculture in Thailand. *Aquaculture Economics & Management* 7(3-4), 249-261.
<https://doi.org/10.1080/13657300309380343>
- Changchai, N., & Saunjit, S. (2014). Occurrence of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in retail raw oysters from the eastern coast of Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 45 (3), 662-9.
- Chen, X., Zhao, H., Jiang, G., Tang, J., Xu, Q., Huang, L., Chen, S., Zou, S., Dong, K., & Li, N. (2020). Responses of free-living *Vibrio* community to seasonal environmental variation in a Subtropical Inland Bay. *Frontiers in Microbiology* 11, 610974.
<https://doi.org/10.3389/fmicb.2020.610974>
- Chen, Y., Liu, X-M., Yan, J-W., Li, X-G., Mei, L-L., Ma, Q-F., & Ma, Y. (2010). Foodborne pathogens in retail oysters in South China. *Biomedical and Environmental Sciences* 23(1), 32–36. [https://doi.org/10.1016/S0895-3988\(10\)60028-1](https://doi.org/10.1016/S0895-3988(10)60028-1)
- Chinnadurai, S., Elavarasan, K., Geethalakshmi, V., Kripa, V., & Mohamed, K.S. (2021) Evaluation of static and flow-through depuration system on depuration of naturally contaminated farmed edible oyster *Crassostrea madrasensis* (Preston, 1916). *Aquaculture* 545, 737141. <https://doi.org/10.1016/j.aquaculture.2021.737141>
- Choudhary, P., Satapathy, P., Mital, A. K., Rustagi, S., Pradhan, K. B., Mohanty, A., Padhi, B. K., & Sah, R. (2023). The rising tide of a deadly bacteria: how warming waters are fueling the spread of *Vibrio vulnificus*. *Microbiology Insights* 16, 11786361231204672.
<https://doi.org/10.1177/11786361231204672>
- Clarke, K. R., & Gorley, R. N. (2006). Primer, 6th edn. PRIMER-E Ltd: Plymouth, UK.
- DePaola, A., Jones, J. L., Woods, J., Burkhardt III, W., Calci, K. R., Krantz, J. A., Kasturi, K., Byars, R. H., Jacobs, E., Williams-Hill, D. & Nabe, K. (2010). Bacterial and viral pathogens in live oysters: 2007 United States market survey. *Applied and Environmental Microbiology* 76 (9), 2754-2768. <https://doi.org/10.1128/AEM.02590-09>
- Department of Fisheries. (2024). Statistics of marine shellfish culture survey. Fisheries statistics group. https://www4.fisheries.go.th/local/index.php/main/view_qr_group/1408/7518

- Department of Medical Sciences. (2017). *Notification on microbiological quality criteria for food and food contact materials (Issue 3)*. Bureau of Quality and Safety of Food, Ministry of Public Health, Thailand. <https://bqsf.dmsc.moph.go.th/en/home>
- European Commission. (2007). *Commission Regulation no. 1441/2007 of 5 December 2007 amending Regulation (EC) no. 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union*. L322, 12–29. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:322:0012:0029:EN:PDF>
- Feng, P., Weagant, S.D., Grant, M.A., & Burkhardt, W. (2020). BAM Chapter 4: Enumeration of *Escherichia coli* and the coliform bacteria. In *Bacteriological Analytical Manual*. U.S. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria>
- Food Standards Australia New Zealand. (2011). *Australia New Zealand food standards code, Part 1.6. Microbiological and processing requirements, Standard 1.6.1. Microbiological limits for food, Compendium of microbiological criteria for food*. Melbourne. <https://www.foodstandards.gov.au/business/microbiological-limits>
- Froelich, B. A., & Noble, R. T. (2016). *Vibrio* bacteria in raw oysters: managing risks to human health. *Philosophical Transactions of the Royal Society B* 371 (1689), 20150209. <https://doi.org/10.1098/rstb.2015.0209>
- Godambe, L. P., Bandekar, J., & Shashidhar, R. (2017). Species specific PCR based detection of *Escherichia coli* from Indian foods. *3 Biotech* 7(2), 130. <https://doi.org/10.1007/s13205-017-0784-8>
- Huss, H. H. (1994). *Assurance of seafood quality*. FAO Fisheries Technical Paper 334. Food and Agriculture Organization, Rome.
- Intarakul, C., Panutrakul, S., & Thungkao, S. (2011). Bacterial contamination in raw shucked oysters in shucking houses and retail shops in Chon Buri Province, Thailand. *Thai Journal of Public Health* 41 (2), 184-198.
- Jeamsripong, S., Chuanchuen, R., & Atwill, E. R. (2018). Assessment of bacterial accumulation and environmental factors in sentinel oysters and estuarine water quality from the Phang Nga estuary area in Thailand. *International journal of environmental research and public health* 15 (9), 1970. <https://doi.org/10.3390/ijerph15091970>

