Marine Environmental Research 212 (2025) 107561

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Environmental factors affecting the presence of coliform bacteria in water and oysters (*Crassostrea cucullata* Born, 1778) in Negombo lagoon, Sri Lanka

Samuel Ayitey [a,b,c,*], Thanne Walawwe Gedera Fathima Mafaziya Nijamdeen [b,g], Harshini Peiris [f], Sunanda Kodikara Arachchilage [e], Isabelle F. George [a], Farid Dahdouh-Guebas [b,c], K.H.M. Ashoka Deepananda [d]

[a] Ecology of Aquatic Systems Research Unit, Brussels School of Bioengineering, Université Libre de Bruxelles, Brussels, Belgium
[b] Systems Ecology and Resource Management Research Unit, Département de Biologie des Organismes, Faculté des Sciences, Université Libre de Bruxelles, Brussels, Belgium
[c] Department of Biology, Faculteit Wetenschappen en Bio ingenieurswetenschappen, Vrije Universiteit Brussel, Brussels, Belgium
[d] Department of Fisheries and Aquaculture, Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna, Matara, Sri Lanka
[e] Department of Botany, Faculty of Science, University of Ruhuna, Matara, Sri Lanka
[f] Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka
[g] Department of Environmental Sciences, Faculty of Science, Open University of the Netherlands, Heerlen, the Netherlands

ARTICLE INFO

Keywords:
Physicochemical parameters
Total coliforms
Fecal indicator bacteria
Escherichia coli
Heterotrophic bacteria
Lagoon water
Oyster tissue
Crassostrea cucullata

ABSTRACT

The recent increase in sewage pollution in the Negombo Lagoon, Sri Lanka, has led to a growing interest in understanding its impact on the local aquatic ecosystem. Physicochemical and general microbiological parameters of the lagoon water (n = 84) were measured at seven sites with contrasted levels of fecal and organic pollution, and their correlation with the presence of total coliform bacteria (TC) was examined. A linear mixed-effect model revealed that heterotrophic bacterial concentrations and electrical conductivity significantly correlated with TC concentrations. Additionally, six individuals of *Crassostrea cucullata* oysters were sampled from five sites (n = 30) to assess their TC levels and compare their variation across sites. Significant differences in TC levels in oysters were observed across the study sites, with oysters from site S7 located in the Northern part of the lagoon being the most contaminated ones. Fecal indicator bacteria, *Escherichia coli*, were found to be present across all the studied sites except S5. Additionally, oysters from five sites tested positive for *E. coli* contamination. The smallest oysters were found at the site most contaminated by microbial load (S2), which may suggest that oysters had decreased filtering activity at the site in response to pollution. Overall, this is the first comprehensive study to provide comparative quantitative data on fecal contamination of oysters in the Negombo Lagoon and its surrounding water.

1. Introduction

The rising human population has led to an increased dependence on coastal ecosystems, as they supply subsistence resources (e.g., seafood, salt), logistical services (e.g., marine transport), and recreational services. These benefits gained from coastal areas have resulted in higher human density in coastal areas than in non-coastal areas (Neumann et al., 2015). Coastal ecosystems encompass a diverse range of habitats, including seagrass meadows, tidal flats, coastal lagoons, and estuaries (Seitz et al., 2013). Coastal lagoons provide both ecological importance (e.g., habitats and spawning grounds for finfish) and economic benefits (e.g., seafood and fuel wood) (Rodrigues-Filho et al., 2023; Nijamdeen et al., 2022), which offer seafood proteins and work opportunities for humans. Human settlements around coastal ecosystems exert multiple anthropogenic pressures (e.g., discharge of effluents from agriculture, animal rearing, aquaculture and domestic households) on the ecosystems, which affect the structure and function of coastal habitats (Newton et al., 2020). Seafood has become one of the most widely consumed sources of protein globally due to its nutritional benefits (long-chain n-3-fatty acids and vitamin D content) and health benefits (anticancer

* Corresponding author. Department of Biology, Faculteit Wetenschappen en Bio ingenieurswetenschappen, Vrije Universiteit Brussel, Brussels, Belgium
E-mail address: sayitey428@gmail.com (S. Ayitey).

https://doi.org/10.1016/j.marenvres.2025.107561
Received 19 December 2024; Received in revised form 30 August 2025; Accepted 16 September 2025
Available online 16 September 2025

properties, improved growth and beneficial for brain function) compared to other sources of protein (Hosomi et al., 2012; Laskowski et al., 2018). The consumption of seafood, such as oysters, in their raw state has become increasingly popular in developed countries (Botta et al., 2020). This is possible due to their strict water quality guidelines, which ensure it is safe for human consumption, and this practice is common in European and American cuisines (Pérez-Lloréns et al., 2021). However, developing countries often lack the necessary infrastructure to manage waste and effectively reduce sewage contamination of aquatic systems. For example, Rondón-Espinoza et al. (2022) performed a study on Yarinacocha lagoon, located in the Peruvian Amazon in Peru. The microbiological quality of the lagoon water was poor, with consequences for the fish marketing chain. They attributed it to the lack of a wastewater treatment system, carry-over of contaminants from anthropogenic activities by the rain, and waste from animal rearing sites surrounding the lagoon. As the consumption of seafood remains a highly valued source of protein, it is necessary to monitor water sources used for seafood production, as well as drinking water and recreational swimming, to ensure human safety. As mentioned earlier, the sources of fecal contamination are multiple. Sewage effluents such as septic leachate or untreated domestic wastewater usually contain substantial amounts of organic materials and fecal bacteria, including total coliforms (TC) (Fouad et al., 2024). TC are a large group of gram-negative, non-spore-forming, lactose-fermenting bacteria mostly derived from human and animal wastes, and their presence in aquatic environments is commonly used as an indicator of fecal contamination. *Escherichia coli* (*E. coli*), a subset of TC, serves as a more specific indicator organism and is frequently used in water quality monitoring (World Health Organization, 2017). The presence of TC in a water body suggests that pathogenic bacteria, such as Shiga-toxin-producing *Escherichia coli*, may also be present (Martin et al., 2016; Some et al., 2021). Downstream sewage outfalls, TC, and more generally, heterotrophic bacteria (HTB, which include TC) tend to survive better or even proliferate due to the organic matter concomitantly released into the environment (He et al., 2022). Unsurprisingly, the abundance of HTB and TC is often positively correlated in aquatic environments (Díaz-Torres et al., 2022; Mondal, 2020). Another source of organic matter and fecal bacteria is surface runoff from agricultural areas into aquatic ecosystems.

Several studies and reviews have established a direct link between TC abundances and environmental parameters (Davies et al., 1995; Hong et al., 2010; Petersen and Hubbart, 2020). Salinity is one of the environmental variables that greatly influences the diversity and dynamics of microorganisms in aquatic environments. High saline environments expose microorganisms to osmotic stress, which affects metabolic processes (Gomaa et al., 2022). A study conducted by Soueidan et al. (2021) on the May River in South Carolina found that TC concentrations were high in the headwaters and decreased towards the mouth of the river, where freshwater mixes with seawater. The authors attributed this decline to the osmotic stress caused by the salinity levels at the mouth of the estuary. Additionally, other environmental factors such as elevated temperatures, non-neutral pH, and high dissolved oxygen alter the decay rates of total coliforms (Hong et al., 2010). For example, An et al. (2002) documented lower monthly *Escherichia coli* concentrations in the marinas of Lake Texoma during the summer season. They explained it through algal photosynthesis, which increased water pH and Dissolved Oxygen (DO) concentrations (An et al., 2002). This phenomenon is enhanced in nutrient-rich (i.e., nitrate- and phosphate-rich) aquatic bodies (An et al., 2002; Van der Steen et al., 2000). High DO levels in combination with UV-radiation result in the formation of reactive free radicals (e.g., singlet oxygen, hydroxyl- and hydroperoxyl-groups) which cause cellular damage to fecal coliforms, including *Escherichia coli* (Hughes, 2003). Conversely, suspended solids promote the survival of fecal indicator bacteria by adsorbing and protecting them from the adverse effects of UV-radiation (Petersen and Hubbart, 2020). Moreover, suspended solids of organic matter favor the survival of heterotrophic bacteria, including coliform bacteria (Amanidaz et al., 2015; Boualam, 2002).

