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Identification and quantification of *Acanthamoeba* spp. within seawater at four coastal lagoons on the east coast of Australia

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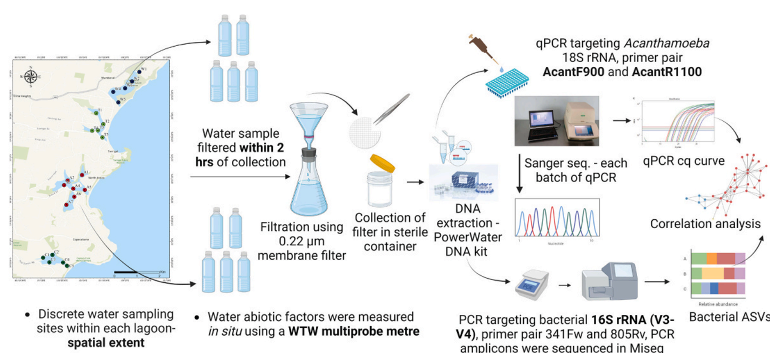
HIGHLIGHTS

- *Acanthamoeba* is an important corneal pathogen, primarily affecting contact lens (CL) wearers.
- A relatively high incidence of *Acanthamoeba* observed in coastal lagoons.
- *Acanthamoeba* incidence was associated with water turbidity, temperature, dissolved O_2 and *intI1* gene ($p < 0.05$).
- Cyanobacteria, *Pseudomonas* spp., *Vibrio pacinii* and *Candidatus* spp. were positively correlation with *Acanthamoeba* in lagoons water.

GRAPHICAL ABSTRACT



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ABSTRACT

Acanthamoeba is an opportunistic free-living heterotrophic protist that is the most predominant amoeba in diverse ecological habitats. *Acanthamoeba* causes amoebic keratitis (AK), a painful and potentially blinding corneal infection. Major risk factors for AK have been linked to non-optimal contact lens hygiene practices and *Acanthamoeba* contamination of domestic and recreational water. This study investigated the incidence and seasonal variation of *Acanthamoeba* spp. within coastal lagoons located on the eastern coast of Australia and then examined the association between *Acanthamoeba* and water abiotic factors and bacterial species within the water.

Water samples were collected from four intermittently closed and open lagoons (ICOLLs) (Wamberal, Terrigal, Avoca and Cockrone) every month between August 2019 to July 2020 except March and April. qPCR was used to target the *Acanthamoeba* 18S rRNA gene, validated by Sanger sequencing. Water abiotic factors were measured *in situ* using a multiprobe metre and 16S rRNA sequencing (V3-V4) was performed to characterise bacterial

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community composition. Network analysis was used to gauge putative associations between *Acanthamoeba* incidence and bacterial amplicon sequence variants (ASVs).

Among 206 water samples analysed, 79 (38.3%) were *Acanthamoeba* positive and *Acanthamoeba* level was significantly higher in summer compared with winter, spring, or autumn ($p = 0.008$). More than 50% (23/45) water samples of Terrigal were positive for *Acanthamoeba* which is a highly urbanised area with extensive recreational activities while about 32% (16/49) samples were positive from Cockrone that is the least impacted lagoon by urban development. All sequenced strains belonged to the pathogenic genotype T4 clade except two which were of genotype clades T2 and T5. Water turbidity, temperature, *intI1* gene concentration, and dissolved O_2 were significantly associated with *Acanthamoeba* incidence ($p < 0.05$). The ASVs level of cyanobacteria, *Pseudomonas* spp., *Candidatus* spp., and marine bacteria of the Actinobacteria phylum and *Acanthamoeba* 18S rRNA genes were positively correlated (Pearson's $r \geq 0.14$). The presence of *Acanthamoeba* spp. in all lagoons, except Wamberal, was associated with significant differences in the composition of bacterial communities (beta diversity).