- Jeamsripong, S., Thaotumpitak, V., Anuntawirun, S., Roongrojmongkhon, N., & Atwill, E. R. (2022). Meteorological and water quality factors associated with microbial diversity in coastal water from intensified oyster production areas of Thailand. *Water* 14 (23), 3838. <https://doi.org/10.3390/w14233838>
- Jones, J. (2014). *Vibrio*. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of food microbiology* (2nd ed., pp. 691–698). Academic Press. <https://www.sciencedirect.com/topics/food-science/vibrio>
- Kanchanamala, C., Yatawara, M., & Ariyawansa, S. (2024). Assessment of microbial quality of oysters in Negombo Lagoon, Sri Lanka. *Sri Lanka Journal of Aquatic Sciences* 29(2). <https://doi.org/10.4038/slj.as.v29i2.7615>
- Kaysner, C.A, DePaola, A., & Jones, J. (2004). BAM Chapter 9: *Vibrio*. In *Bacteriological Analytical Manual*, U.S. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-vibrio>
- Kim, Y. M., Choi, E., Cho, T. J., Rhee, M. S., & Kim, S. A. (2024). Microbial profiling of oysters from a processing plant and retail products: Analysis based on culture-dependent methods and 16S rRNA gene sequencing. *Food Research International* 196, 115096. <https://doi.org/10.1016/j.foodres.2024.115096>
- Love, D. C., Lane, R. M., Kuehl, L. M., Hudson, B., Harding, J., Clancy, K., & Fry, J. P. (2020). Performance and conduct of supply chains for United States farmed oysters. *Aquaculture*, 515, 734569. <https://doi.org/10.1016/j.aquaculture.2019.734569>
- Maturin, L., & Peeler, J. T. (2001). BAM Chapter 3: Aerobic Plate Count. In *Bacteriological Analytical Manual*. U.S. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count>
- Mignani, L., Barbieri, E., Marques, H. L. D. A., & Oliveira, A. J. F. C. D. (2013). Coliform density in oyster culture waters and its relationship with environmental factors. *Pesquisa agropecuária brasileira* 48, 833-840. <https://doi.org/10.1590/S0100-204X2013000800004>
- Mudadu, A. G., Spanu, C., Pantoja, J. C. F., Dos Santos, M. C., De Oliveira, C. D., Salza, S., Piras, G., Uda, M. T., Virgilio, S., Giagnoni, L., Pereira, J. G. & Tedde, T. (2022). Association between *Escherichia coli* and *Salmonella* spp. food safety criteria in live