Studies have shown that shellfish such as mussels and oysters can remove excess nutrients and suspended particulate matter from the water column through their filter-feeding behaviour (Dong, 2023c; Filippini et al., 2022; Sauvey et al., 2021). Some studies reported that this process can improve water quality locally (Gray et al., 2021). Thanks to their filtration activity, shellfish also remove bacteria from the water column, as reported by Hajisafarali et al. (2021) for the fish pathogen *Flavobacterium columnare*. This cleaning behaviour could impact the TC contamination levels in the water column. This has driven research to explore their potential use as bioremediators in aquatic environments (Li et al., 2019; Silva et al., 2012).

The Negombo lagoon is a coastal lagoon subjected to fecal contamination by shoreline populations (Ayitey et al., 2024; Kanchanamala et al., 2024). Shellfish, such as oysters, utilize these ecosystems as their habitat, making them suitable for exploring how environmental factors influence the presence of TC and investigating the potential use of oysters to remediate TC in aquatic systems. Studies on fecal contamination of water bodies in Sri Lanka have primarily focused on freshwater systems (Mahagamage et al., 2019, 2020; Thilakarathna et al., 2023). Therefore, a knowledge gap exists regarding TC in coastal lagoons in the country. This study aims to assess TC contamination levels in the water and oysters of the Negombo Lagoon, Sri Lanka, and to address the influence of environmental factors on TC abundance. It was hypothesized that i) TC abundances in lagoon water are correlated with some physicochemical parameters, ii) TC numbers in lagoon water and oysters are higher in areas that experience greater anthropogenic pressure, and iii) fecal indicator bacteria, *Escherichia coli*, contamination were present across all sites in the lagoon water. Those hypotheses aim to advance our understanding of the environmental drivers of microbial contamination and the potential use of oysters in bioremediation efforts within lagoon ecosystems.

2. Materials and methods

2.1. Study area description

The Negombo Lagoon is a coastal lagoon located on the west coast of Sri Lanka. Some parts of the lagoon are surrounded by residential households and industries, which significantly influence the ecological dynamics of the ecosystem. The northern part of the lagoon is heavily impacted by high levels of anthropogenic pollution. In contrast, the southern part of the lagoon is a protected, less disturbed area.

2.2. Sample collection

Ethics clearance (*Reference: 2021.08.17,* April 25, *2022*) was obtained from the Ethics Review Committee of the Faculty of Allied Health Sciences of the University of Ruhuna, Sri Lanka. Seven sample sites (S1-S7, coordinates in Supplementary 1) were identified and chosen for the contrasted levels of fecal contamination. S1, S2, and S7 are all located in the northern section of the lagoon and receive mainly domestic and agricultural effluents (Fig. 1). S3 is located in the western section of the lagoon and receives effluents mainly from shrimp farms. S6 is located on the eastern section of the lagoon and receives effluent from industries. S4 and S5 are in the southern part close to the Dutch canal and the Dandugam-Oya River, respectively. At each sampling site, triplicate water samples were aseptically collected for microbial assessment of HTB and TC in the laboratory. The samples were kept in sterile containers and transported at 2–6 °C to the laboratory for analysis. Out of seven sites that were sampled (S1-S7), oysters were only found in five sites (i.e., S1, S2, S3, S6, and S7). Six oysters were randomly handpicked from the five sites (n = 30) with the assistance of a local fisherman, to assess their microbial load. The oysters were stored in sterile plastic bags and kept in ice coolers at 2–6 °C before being transported to the laboratory.

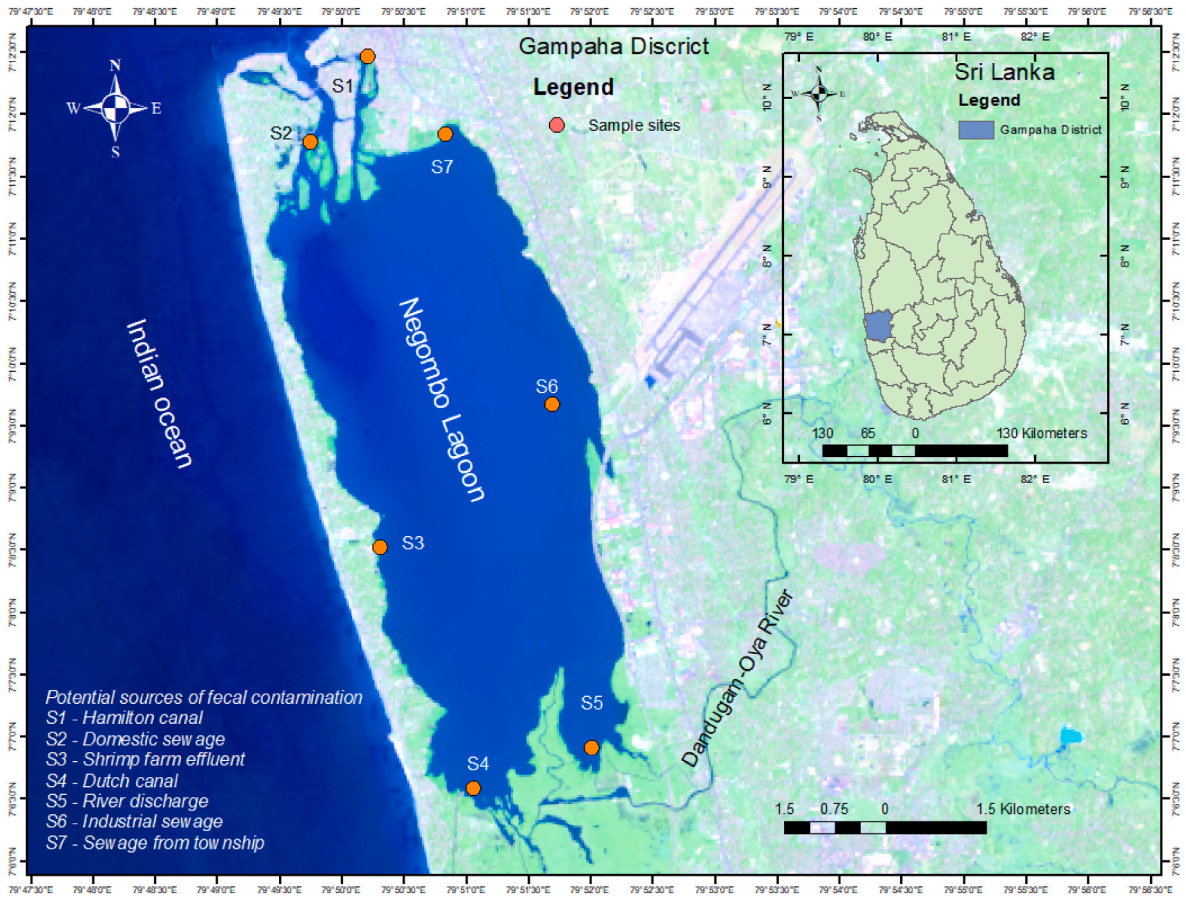

Fig. 1. A study map showing the different sampling sites in the Negombo Lagoon, Sri Lanka and associated potential sources of contamination. The Landsat image was obtained from Google Earth Explorer.

2.3. Physical characterization of oysters

The shell length (mm) and weight (g) of the oysters were determined using a measuring board and a weighing balance, respectively. After shucking the oyster meat, the shell morphological features were examined using a shellfish identification guide as described in our previous study (Ayitey et al., 2024). Based on morphological features, the oysters were identified to the genus and species level as *Crassostrea cucullata*.