The results of this study suggest that coastal lagoons, particularly those in urbanised regions with extensive water recreational activities, may pose an elevated risk to human health due to the relatively high incidence of pathogenic *Acanthamoeba* in the summer. These findings underscore the importance of educating the public about the rare yet devastating impact of AK on vision and quality of life, highlighting the need for collaborative efforts between public health officials and educators to promote awareness and preventive measures, especially focusing lagoons residents and travellers.

1. Introduction

Acanthamoeba is a free-living heterotrophic protist found in diverse climatic regions and ecological habitats such as soil, sewage, domestic water supplies, ponds, seawater, and water reservoirs (Khan, 2006; Koyun et al., 2020). *Acanthamoeba* adopts a biphasic life cycle consisting of an active and feeding trophozoite and a dormant double-walled polygonal cyst (Marciano-Cabral and Cabral, 2003). Exposure of trophozoites to harsh conditions results in transformation into the resilient cyst, and the cysts can remain viable for many years (>20 years) (Sriram et al., 2008). Cysts possess ostioles that can sense changes in their environment to allow excystment to occur under suitable conditions such as abundant nutrient supply, optimal temperature (30–32 °C) and osmolarity (50–80 mOsmol) (Garajová et al., 2019). *Acanthamoeba* strains are broadly classified into three groups (I–III) based on cyst shape and size, although this can be influenced by the source of isolation and *in vitro* culture conditions, and these groups consist of over 25 nominal species (Page, 1967). At least 23 genotypes (T1–T23) have been identified based on the 18S rRNA gene sequence of *Acanthamoeba* (Putaporntip et al., 2021), and inter-strain variations in the 18S rRNA subunit (*Rns*) gene sequence is used to identify sub-generic genotypes (Fuerst et al., 2015).

Data indicates that the number of severe and life-threatening infections in humans caused by *Acanthamoeba* has been increasing in recent years (Antonelli et al., 2018; Carnt et al., 2018a; Damhorst et al., 2022; Randag et al., 2019). Immune-suppressed individuals, including HIV infected and tuberculosis patients, are particularly susceptible to *Acanthamoeba* associated brain and lungs infections (Aichelburg et al., 2008). *Acanthamoeba* spp. are the causative agent of encephalitis (typically defined as granulomatous amoebic encephalitis), amoebic keratitis (AK), and have been infrequent causes of sinusitis, cutaneous lesions and *Acanthamoeba* pneumonia which may progress to a disseminated acanthamoebiasis (Aichelburg et al., 2008; Kot et al., 2021). *Acanthamoeba*-associated central nervous system infection is sporadic, but has over 90% mortality (Damhorst et al., 2022). AK causes 2% of global corneal infections and, perhaps due to increase in contact lens use globally, *Acanthamoeba* corneal infections are increasing in many countries (Antonelli et al., 2018; List et al., 2021; Randag et al., 2019; Rayamajhee et al., 2022a). AK is a severe form of infectious keratitis and a significant challenge to clinicians. The diagnosis and treatment of AK patients is difficult, resulting in prolonged medication, late diagnosis and misdiagnosis (Lee et al., 2020), and leading to substantial vision loss (Carnt et al., 2018b; Kaufman and Tu, 2022). *Acanthamoeba* often introduced to the eye through contact lens due to inadequate contact lens hygiene habits. Wearing of contact lens during showering or recreational activities such as swimming or surfing is a major risk factor of AK in developed countries (Hassan et al., 2019; McAllum et al., 2009). Recreational activities, especially in coastal water, increase humans' exposure to *Acanthamoeba* spp. (Mohd Hussain et al., 2022). A retrospective study conducted in Australia showed 17.6% (6/34) of patients acquired AK infection after swimming with their soft contact lenses in fresh or seawater (Höllhumer et al., 2020). There are substantial awareness campaigns such as “no-water stickers” on contact lens storage cases to inform the importance of avoiding water contact with contact lens at all times irrespective of water sources (Arshad et al., 2021). In developing countries, the vast majority of AK cases occur after ocular contamination from water and soil following ocular trauma (Mascarenhas et al., 2014).