- bivalve molluscs from wholesale and retail markets. *Food Control*, 137, 108942. <https://doi.org/10.1016/j.foodcont.2022.108942>
- Nuñal, S. N., Monaya, K. J. M., Mueda, C. R. T., & Santander-De Leon, S. M. (2023). Microbiological quality of oysters and mussels along its market supply chain. *Journal of Food Protection* 86 (3), 100063. <https://doi.org/10.1016/j.jfp.2023.100063>
- Pakingking Jr, R., Hualde, M. L., Peralta, E., Faisan, J., & Usero, R. (2022). Microbiological quality and heavy metal concentrations in slipper oyster (*Crassostrea iredalei*) cultured in major growing areas in Capiz Province, Western Visayas, Philippines: Compliance with international shellfish safety and sanitation standards. *Journal of Food Protection* 85 (1), 13-21. <https://doi.org/10.4315/JFP-21-257>
- Rafiq, S., Vinitnantharat, S., Plaimart, J., Pattanachan, P., Neamchan, R., Saithong, A., Tirachulee, B. & Werner, D. (2025). Discrepancies in Asia–Pacific standards for water quality in aquaculture and comparison with reported coastal surface water quality in central Thailand. *Current Pollution Reports* 11(1), 57. <https://doi.org/10.1007/s40726-025-00388-0>
- Richards, G. P., McLeod, C., & Le Guyader, F. S. (2010). Processing strategies to inactivate enteric viruses in shellfish. *Food and Environmental Virology* 2, 183-193. <https://doi.org/10.1007/s12560-010-9045-2>
- Ryder, J., Karunasagar, I., & Ababouch, L. (Eds.). (2014). Assessment and management of seafood safety and quality: Current practices and emerging issues. FAO Fisheries and Aquaculture Technical Paper No. 574. Rome: FAO. <https://openknowledge.fao.org/server/api/core/bitstreams/55d84af9-b252-4334-9798-f222a92120c1/content>
- Shuping, L. S., Human, I. S., Lues, R., & Paulse, A. N. (2023). The prevalence of bacteria commonly related to the production of mussels and oysters in Saldanha Bay. *Aquaculture Research* 2023(1), 7856515. <https://doi.org/10.1155/2023/7856515>
- Simma, D., Padovan, A., Kaestli, M., & Gibb, K. (2025). Wet season and monsoon rain events are associated with increased abundance of potentially pathogenic *Vibrio* spp. in a tropical estuary. *The Microbe* 7, 100311. <https://doi.org/10.1016/j.microb.2025.100311>

- Srisunont, T., & Srisunont, C. (2022). Water quality and sediment quality of cockle culture area in the upper Gulf of Thailand. *Current Applied Science and Technology* 22(3), 10-20.
<https://doi.org/10.55003/cast.2022.03.22.003>
- Strickland, J. D. H., & Parsons, T. R. (1972). A practical handbook of seawater analysis (2nd ed., Bulletin No. 167). Fisheries Research Board of Canada.
https://epic.awi.de/id/eprint/39262/1/Strickland-Parsons_1972.pdf
- Szuster, B. W., Chalermwat, K., Flaherty, M. & Intacharoen, P. (2008). Peri-urban oyster farming in the upper Gulf of Thailand. *Aquaculture Economics & Management* 12 (4), 268-288. <https://doi.org/10.1080/13657300802494438>
- Thai Agricultural Standard (TAS 7022-2012). (2012). *Live and raw bivalve molluscs*. National Bureau of Agricultural Commodity and Food Standards. <https://agristandards.acfs.go.th/>
- U.S. Food and Drug Administration. (2009). *Bacteriological examination of shellfish shipments decision tree, Sect. IV, Chap. II*. In National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish. Washington, DC.
https://www.issc.org/sites/default/files/uploads/2009_nssp_guide/section_iv_chapter_ii-12.pdf
- Ukwo, S. P., Ndah, L. N., & Inyang, M. E. (2024). Prevalence of bacterial pathogens in bivalve shellfish harvested from the brackish waters of Niger Delta, Nigeria. *Research Journal of Food Science and Nutrition* 9 (1), 1-8. <https://doi.org/10.31248/RJFSN2023.159>
- Villicaña, C., Amarillas, L., Soto-Castro, L., Gómez-Gil, B., Lizárraga-Partida, M. L., & León-Félix, J. (2019). Occurrence and abundance of pathogenic *Vibrio* species in raw oysters at retail seafood markets in northwestern Mexico. *Journal of Food Protection* 82 (12), 2094-2099. <https://doi.org/10.4315/0362-028X.JFP-19-237>
- Yu, M., Wang, X., & Yan, A. (2021). Microbial profiles of retail pacific oysters (*Crassostrea gigas*) from Guangdong province, China. *Frontiers in Microbiology* 12, 689520.
<https://doi.org/10.3389/fmicb.2021.689520>