2.4. Measurement of physicochemical parameters

All samples were randomly collected in triplicate from each sampling site. The parameters measured *in situ* included temperature, electrical conductivity (EC), dissolved oxygen (DO), pH, total dissolved solids (TDS), and salinity. They were measured by immersing a multi-parametric probe (Hanna Instrument-HI 98194, Woonsocket, USA) into the water and waiting a few minutes for the value to stabilize before recording it. The remaining environmental parameters, i.e. biological oxygen demand (BOD_5), nitrate and phosphate concentrations, were measured *ex situ* in the laboratory. Nitrate and phosphate concentrations were determined by spectrophotometry (Cole-Parmer-Jenway 6405, Staffordshire, UK). Two vials were filled with 1 mL of the lagoon water. One of the vials was used to calibrate the spectrophotometer, and the other vial was mixed with a reagent pack (Hach) to determine the concentration of nutrients in the sample using the spectrophotometer. Additionally, for the calculation of the biological oxygen demand (BOD_5), the Winkler titration method was used to determine the dissolved oxygen levels of the water samples initially and after 5 days of incubation at room temperature, described in Ayitey et al. (2024). The BOD_5 was calculated as follows:

$$BOD_5 = (DO)_i - (DO)_f$$

Where $(DO)_i$ is the initial dissolved oxygen level in the water sample, and $(DO)_f$ is the final oxygen level in the water sample.

2.5. Enumeration of total coliform (MPN/100g) in oyster samples

Crassostrea cucullata oysters were shucked, and their meat was homogenized using a sterile mortar and pestle. About 22.3 g of the homogenized oyster meat was diluted in 200 mL of phosphate-buffered saline solution (PBS) ("oyster diluent") to be used for the analysis. Three different volumes of the oyster diluent (i.e., 10 mL, 1 mL and 0.1 mL) were pipetted 5 times into test tubes (n = 15 per oyster) containing double strength (10 mL of Lauryl Tryptose Broth (LTB) for 10 mL pipetted sample) and single strength (9 mL and 10 mL of LTB for 1 and 0.1 mL pipetted samples, respectively) broth with inverted Durham tubes to test for coliform-containing samples. These tubes were incubated at 37 °C for 24–48 h, after which the positive tubes (i.e., those showing growth and gas production) were counted and compared with the MPN chart to enumerate TC concentration (MPN/g) (Blodgett, 2010). Afterwards, the TC values obtained from the MPN chart were divided by 11.15 g and multiplied by 100 to obtain TC concentrations in MPN/100g units.

2.6. Enumeration of total coliforms in lagoon water samples

TC were enumerated using the most probable number (MPN) method. Three different volumes of lagoon water (i.e., 10 mL, 1 mL and 0.1 mL) were pipetted five times into test tubes (n = 15 per sample)

containing double strength (10 mL of Lauryl Tryptose Broth (LTB) for the 10 mL pipetted sample) and single strength (9 mL and 10 mL of LTB for 1 and 0.1 mL pipetted samples, respectively) broth with inverted Durham tubes to test for samples containing coliforms as performed in Ayitey et al. (2024). The tubes were incubated at 37 °C for 24–48 h, after which the positive tubes (i.e., those showing growth and gas production) were counted and compared with the MPN chart to calculate TC concentration per 100 mL (Ballance and Bartram, 1996).

2.7. Measurement of heterotrophic bacterial abundance in water and oysters samples

HTB were enumerated in the lagoon samples using the standard plate count technique with nutrient agar (Sisco Research Laboratories) as the culture medium. Seven test tubes were filled with 9 mL of distilled water or phosphate-buffered saline (PBS) solution and then autoclaved. To determine the abundance of HTB in lagoon water, the tubes containing distilled water were inoculated with 1 mL of the lagoon water sample, using a 10-fold dilution factor (10^{-1} to 10^{-7}-fold dilutions). 0.1 mL of each dilution was poured onto the nutrient agar and spread uniformly. The plates were then incubated at 37 °C for 24 h. After incubation, the number of colonies within the range of 30–300 colony-forming units (CFU/mL) was counted and recorded (Alo et al., 2012). However, in determining the abundance of HTB in lagoon oysters, the tubes containing PBS were inoculated with 1 mL of the oyster diluents, using a 10-fold dilution factor. We then transferred and spread uniformly, 0.1 mL of the dilution onto the nutrient agar. The plates were then incubated at 37 °C for 24 h. The number of colonies was counted and their abundance computed as colony-forming units per gram (CFU/g). The abundance of bacteria was estimated by applying the formula below.

$$\text{Bacterial load} = \frac{c\ xd}{0.1} \quad \text{(eq 1)}$$

Where c is the number of colonies counted, and d is the dilution factor of the sample.

2.8. Assessment of the presence of Escherichia coli in water and oyster samples

An inoculating loop was used to collect samples from the tubes that tested positive for the presence of coliform bacteria. These samples were then streaked onto Eosin Methylene Blue (EMB) agar plates to determine the presence of *E. coli*. The streaked plates were then incubated at 37 °C for 24 h. Afterwards, the plates were observed for the formation of green metallic sheen colonies (i.e., an indication of the presence of *E. coli*) (Fig. 2A). Furthermore, biochemical tests (indole test) were performed on colony samples that did not test positive on the EMB plates (Fig. 2B). This was achieved by taking a clean bacterial colony from these plates, inoculating it into tryptone water, and incubating it at 37 °C for 24 h. Afterwards, five drops of Kovac's reagent were added to the test tubes to confirm the presence of *E. coli* in the samples. The positive indole test showed purple colouration, while the negative tubes displayed yellowish colour (Fig. 2C & D).

2.9. Data analysis

Microsoft Excel and RStudio 4.2.0 were used for statistical analyses. A linear mixed model with sampling site as a blocking factor was used to determine the environmental variables which had a significant relationship with TC in the lagoon water environment. Both predictors (HTB, temperature, TDS, DO, BOD_5, nitrate, phosphate, EC, salinity, and pH) and response (TC) variables were log-transformed to meet the parametric assumption of homogeneity and homoscedasticity before running the model in R. The ggplot tool in R was used to visualize our ANOVA and regression results. The variation of TC concentration in oysters across the sampling sites was examined using a one-way ANOVA test. The response variables (i.e., TC) were square root transformed before running the ANOVA test to adhere to the parametric assumptions of homogeneity and homoscedasticity. Finally, a post hoc test (Tukey HSD) was used to identify pairs of sampling sites for which differences in TC concentrations in oysters were significant. A Pearson correlation was used to evaluate the relationship between the mean TC in lagoon water and oysters.

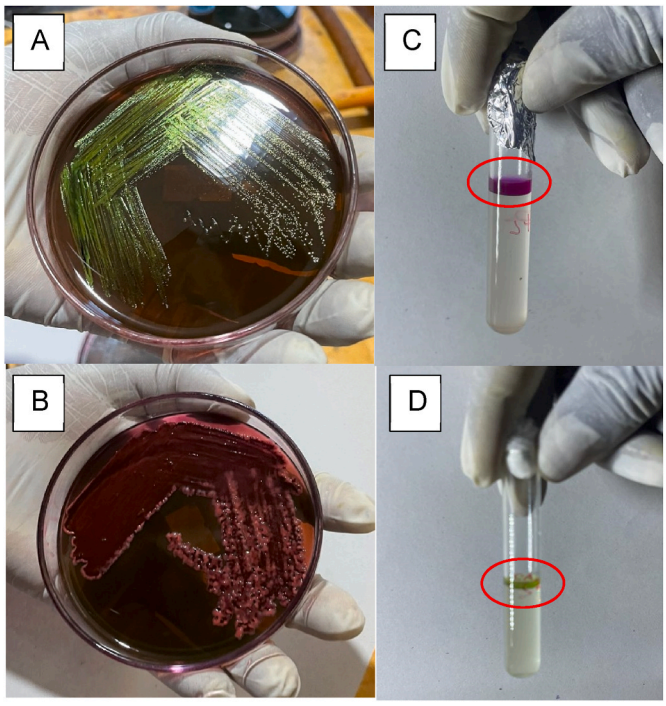

Fig. 2. The following test results indicate A) presence of *E. coli* on culture plate media, B) absence of *E. coli* on culture plate media, C) presence of *E. coli* in tryptone water, D) absence of *E. coli* in tryptone water.