Acanthamoeba plays dual role as a phagocytic predator and an environmental host of other microbes, including bacteria, fungi, and viruses (Espinoza-Vergara et al., 2019). Trophozoites consume microorganisms, especially bacteria including potential human pathogens, to fulfil their nutritional needs by taking up microbes through phagocytosis and digesting them in acidic phagolysosomes (Michalek et al., 2013). Predation by *Acanthamoeba* represents a major driving force shaping bacterial population structure and density in ecological niches and may account for eradication of around 60% of the bacterial populations in an environment (Jürgens and Matz, 2002; Mungroo et al., 2021). However, bacterial species have developed antipredator strategies to respond this predation pressure that enhance their survival and persistence in the environment (Matz and Kjelleberg, 2005). A group of bacteria, amoeba-resisting bacteria (ARB), have evolved to resist destruction by free-living amoebae (FLA) such as *Acanthamoeba*. Some of these can exploit amoebae as a host (Shi et al., 2021). Due to the random feeding feature of *Acanthamoeba*, the intracellular microbiota in the same food vacuole could serve as a ‘genetic melting pot’ and enhance the emergence of microbes with increased abilities to survive intracellularly in protists as well as cells of higher organisms (Rayamajhee et al., 2022b). Bacteria that have evolved strategies to overcome the microbicidal milieu in amoeba can be better equipped to escape destruction by human immune cells (Amaro and Martín-González, 2021).

Estuaries and lagoons are highly valued systems and are often used for recreational purposes (Dolbeth et al., 2016; Lau et al., 2019). However, particularly in urban settings, these ecosystems often experience poor water quality, posing a substantial public health risk (Williams et al., 2022b). Contamination of the water in lagoons is usually from diverse sources, including human and animal faeces and rural, industrial and/or urban stormwater runoff. Several studies have reported pathogenic bacteria within urbanised coastal environments (Numberger et al., 2022; Williams et al., 2022b), but studies on *Acanthamoeba* incidence in

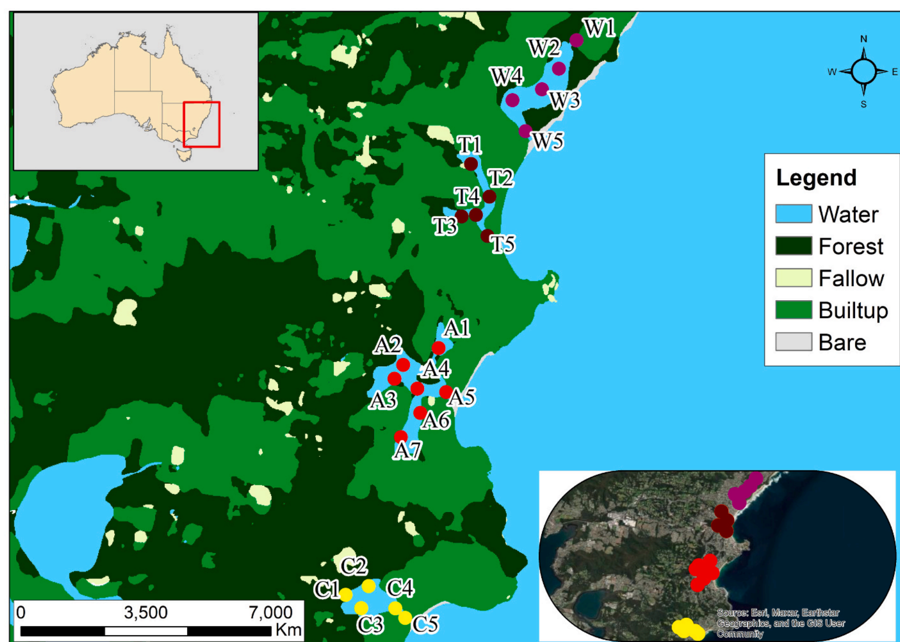

Fig. 1. Map showing sampling sites in the four lagoons on the NSW, Central Coast, Australia. Each circle represents one sampling point within the lagoons. Purple circles refer to five sampling points selected in Wamberal, maroon circles refer to Terrigal, and yellow circles refer to sites within Cockrone, but seven points were selected in Avoca represented by red circles. The map was created using ArcGIS (Esri, GIS, California, USA).