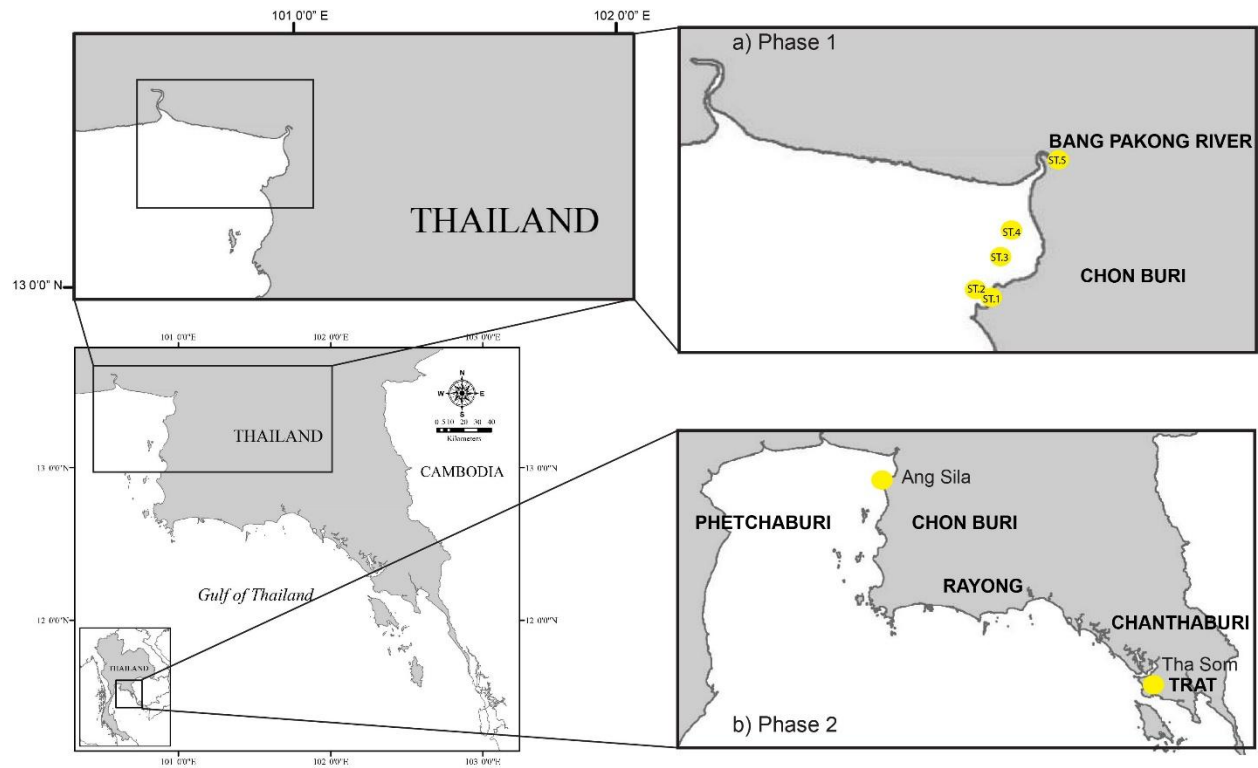


Fig. 1 Map of eastern Thailand showing the study sites for the collection of farmed oysters and surrounding water samples at Stations 1–5 (a), and location of the Ang Sila–Khao Sam Muk community in Chon Buri Province and the Tha Som community in Trat Province (b).