3. Results

3.1. Physicochemical and microbiological parameters of lagoon water

Temperatures of lagoon water ranged between 30.61 and 34.64 °C across all sampling sites (Table 1). Salinity ranged between 15.64 and 32.82 ppt, with the lowest mean values recorded at S4 (i.e., 20.98 ppt) and S5 (i.e., 20.92 ppt) (Table 1). The sample site S5 recorded the highest mean DO concentration (1.03 mg/L) and the lowest BOD_5 level (<2.2 mg/L). Mean phosphate concentration was found to be highest at S4 (i.e., 0.26 mg/L) and that of nitrate (i.e., 2.5 mg/L) at S7. Mean EC, pH and TDS values were found to be highest at S2 (42.79 μS/cm, 8.04 and 21.39 ppt, respectively). The microbial parameters of lagoon water, such as HTB and TC concentrations, were found to be highest at S1 (mean values: 2.18×10^7 CFU/mL for HTB and 1129 MPN/100 mL for TC) and S2 (mean values: 2.10×10^7 CFU/mL for HTB and 1826 MPN/100 mL for TC) (Table 1). Moreover, high variations in concentrations were observed over weeks, not just across sampling stations (Supplementary 2 & 3).

3.2. Relationship between environmental variables and total coliform abundance in lagoon water

A linear mixed-effect model showed that HTB and EC were the only variables that had a significant effect ($p < 0.05$) on TC concentrations across the different sample sites (Table 2). After $\log_{10}$ transformation to ensure normality of the data, HTB showed a positive correlation with the

Table 1
Mean (±SE), minimal and maximal values of microbiological and physicochemical parameters that were measured in the waters of the Negombo Lagoon, Sri Lanka.

Parameters	HTB(CFU/mL)*10^7	TC(MPN/100 mL)	Temperature(°C)	Salinity(ppt)	DO(mg/L)	TDS(ppt)	pH	BOD_5(mg/L)	Nitrate(mg/L)	Phosphate(mg/L)	EC(μS/cm)
S1											
Mean (±SE)	2.18 ± 0.107	1129.43 ± 113.288	31.19 ± 0.104	26.63 ± 1.344	0.54 ± 0.146	17.91 ± 1.567	7.64 ± 0.047	6.18 ± 0.561	1.95 ± 0.352	0.21 ± 0.048	31.3 ± 3.208
Min - Max	1.64–2.73	540–1749.63	30.61–31.91	17.8–31.45	0.23–1.78	7.05–23.8	7.26–7.83	3.84–8.48	0.96–5.39	0.05–0.42	7.55–39.46
S2											
Mean (±SE)	2.1 ± 0.146	1826.17 ± 279.628	30.99 ± 0.050	27.41 ± 1.630	0.56 ± 0.078	21.39 ± 1.149	8.04 ± 0.005	7.38 ± 0.297	1.91 ± 0.157	0.11 ± 0.018	42.79 ± 2.305
Min - Max	1.42–2.9	350–3454.22	30.73–31.26	20.19–32.82	0.37–1.21	16.32–25.2	8.01–8.08	4.88–10.08	1.23–3.08	0.03–0.23	32.66–50.41
S3											
Mean (±SE)	1.27 ± 0.122	879.41 ± 160.244	31.72 ± 0.056	23.2 ± 1.595	0.57 ± 0.126	18.65 ± 1.060	7.82 ± 0.040	8.33 ± 0.383	2.17 ± 0.115	0.12 ± 0.016	37.23 ± 2.450
Min - Max	0.53–1.98	280–1749.63	31.43–32	17–28.58	0.21–1.64	13.9–22.27	7.56–7.96	6.24–10.48	1.41–2.72	0.05–0.23	27.89–49.45
S4											
Mean (±SE)	1.89 ± 0.116	411.67 ± 80.064	31.7 ± 0.120	20.98 ± 1.001	0.82 ± 0.172	18.2 ± 0.347	7.68 ± 0.054	5.9 ± 0.311	1.84 ± 0.173	0.26 ± 0.048	38 ± 0.394
Min - Max	1.18–2.64	70–920	31.12–32.65	17.16–24.73	0.39–2.24	16.25–19.57	7.26–7.91	4.4–7.84	0.93–2.96	0.05–0.48	35.26–39.63
S5											
Mean (±SE)	0.89 ± 0.092	91.22 ± 25.024	32.15 ± 0.216	20.92 ± 1.348	1.03 ± 0.229	18.84 ± 0.434	7.61 ± 0.061	2.22 ± 0.222	2.07 ± 0.166	0.13 ± 0.020	37.76 ± 0.832
Min - Max	0.33–1.36	7.8–280	31.25–33.61	15.64–25.47	0.5–2.71	16.25–20.3	7.14–7.96	1.28–3.84	1.05–2	0.04–0.24	32.61–40.6
S6											
Mean (±SE)	1.94 ± 0.122	791.93 ± 169.247	32.34 ± 0.309	25.96 ± 1.190	0.75 ± 0.096	20.42 ± 0.835	7.77 ± 0.044	6.41 ± 0.670	2.25 ± 0.207	0.12 ± 0.023	40.84 ± 2.478
Min - Max	1.43–2.86	220–1749.63	31.14–34.63	21.39–30.14	0.47–1.35	17.22–23.38	7.54–8.03	4.64–8.32	0.96–3.65	0.01–0.25	34.41–46.77
S7											
Mean (±SE)	2.02 ± 0.149	588.33 ± 156.649	32.74 ± 0.313	26.36 ± 1.567	0.88 ± 0.099	21.14 ± 1.306	7.86 ± 0.076	10.06 ± 0.690	2.5 ± 0.146	0.15 ± 0.019	41.45 ± 2.216
Min - Max	1.22–2.96	70–1600	31.64–34.02	20.28–31.92	0.55–1.52	16.42–29.64	7.54–8.39	8.4–12.72	1.62–3.29	0.08–0.24	32.82–49.25

HTB = Heterotrophic Bacteria, TDS = Total Dissolved Solids, TC = Total Coliform, EC = Electrical Conductivity, DO = Dissolved Oxygen, BOD_5 = Biological Oxygen Demand, SE. = Standard Error. ppt = Parts Per Thousand, Min = Minimum, Max = Maximum.

Table 2
A linear-mixed effect model showing the correlation between total coliforms and environmental variables. All data were log_{10}-transformed before running the model.

	Linear Mixed-Effect Model		
Predictors	Estimates	CI	P-value
(Intercept)	−11.08	−31.68–9.52	0.287
HTB	0.73	0.00–1.45	**0.049**
Temperature	1.52	−9.38–12.41	0.782
Nitrate	−0.14	−0.77–0.49	0.65
Phosphate	0.28	−0.22–0.77	0.27
pH	7.22	−3.02–17.46	0.164
Dissolved oxygen	−0.08	−0.61–0.45	0.766
Electrical Conductivity	−1.59	−3.06–−0.13	**0.033**
Salinity	0.15	−1.85–2.15	0.885
Biological Oxygen Demand	0.16	−0.51–0.82	0.642
Total Dissolved Solids	1.75	−0.32–3.82	0.097
Random Effects			
σ^2	0.13		
$\tau_{00\ Stations}$	0.11		
ICC	0.45		
$N_{\ Stations}$	7		
Observations	84		
Marginal R^2/Conditional R^2	0.193/0.555		

abundance of TC, while EC showed a negative correlation (Table 2). Our model demonstrated weak explanatory power for fixed effects, capturing only 19 % of the variation (Marginal $R^2 = 0.19$), while a large portion (81 %) remained unexplained.

3.3. Inter-site variations in total coliform concentrations in oyster tissues

No oysters were found at the sampling sites S4 and S5, i.e. those with the lowest salinity. Oysters between 70 mm and 85 mm in length were dominant across the five other sampling sites. The largest oysters were found in sites 1, 3, and 7 (Fig. 3). These sites are in the northern and western parts of the Negombo lagoon. There was no significant difference in oyster length and weight across the five sites that were studied (Fig. 3). A Pearson correlation coefficient revealed a significant relationship between length and weight of oysters (*R=0.64, p=0*) (Supplementary 4). A one-way ANOVA test indicated a significant difference ($F_{4,\ 25} = 6.32$; *p = 0.00118*) of TC concentrations in oysters across sites. A Tukey HSD test revealed that TC concentrations in oysters were significantly different at S7 from the other sites (except site S1). The concentrations were highest at site S7, and lowest at S2, S3, and S6 (Fig. 4).