lagoon ecosystems are limited. The Central Coast urbanised catchments reduced water quality is often linked with high faecal indicator bacteria and seasonal algal blooms (DPIE, 2020). Therefore, this study was designed with the principal goal to investigate the incidence and seasonal variation of *Acanthamoeba* within four coastal lagoons located on the south-eastern coast of Australia. The study also examined the association between *Acanthamoeba* and water physiochemical parameters and bacterial species within the water.

2. Methods

2.1. Sampling sites

The Central Coast is located on the Tasman Sea of New South Wales (NSW), Australia, about 80 km north of Sydney, Australia's largest city. Along the 87 km of coastline, estuaries including coastal lakes and lagoons constitute 13% of the region. These water resources are used for a wide range of recreational activities such as surfing, swimming, and kayaking, especially during the summer season.

Water samples were collected from four coastal lagoons (Wamberal, Terrigal, Avoca and Cockrone) on the Central Coast (Fig. 1) between August 2019 to July 2020 (except March and April 2020 due to COVID19 restrictions). These lagoons are predominantly closed to the ocean and retain much of the stormwater inflow and local councils have to open the lagoons for flood mitigation that impacts the beaches, swimmers, and surfers. Within each lagoon, distinct water samples were collected from 5 to 7 sites to evaluate the spatial extent of water quality (Map 1, supplementary material). This sampling procedure allowed for collection of samples across seasonal variations and during two wet-weather events, in February 2020 and October 2019, when 65 and 40 mm of rainfall was recorded five days prior to sample collection, respectively. Except for June and July of 2020, where water sampling was preceded by 7 and 8.6 mm of rainfall, respectively, <5 mm of rain was recorded in the five days preceding all other sampling episodes (Table 1, and supplementary material-S1).

2.2. Sample processing and analyses

2.2.1. Water sample collection and filtration

At each sampling site, water samples were collected using pre-sterilised 2 litre plastic bottles in an extendable pole sampler to collect surface water to 1 m depth water. Within 2 h of collection, each sample was filtered using hydrophilic polyvinylidene fluoride (PVDF) membrane filters (Durapore, 47 mm diameter with 0.22 μm pore-size, Merck

Table 1
Sampling dates, time, and recorded rainfall information.

S.N.	Sampling date	Sampling time	Rainfall information of 5 days preceding sample collection times
1.	13/08/2019	10:33–14:22	<5 mm
2.	15/08/2022	10:34–14:46	<5 mm
3.	12/09/2019	8:50–14:16	<5 mm
4.	12/10/2019	8:35–13-05	40 mm
5.	11/11/2019	9:59–15:59	<5 mm
6.	04/12/2019	8:44–15:20	<5 mm
7.	14/01/2020	7:38–15:57	<5 mm
8.	07/02/2020	7:10–13:21	65 mm
9.	21/05/2020	7:43–14:35	<5 mm
10.	16/06/2020	8:48–15:51	7 mm
11.	09/07/2020	7:49–15:27	8.6 mm