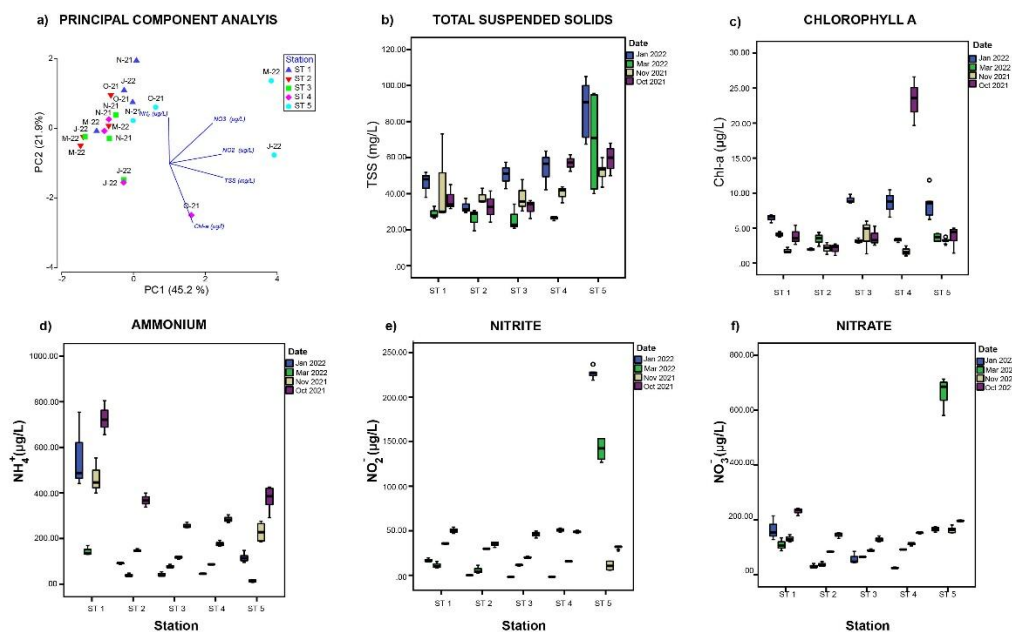


Fig. 2 Principal component analysis (PCA) of environmental variables measured at five sampling stations in Chon Buri Bay (a). Boxplots showing the spatial and seasonal variability of total suspended solids (TSS) (b), chlorophyll *a* (c), ammonium (NH_4^+) (d), nitrite (NO_2^-) (e), and nitrate (NO_3^-) (f).