3.4. Assessment of Escherichia coli presence in water and oyster samples

The overall tests revealed that the sampling sites (except S5) were contaminated with *E. coli* in the water (Supplementary 5A). However, the oysters from all five studied sites tested positive for *E. coli* contamination (Supplementary 5B). Additionally, no significant correlation was observed between mean TC concentrations in water and those in oysters (Pearson correlation, p = 0.848) (Fig. 5).

4. Discussion

Environmental parameters in aquatic environments are constantly influenced by both natural phenomena (e.g., tidal inundation and rainfall) and anthropogenic factors (e.g., sewage, aquaculture, and agriculture). In this study, dissolved oxygen (DO) levels were found to be extremely low at all sites, suggesting the influence of factors such as reduced oxygen solubility and elevated decomposition activity by aerobic bacteria. Surface water temperatures ranged from 30.61 °C to 34.63 °C, which was consistent with a previously reported study for the Negombo lagoon with recorded temperatures between 26.0 and 34.1 °C

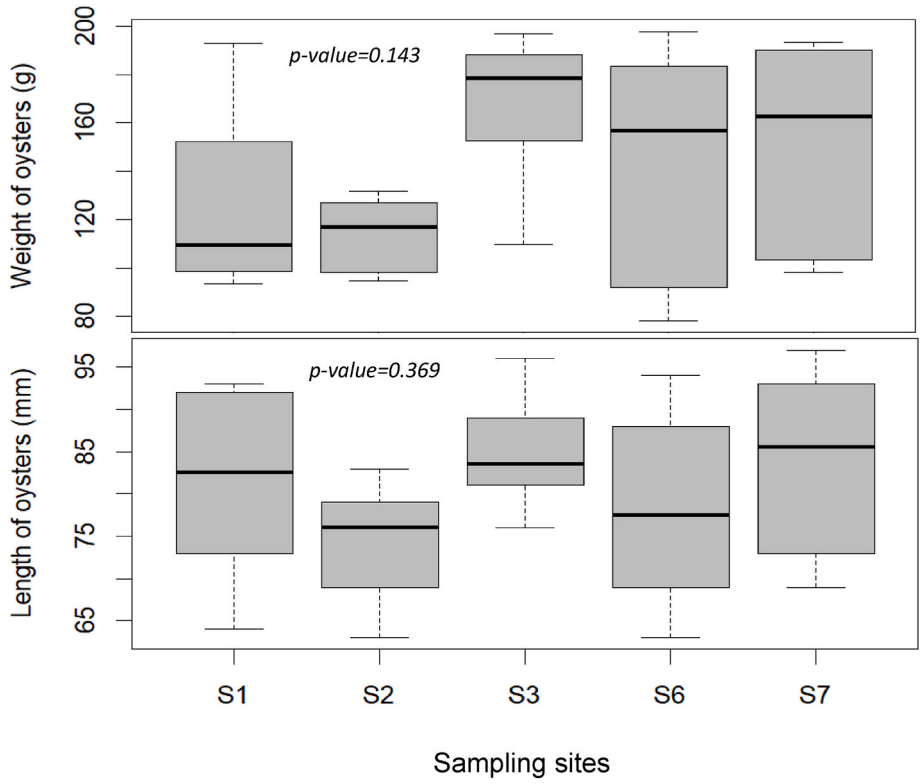

Fig. 3. A boxplot showing the length and weight of oysters across the five sampling sites. The horizontal lines in the boxplot represent the median. The lower and upper hinges represent the first and third quartiles. The sample size (n) is 30.

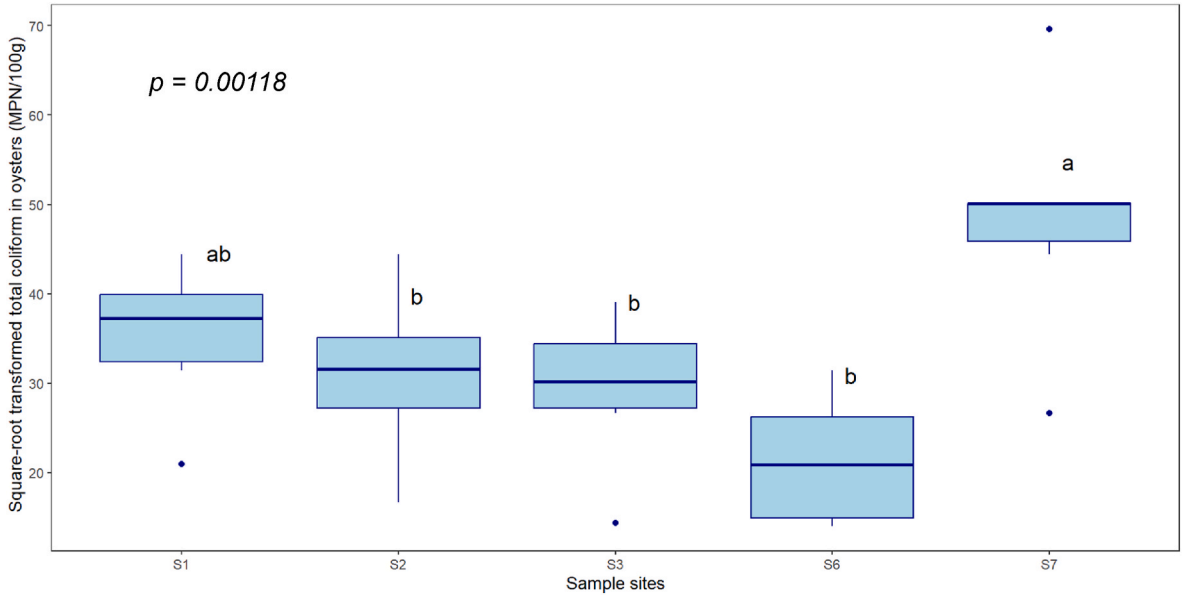

Fig. 4. Total coliform concentrations in oysters across the different sample sites compared by a one-way ANOVA. Identical letters on top of boxplots represent no significant difference. The horizontal lines in the boxplot represent the median. The lower and upper hinges represent the first and third quartiles. The dots represent variables that are outliers.

(Gammanpila, 2013). Elevated temperatures are known to reduce the solubility of oxygen in water (Hsieh et al., 2021), leading to oxygen loss into the atmosphere. This may have contributed to the extremely low DO levels that were recorded. Similarly, Hsieh et al. (2021) reported low mean DO concentrations (2.76 mg/L) at the Dandugam Oya River outflow of the Negombo lagoon. The authors further observed that DO levels occasionally dropped below 1 mg/L in the Hamilton Canal. They attributed the trend in observations to the discharge of sewage from anthropogenic sources into the Negombo lagoon. Concurrently, the mean biological oxygen demand (BOD_5) values were moderately high (<3 mg/L, Hu et al., 2022) across the sampling sites (except site S5), which is possibly due to sewage discharge into those sites. Given the exposure of our sampling sites to sewage pollution, it is possible that the influx of organic waste contributed to the low DO levels observed.