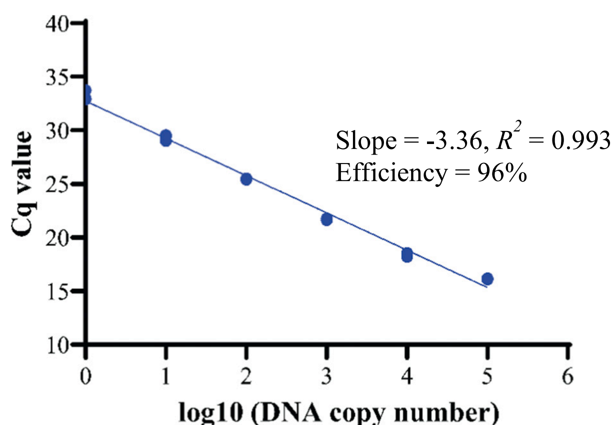

Fig. 2. Standard curve of qPCR Cq values against the *Acanthamoeba* gene copy concentration per microliter (R^2 = 0.993). Tenfold serial dilutions of positive control strain *A. castellanii* ATCC 30868 DNA copies were amplified, and the standard curve was plotted by a linear regression.

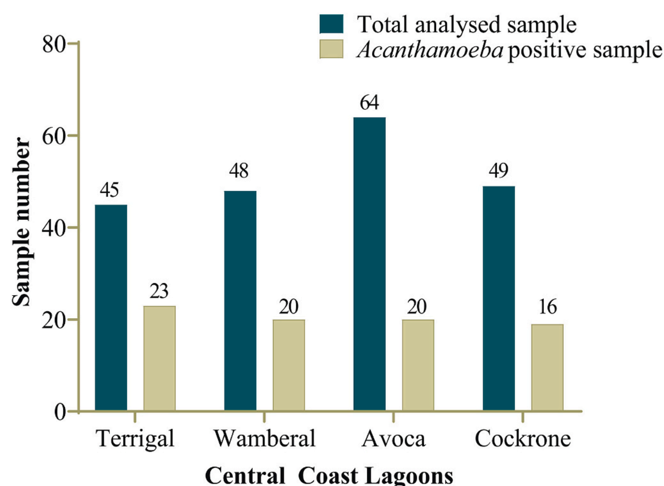

Fig. 3. *Acanthamoeba* 18S rRNA detection results in water samples collected from the Central Coast Lagoons, bar graph represents total sample analysed and *Acanthamoeba* positive samples.

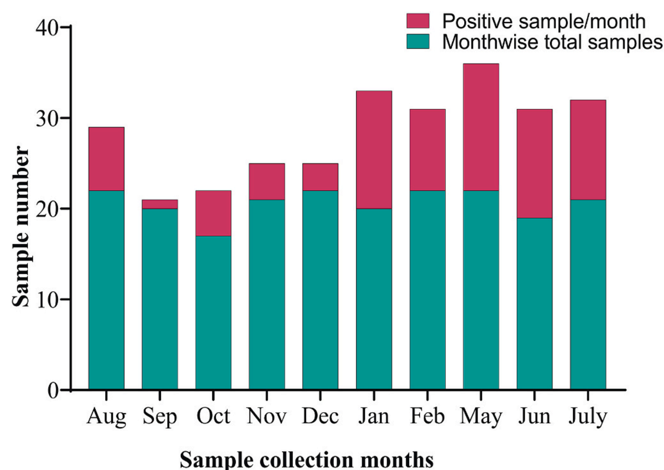

Fig. 4. Graph showing total number of samples processed across ten months of study period and *Acanthamoeba* positive sample in each month. Colour scale denotes number of total water samples analysed and *Acanthamoeba* 18S rRNA positive in qPCR assay.

Millipore Ltd., Cork, Ireland) through a sterile peristaltic pump system (100 rpm). Between each sample, 10% bleach was passed through the pump followed by sterile MiliQ water and then the sample to wash any MiliQ water from the filtration system before entering new filter. The wet filters were cut into small pieces using sterile scissors and collected in 5 ml sterile vial consisting of phosphate-buffered saline (PBS; 3.3 mM NaH_2PO_4, 7.5 mM Na_2HPO_4, 108 mM NaCl, pH 7.2). The vials containing the collected filters were transported on dry ice and stored at −80 °C until DNA was extracted, which was carried out within two weeks of the filters' collection (Kao et al., 2014).