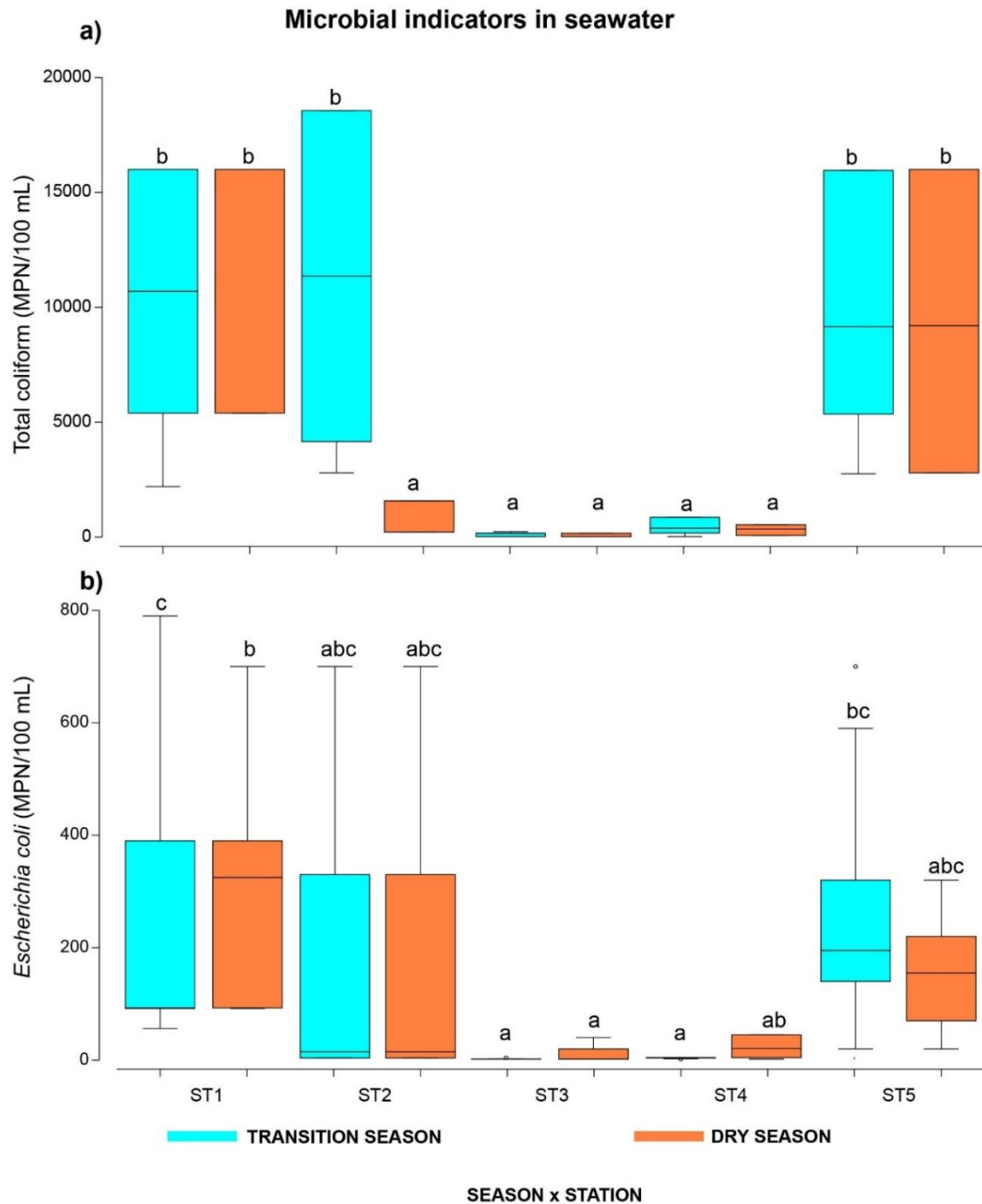


Fig. 3 Boxplots showing the concentrations of total coliforms (a) and *Escherichia coli* (b) in seawater samples collected from five sampling stations in Chon Buri Bay between October 2021 and March 2022. Different letters above error bars indicate statistical groupings among sampling stations and/or sampling periods ($P < 0.05$). Each boxplot shows the median, interquartile range (IQR), and overall variability of microbial counts, with whiskers representing data dispersion and points indicating outliers.