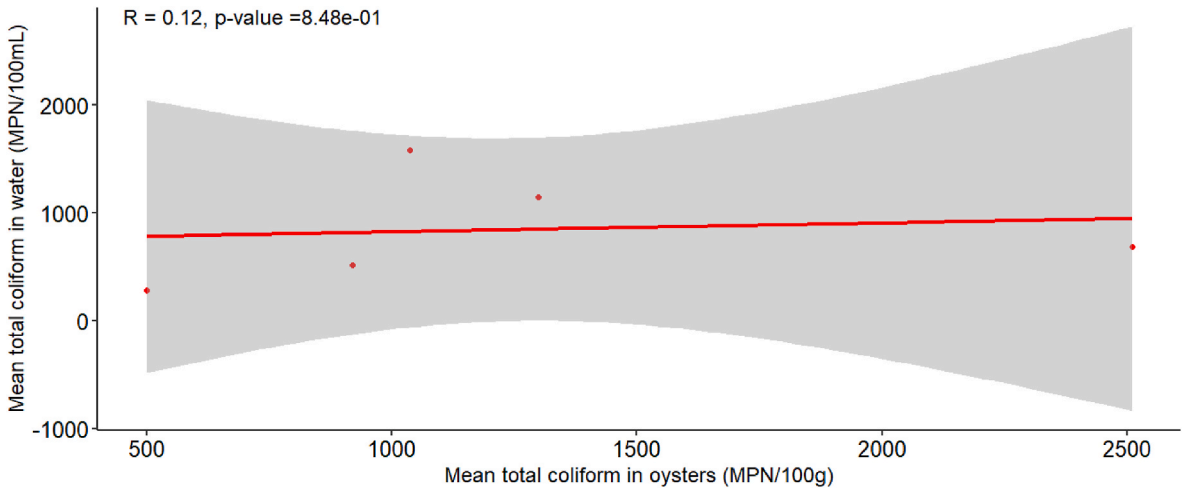

Fig. 5. Pearson correlation between mean total coliform concentrations in lagoon water and oysters in the Negombo lagoon, Sri Lanka.

This study examined several environmental factors that may influence the total coliform (TC) bacteria concentration in the Negombo lagoon. Two were identified by a linear mixed-effects model: heterotrophic bacteria (HTB) and electrical conductivity (EC). TC concentration was inversely correlated with EC, suggesting that higher levels of dissolved ions (e.g., salt) were associated with lower TC concentrations. Generally speaking, high levels of dissolved ions (e.g., Na^{+}, Mg^{2+}, Cl^{-}) in aquatic environments are detrimental to coliform bacteria as they are responsible for an osmotic stress and affect their enzymatic activity (Gomaa et al., 2022). A similar finding was reported by Bordalo et al. (2002), where the authors simulated conditions in the Bangpakong River, Thailand, to examine how varying salinity and light exposure affect microbial die-off. Their results revealed that fecal coliforms had the highest survival rates in experimental tanks with the lowest salinity levels. Additionally, a study conducted by Kanchanamala et al. (2024) on the Negombo lagoon overlapped our sampling period. They reported an inverse relationship between salinity levels and TC concentrations over time. In April, when salinity levels were relatively low, TC concentrations were comparatively high. Conversely, in August, high salinity levels corresponded with lower TC concentrations. The authors attributed elevated TC concentration levels to sewage discharges from nearby fish processing facilities and restaurants located close to the sampling sites.

The positive linear relationship between TC and HTB, as revealed by our mixed model, can be attributed to the fact that coliform bacteria are a subset of heterotrophic bacteria in aquatic environments. Spatial variations in both HTB and TC can be attributed to the fact that the northern part of the lagoon has higher anthropogenic contamination levels compared to the southern part. Additionally, the introduction of both heterotrophic bacteria and their associated coliform bacteria into receiving water bodies can be influenced by rainfall patterns. This natural phenomenon elevates the bacterial loads in the environment (Díaz-Torres et al., 2022). Our study was conducted during the pre-monsoon season, which was marked by rainfall in late April extending into May 2022 (personal observation). This corresponds to observations made by Kanchanamala et al. (2024) on the Negombo lagoon, where the authors noted a salinity drop in April 2022 (likely due to rainfall) and a concomitant increase in microbial concentration. This suggests that rainfall may have contributed to the elevated TC loads, which exceeded the compliance threshold recommended by the Food and Drug Administration of the United States, observed in the lagoon (<43MPN/100 mL compliance for shellfish waters, US FDA, 2017).

This study further assessed TC contamination levels in oyster tissue across the various sampling sites. They were significantly different according to the one-way ANOVA test, with the highest mean TC numbers observed in oysters from S7, followed by S1, both located in the northern part of the Negombo lagoon. Site S7 receives sewage effluents primarily from the city's major market and surrounding domestic households, as reflected in its mean BOD_5 levels. Similarly, S1 is influenced by sewage discharges from the Hamilton Canal. However, the oysters at S1 were not statistically more contaminated than those at S2 (also located in an area exposed to considerable anthropogenic pollution) or S3 and S6 (located in less contaminated areas). Oysters facing high levels of sewage pollution may adopt strategies such as reduced filter-feeding behavior and active purging of contaminants in response to the contamination levels (Bringer et al., 2021; Gökoğlu, 2021; Salama et al., 2021). Such a process may represent a form of homeostasis where oysters regulate internal conditions despite external pollution levels. An additional protective mechanism against pollution might be the egestion of waste in the form of pseudo-feces (mucus-bound masses which are ejected by bivalves as waste), which are subsequently buried by sediment through siltation dynamics, as reported by Craig et al. (2022) for *Crassostrea virginica*. In their study, the egestion process helped reduce the concentrations of contaminants (microplastics) within the oyster tissues. Moreover, our study did not observe a significant relationship between TC in lagoon water and TC in oysters. Contrariwise, Kanchanamala et al. (2024) reported a significant and strong positive correlation between *Escherichia coli* (the main species of fecal coliforms) in oysters and their corresponding water samples in the Negombo lagoon. This suggests that oysters filter fecal bacteria into their tissues, reflecting the fecal contamination levels in the surrounding water, which partly aligned with our observations.

Of note, the smallest average oyster length (74 mm) and weight (114g) were recorded at site S2, located in a contaminated area of the lagoon. Oysters generally thrive in good water quality conditions and are used as sentinel species to monitor sewage pollution in aquatic environments (Volety et al., 2014). Therefore, site S2, which was reported to experience a high microbial contamination load, may contribute to the stunted oyster development that was observed. The lagoon water at the studied sites (except S5) was contaminated with fecal indicator bacteria, *Escherichia coli*. These sites were characterized by anthropogenic activities (e.g., domestic sewage, piggery, aquaculture, fish processing waste, hospital effluence), which contribute fecal bacteria into the lagoon ecosystem (Ayitey et al., 2024; Kanchanamala et al., 2024). The absence of fecal indicator bacteria from S5 can be attributed to the fact that the site mainly receives freshwater discharge from the Dandugam-Oya River, which is less likely to contain fecal contaminants (Personal observation). However, fecal indicator bacteria were recorded in oysters from the five sites that were studied. This can be attributed to the discharge of human and animal waste effluence from the densely

populated communities surrounding those sites.

5. Conclusion

Microbial contamination of coastal water bodies, particularly lagoons, remains a major public health concern. Elevated total coliform levels indicate the risk of fecal contamination by fecal indicator organisms, *Escherichia coli*. Sewage discharge into the Negombo lagoon ecosystem plays a critical role in driving these contaminations. This emphasizes the need for improved waste management systems within the city. Strengthening such interventions would help improve ecosystem health and reduce the risk of fecal pollution. Future studies should focus on identifying pathogenic microbes that are present in the lagoon ecosystem. Furthermore, research would be needed to explore the potential active purging of coliform bacteria by oysters, which would fit into their use in bioremediation of fecal contamination in aquatic environments. Environmental parameters, such as heterotrophic bacteria and dissolved ions, may serve as early warning indicators of contamination, providing valuable insights for resource managers in their monitoring efforts. Future studies should investigate the long-term interactions between rainfall patterns, waste management practices, and microbial pollution in the Negombo lagoon to better understand the contribution of rainfall-driven contamination to the ecosystem.

CRediT authorship contribution statement

Samuel Ayitey: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thanne Walawwe Gedera Fathima Mafaziya Nijamdeen:** Writing – review & editing, Validation, Supervision. **Harshini Peiris:** Writing – review & editing, Validation, Supervision, Resources, Methodology. **Sunanda Kodikara Arachchilage:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition. **Isabelle F. George:** Writing – review & editing, Validation, Supervision, Methodology. **Farid Dahdouh-Guebas:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition. **K.H.M. Ashoka Deepananda:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology.