2.2.2. DNA extraction

The PowerWater DNA isolation Kit (QIAGEN GmbH, Hilden, Germany) was used to extract total genomic DNA (gDNA) following the manufacturer's instructions manual. DNA extraction was performed in batches of 96 or 48, with every batch consisting of three blank kits, which were consequently included in all qPCR assays. A Nano Drop UV–Vis spectrophotometer (Thermo Fisher Scientific) was used to measure the amount of extracted dsDNA, and the gDNA vials were subsequently stored at −20 °C until further use.

2.3. Assessment of water physicochemical parameters

A WTW multiprobe meter (Geotech Environmental Equipment, Inc., Wertheim, Germany) was used to access the water physiochemical parameters, including dissolved O_2, temperature, pH, and salinity *in situ* at each sampling point. To determine the chlorophyll-a (Chl-a) concentration, a 0.45 μm glass fibre filter was employed to filter 110 ml of water sample, and the resulting filter was immediately frozen and transported to the laboratory. Chl-a levels were then quantified using a modified version of the APHA Method 10,200-H, as previously described (Eaton and Franson, 2005).

For nutrient assessment, three extra samples were collected from all sampling sites, with one of the samples collected in a 30 ml sterile vial specifically for the total nutrient study. Two of the three samples were filtered through a 0.45 μm cellulose acetate syringe filter to analyse dissolved inorganic and total nutrients. All samples collected for nutrient analysis were instantly frozen prior to measurement using standard techniques (Eaton and Franson, 2005; Williams et al., 2022a); Ammonium N (APHA 4500-NH3-H: Phenate method), Nitrate and Nitrite (APHA 4500-NO3-I -Cadmium reduction method), Total Phosphate (TP), Total Nitrogen (TN), Total Dissolved Phosphate (TDP), Total Dissolved Nitrogen (TDN), (APHA 4500-P-J: Persulfate digestion method), and filterable reactive phosphorus (FRP) (APHA 4500-P-E-Ascobic acid method).

2.4. Quantitative PCR (qPCR) analysis

Quantitative PCR (qPCR) assay targeting the *Acanthamoeba* 18S rRNA was used to analyse the amount of *Acanthamoeba* in each sample. The qPCR assay was performed using the primer pair AcantF900 (5’-CCCAGATCGTTTACCGTGAA-3′) and AcantR1100 (5’-TAAATATTAATGCCCCCAACTATCC-3′) which yields a fragment of ~180 bp (Qvarnstrom et al., 2006). qPCR was performed using a CFX384 real-time PCR system (Biorad, Hercules, California, USA) with the following cycling conditions: 95 °C for 3 min followed by 45 cycles of 95 °C for 15 s and 56 °C for 1 min, followed by a melting curve (efficiency, R^2 = 0.993). Each reaction volume of 5 μl contained 2.5 μl of SYBR Green Master Mix (Biorad), 0.2 μl of each primer, 1.1 μl of molecular grade water and 1 μl of extracted DNA. The SYBR Green fluorescence intensity was measured during the annealing/extension step and quantified against a seven point calibration curve prepared from genomic DNA that was extracted from a known number of cells. Molecular grade water instead of template DNA was used as a negative control. DNA extracted from 3.15 × 10^6 cells of *A. castellanii* ATCC 30868 was used as a reference sample to optimise the qPCR assay and for