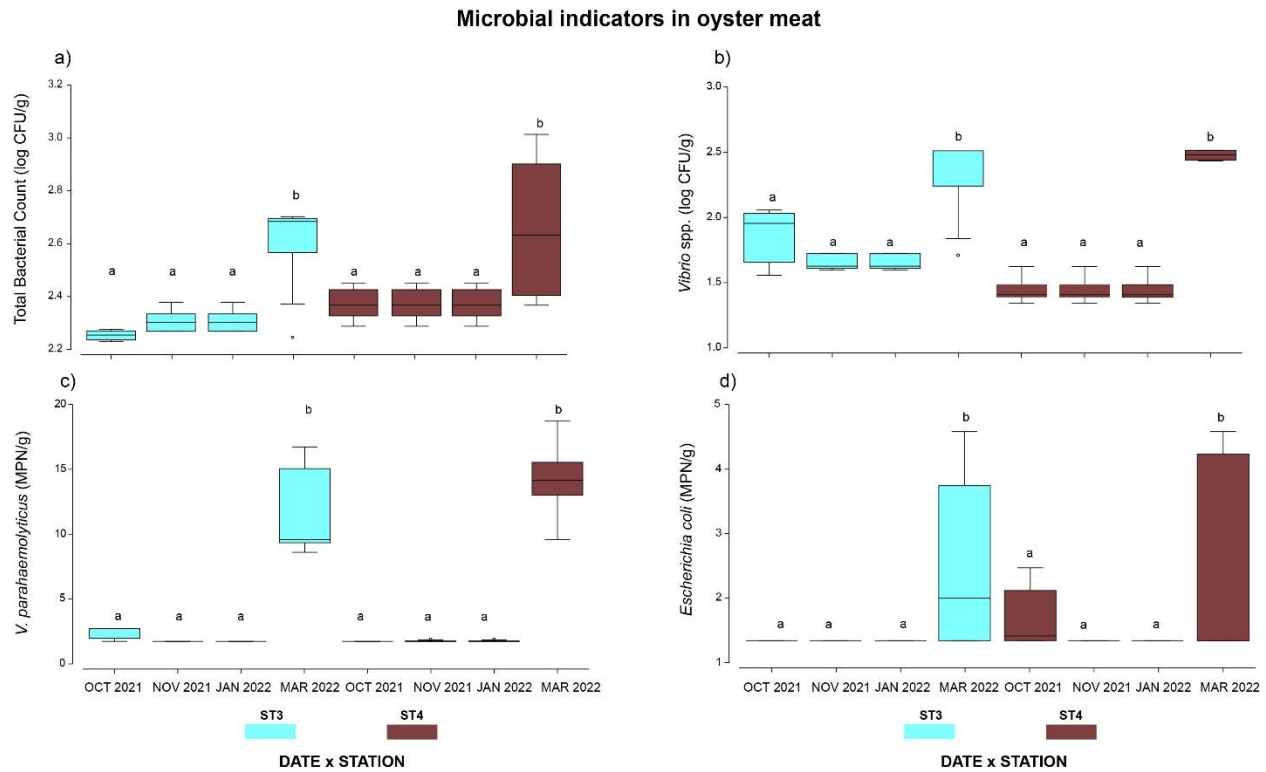


Fig. 4 Boxplots showing the concentrations of total bacterial counts (a), *Vibrio* spp. (b), *Vibrio parahaemolyticus* (c), and *Escherichia coli* (d) in oyster samples collected from Stations 3 and 4 in Chon Buri Bay between October 2021 and March 2022. Each boxplot shows the median, interquartile range (IQR), and overall variability of microbial counts, with whiskers representing data dispersion and points indicating outliers. Different letters above error bars indicate statistical groupings among sampling stations and/or sampling periods ($P < 0.05$).

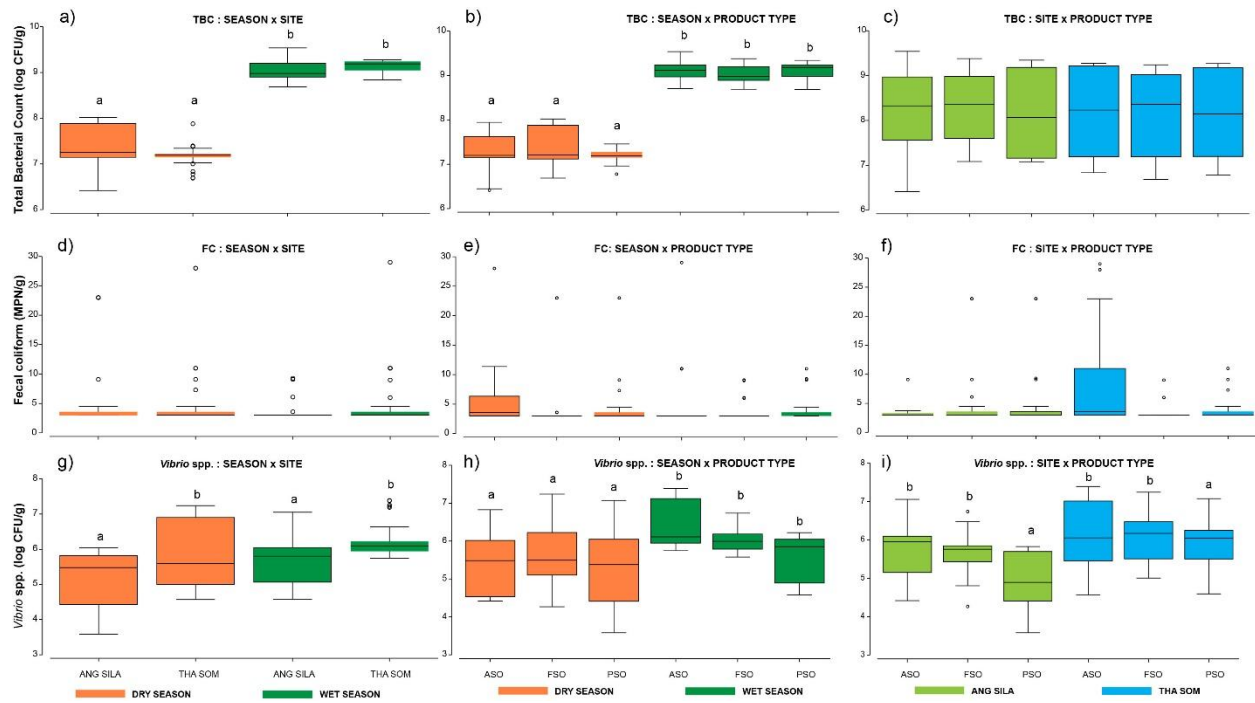


Fig. 5 Combined multi-panel boxplots showing the distributions of total bacterial counts (TBC) (a-c), fecal coliforms (FC) (d-f), and *Vibrio* spp. (g-i) in oyster samples according to season, sampling site, and oyster product type. Each boxplot shows the median, interquartile range (IQR), and overall variability of microbial counts, with whiskers representing data dispersion and points indicating outliers. Different letters above the error bars indicate statistical grouping among seasons, sampling sites, and/or oyster product types ($P < 0.05$).