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

The authors would like to express their sincere gratitude to the European Commission of the European Union for its support through the Erasmus Mundus Joint Master Degree in Tropical Biodiversity and Ecosystems (TROPIMUNDO), and to the FSPI-SEDRIC Project of the Department of Botany at the University of Ruhuna (UoR), Sri Lanka, for providing funding for this research. We also thank the Faculty of Allied Health Sciences, the Faculty of Fisheries and Marine Sciences & Technology, and the Department of Botany at UoR for providing laboratory space and research facilities. Special thanks are extended to Vidusana P. G.G.S. of the Faculty of Allied Health Sciences, UoR, Sri Lanka, for his support in conducting the microbiological aspects of this study, and to Wijesundara W.M.I.C. and Ranawaka D.P.D. from the Department of Botany, UoR, Sri Lanka, for their assistance in conducting interviews with the local community in Negombo, Sri Lanka.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107561.

Data availability

Data will be made available on request.

References

Alo, M.N., Eze, U.A., Eda, N.E., 2012. Microbiological qualities of burukutu produced from a mixture of sorghum and millet. Am. J. Food Nutr. 2, 96–102. https://doi.org/10.5251/ajfn.2012.2.4.96.102.

Amanidaz, N., Zafarzadeh, A., Mahvi, A.H., 2015. The interaction between heterotrophic bacteria and coliform, fecal coliform, fecal streptococci bacteria in the water supply networks. PubMed 44 (12), 1685–1692. https://pubmed.ncbi.nlm.nih.gov/26811820.

An, Y., Kampbell, D.H., Breidenbach, G.P., 2002. Escherichia coli and total coliforms in water and sediments at lake marinas. Environ. Pollut. 120 (3), 771–778. https://doi.org/10.1016/s0269-7491(02)00173-2.

Ayitey, S., Nijamdeen, T.M., Peiris, H., Arachchilage, S.K., George, I., Dahdouh-Guebas, F., Deepananda, K.H.M.A., 2024. Human health risk attributed to consumption of seafood and recreation swimming in Negombo Lagoon, Sri Lanka: an assessment on lagoon water and inhabitant oysters (Crassostrea cucullata born, 1778). Mar. Pollut. Bull. 201, 116189. https://doi.org/10.1016/j.marpolbul.2024.116189.

Ballance, R., Bartram, J., 1996. Water Quality Monitoring. CRC Press eBooks. https://doi.org/10.4324/9780203476796.

Blodgett, R., 2010. Laboratory methods–BAM Appendix 2: Most Probable Number from Serial Dilutions. WebContent. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm.

Bordalo, A., Onrassami, R., Dechsakulwatana, C., 2002. Survival of faecal indicator bacteria in tropical estuarine waters (Bangpakong River, Thailand). J. Appl. Microbiol. 93 (5), 864–871. https://doi.org/10.1046/j.1365-2672.2002.01760.x.

Botta, R., Asche, F., Borsum, J.S., Camp, E.V., 2020. A review of global oyster aquaculture production and consumption. Mar. Pol. 117, 103952. https://doi.org/10.1016/j.marpol.2020.103952.

Boualam, M., 2002. Relationship between coliform culturability and organic matter in low nutritive waters. Water Res. 36 (10), 2618–2626. https://doi.org/10.1016/s0043-1354(01)00460-2.

Bringer, A., Thomas, H., Dubillot, E., Floch, S.L., Receveur, J., Cachot, J., Tran, D., 2021. Subchronic exposure to high-density polyethylene microplastics alone or in combination with chlortoluron significantly affected valve activity and daily growth of the Pacific oyster, Crassostrea gigas. Aquat. Toxicol. 237, 105880. https://doi.org/10.1016/j.aquatox.2021.105880.

Craig, C.A., Fox, D.W., Zhai, L., Walters, L.J., 2022. In-situ microplastic egestion efficiency of the eastern oyster, *Crassostrea virginica*. Mar. Pollut. Bull. 178, 113653. https://doi.org/10.1016/j.marpolbul.2022.113653.

Davies, C.M., Long, J.A., Donald, M., Ashbolt, N.J., 1995. Survival of fecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol. 61 (5), 1888–1896. https://doi.org/10.1128/aem.61.5.1888-1896.1995.

Díaz-Torres, O., Lugo-Melchor, O.Y., De Anda, J., Pacheco, A., Yebra-Montes, C., Gradilla-Hernández, M.S., Senés-Guerrero, C., 2022. Bacterial dynamics and their influence on the biogeochemical cycles in a subtropical hypereutrophic lake during the rainy season. Front. Microbiol. 13. https://doi.org/10.3389/fmicb.2022.832477.

Dong, S., 2023. Biological regulation of water quality in aquaculture. In: Biological Regulation of Water Quality in Aquaculture, pp. 249–307. https://doi.org/10.1007/978-981-19-5486-3_7.

Filippini, G., Dafforn, K.A., Bugnot, A.B., 2022. Shellfish as a bioremediation tool: a review and meta-analysis. Environ. Pollut. 316, 120614. https://doi.org/10.1016/j.envpol.2022.120614.

Fouad, M.S., Mustafa, E.F., Hellal, M.S., Mwaheb, M.A., 2024. A comprehensive assessment of water quality in fayoum depression, Egypt: identifying contaminants, antibiotic pollution, and adsorption treatability study for remediation. Sci. Rep. 14 (1). https://doi.org/10.1038/s41598-024-68990-8.

Gammanpila, M., 2013. Hydrography, nutrients and abundance and distribution of zooplankton in negombo lagoon, Sri Lanka. Sri Lanka J. Aqu. Sci. 15, 13–24. https://doi.org/10.4038/sljas.v15i0.5447.

Gökoğlu, N., 2021. Shellfish Processing and Preservation. Springer eBooks. https://doi.org/10.1007/978-3-030-60303-8.

Gomaa, H.E., Charni, M., Alotibi, A.A., AlMarri, A.H., Gomaa, F.A., 2022. Spatial distribution and hydrogeochemical factors influencing the occurrence of total coliform and E. coli bacteria in groundwater in a hyperarid area, Ad-Dawadmi, Saudi Arabia. Water 14 (21), 3471. https://doi.org/10.3390/w14213471.

Gray, M.W., Pinton, D., Canestrelli, A., Dix, N., Marcum, P., Kimbro, D., Grizzle, R., 2021. Beyond residence time: quantifying factors that drive the spatially explicit filtration services of an abundant native oyster population. Estuaries Coasts 45 (5), 1343–1360. https://doi.org/10.1007/s12237-021-01017-x.

Hajisafarali, M., Aaltonen, S., Pulkkinen, K., Taskinen, J., 2021. Does the freshwater mussel Anodonta anatina remove the fish pathogen Flavobacterium columnare from water? Hydrobiologia 849 (4), 1067–1081. https://doi.org/10.1007/s10750-021-04769-6.

He, Q., Xiao, Q., Fan, J., Zhao, H., Cao, M., Zhang, C., Jiang, Y., 2022. The impact of heterotrophic bacteria on recalcitrant dissolved organic carbon formation in a typical karstic river. Sci. Total Environ. 815, 152576. https://doi.org/10.1016/j.scitotenv.2021.152576.

Hong, H., Qiu, J., Liang, Y., 2010. Environmental factors influencing the distribution of total and fecal coliform bacteria in six water storage reservoirs in the Pearl river Delta region, China. J. Environ. Sci. 22 (5), 663–668. https://doi.org/10.1016/s1001-0742(09)60160-1.

Hosomi, R., Yoshida, M., Fukunaga, K., 2012. Seafood consumption and components for health. Global J. Health Sci. 4 (3). https://doi.org/10.5539/gjhs.v4n3p72.

Hsieh, H., Chuang, M., Shih, Y., Weerakkody, W.S., Huang, W., Hung, C., Muller, F.L.L., Ranatunga, R.R.M.K.P., Wijethunga, D.S., 2021. Eutrophication and hypoxia in tropical Negombo Lagoon, Sri Lanka. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.678832.

Hu, L., Chen, L., Li, Q., Zou, K., Li, J., Ye, H., 2022. Water quality analysis using the CCME-WQI method with time series analysis in a water supply reservoir. Water Sci. Technol. Water Supply 22 (7), 6281–6295. https://doi.org/10.2166/ws.2022.245.

Hughes, K.A., 2003. Influence of seasonal environmental variables on the distribution of presumptive fecal coliforms around an antarctic research station. Appl. Environ. Microbiol. 69 (8), 4884–4891. https://doi.org/10.1128/aem.69.8.4884-4891.2003.

Kanchanamala, C., Yatawara, M., Ariyawansa, S., 2024. Assessment of microbial quality of oysters in Negombo Lagoon, Sri Lanka. Sri Lanka J. Aqu. Sci. 29 (2), 57–66. https://doi.org/10.4038/sljas.v29i2.7615.

Laskowski, W., Górska-Warsewicz, H., Kulykovets, O., 2018. Meat, meat products and seafood as sources of energy and nutrients in the average Polish diet. Nutrients 10 (10), 1412. https://doi.org/10.3390/nu10101412.

Li, M., Callier, M.D., Blancheton, J., Galès, A., Nahon, S., Triplet, S., Geoffroy, T., Menniti, C., Fouilland, E., D'orbcastel, E.R., 2019. Bioremediation of fishpond effluent and production of microalgae for an oyster farm in an innovative recirculating integrated multi-trophic aquaculture system. Aquaculture 504, 314–325. https://doi.org/10.1016/j.aquaculture.2019.02.013.

Mahagamage, M., Manage, P.S., Manage, P.M., 2019. Water quality and microbial contamination status of groundwater in Jaffna peninsula, Sri Lanka. J. Water Land Dev. 40 (1), 3–12. https://doi.org/10.2478/jwld-2019-0001.

Mahagamage, M., Pathirage, M., Manage, P.M., 2020. Contamination status of Salmonella spp., Shigella spp. and Campylobacter spp. in surface and groundwater of the Kelani River Basin, Sri Lanka. Water 12 (8), 2187. https://doi.org/10.3390/w12082187.

Martin, N.H., Trmčić, A., Hsieh, T., Boor, K.J., Wiedmann, M., 2016. The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01549.

Mondal, D., 2020. Seasonal variations in abundance and distribution of pollution indicator bacteria of an eastern Himalayan lake of India. Adv. Zool. Botan. 8 (5), 383–391. https://doi.org/10.13189/azb.2020.080502.

Neumann, B., Vafeidis, A.T., Zimmermann, J., Nicholls, R.J., 2015. Future coastal population growth and exposure to Sea-level rise and coastal flooding - a global assessment. PLoS One 10 (3), e0118571. https://doi.org/10.1371/journal.pone.0118571.

Newton, A., Icely, J., Cristina, S., Perillo, G.M.E., Turner, R.E., Ashan, D., Cragg, S., Luo, Y., Tu, C., Li, Y., Zhang, H., Ramesh, R., Forbes, D.L., Solidoro, C., Béjaoui, B., Gao, S., Pastres, R., Kelsey, H., Taillie, D., Kuenzer, C., 2020. Anthropogenic, direct pressures on coastal wetlands. Front. Ecol. Evol. 8. https://doi.org/10.3389/fevo.2020.00144.

Nijamdeen, T.M., Ephrem, N., Hugé, J., Kodikara, K.a.S., Dahdouh-Guebas, F., 2022. Understanding the ethnobiological importance of mangroves to coastal communities: a case study from Southern and North-western Sri Lanka. Mar. Pol. 147, 105391. https://doi.org/10.1016/j.marpol.2022.105391.

Pérez-Lloréns, J.L., Acosta, Y., Brun, F.G., 2021. Seafood in mediterranean countries: a culinary journey through history. Int. J. Gastron. Food Sci. 26, 100437. https://doi.org/10.1016/j.ijgfs.2021.100437.

Petersen, F., Hubbart, J.A., 2020. Physical factors impacting the survival and occurrence of Escherichia coli in secondary habitats. Water 12 (6), 1796. https://doi.org/10.3390/w12061796.

Rodrigues-Filho, J.L., Macêdo, R.L., Sarmento, H., Pimenta, V.R.A., Alonso, C., Teixeira, C.R., Pagliosa, P.R., Netto, S.A., Santos, N.C.L., Daura-Jorge, F.G., Rocha, O., Horta, P., Branco, J.O., Sartor, R., Muller, J., Cionek, V.M., 2023. From ecological functions to ecosystem services: linking coastal lagoons biodiversity with human well-being. Hydrobiologia 850 (12–13), 2611–2653. https://doi.org/10.1007/s10750-023-05171-0.

Rondón-Espinoza, J., Gavidia, C.M., González, R., Ramos, D., 2022. Water quality and microbiological contamination across the fish marketing chain: a case Study in the Peruvian amazon (Lagoon Yarinacocha). Water 14 (9), 1465. https://doi.org/10.3390/w14091465.

Salama, Y., Salama, O., Amraoui, M.E., Chennaoui, M., 2021. Assessment of bacterial contamination of Mytilus galloprovincialis, Cardium edule and water quality in Bouregreg estuary (Morocco, Atlantic Ocean). SN Appl. Sci. 3 (1). https://doi.org/10.1007/s42452-020-04008-y.

Sauvey, A., Denis, F., Hégaret, H., Roy, B.L., Lelong, C., Jolly, O., Pavie, M., Fauchot, J., 2021. Interactions between filter-feeding bivalves and toxic diatoms: influence on the feeding behavior of Crassostrea gigas and Pecten maximus and on toxin production by pseudo-nitzschia. Toxins 13 (8), 577. https://doi.org/10.3390/toxins13080577.

Seitz, R.D., Wennhage, H., Bergström, U., Lipcius, R.N., Ysebaert, T., 2013. Ecological value of coastal habitats for commercially and ecologically important species. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 71 (3), 648–665. https://doi.org/10.1093/icesjms/fst152.

Silva, C., Yáñez, E., Martín-Díaz, M., DelValls, T., 2012. Assessing a bioremediation strategy in a shallow coastal system affected by a fish farm culture – application of GIS and shellfish dynamic models in the Rio San Pedro, SW Spain. Mar. Pollut. Bull. 64 (4), 751–765. https://doi.org/10.1016/j.marpolbul.2012.01.019.

Some, S., Mondal, R., Mitra, D., Jain, D., Verma, D., Das, S., 2021. Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. Energy Nexus 1, 100008. https://doi.org/10.1016/j.nexus.2021.100008.

Soueidan, J., Warren, A., Pearson, M., Montie, E.W., 2021. A changing estuary: understanding historical patterns in salinity and fecal coliform levels in the May River, SC. Mar. Pollut. Bull. 168, 112384. https://doi.org/10.1016/j.marpolbul.2021.112384.

Thilakarathna, P.T.A., Fareed, F., Athukorala, S., Premachandra, T.N., Noordeen, F., Makehelwela, M., Fernando, R., Gamage, C., Rajapakse, M., Weragoda, S.K., Karunaratne, S.H.P.P., 2023. Monitoring coliform contamination at Mahaweli river water intakes to ensure safe drinking water supply. Sri Lankan J. Infect. Dis. 13. https://doi.org/10.4038/sljid.v13i5.8649. PP15-A28.

US FDA, 2017. National Shellfish sanitation program (NSSP) - guide for the control of Molluscan shellfish, 2017 revision. https://www.fda.gov/media/117080/download, 2017.

Van Der Steen, P., Brenner, A., Shabtai, Y., Oron, G., 2000. Improved fecal coliform decay in integrated duckweed and algal ponds. Water Sci. Technol. 42 (10–11), 363–370. https://doi.org/10.2166/wst.2000.0682.

Volety, A.K., Haynes, L., Goodman, P., Gorman, P., 2014. Ecological condition and value of oyster reefs of the Southwest Florida shelf ecosystem. Ecol. Indic. 44, 108–119. https://doi.org/10.1016/j.ecolind.2014.03.012.

World Health Organization, 2017. Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum. https://www.who.int/publications/i/item/9789241549950.