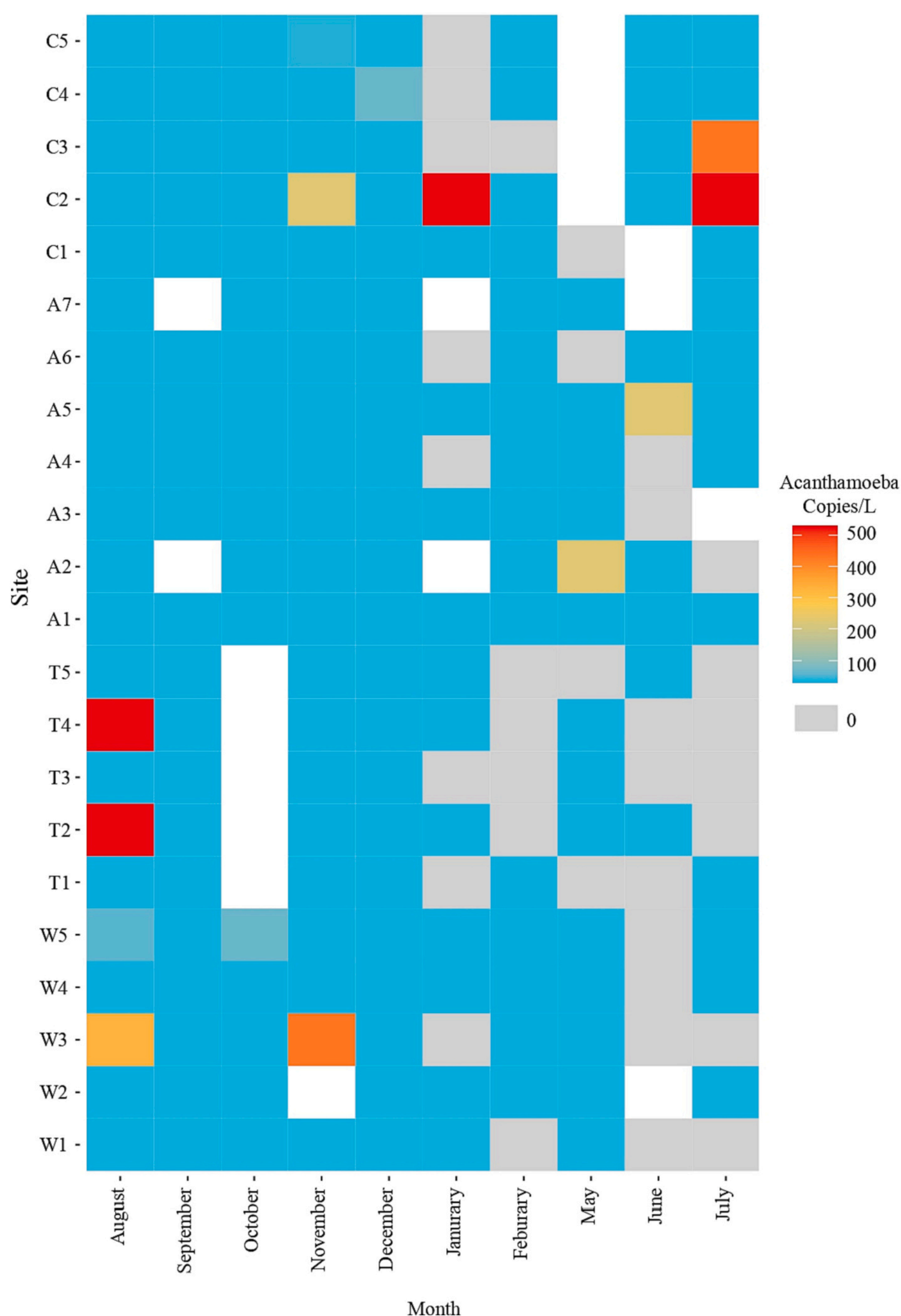

Fig. 5. Heatmap displaying the distribution of *Acanthamoeba* 18S rRNA across four lagoons in Y-axis (W = Wamberal, T = Terrigal, A = Avoca, and C = Cockrone) and sampling months (X-axis). Colour scale denotes *Acanthamoeba* copy numbers/litre quantified using optimised qPCR. Grey cells represent samples below detection limit by optimised qPCR assay. White cells correspond no samples collected.

ten-fold serial dilution from 10^5 to 10^{-1}. All tests were performed in triplicate to gauge reproducibility of the assay.

Following the qPCR melt curve analysis, environment samples with peak slightly different from the standard curve were sent to sanger sequencing (Ramaciotti Centre for Genomics, UNSW, Sydney, Australia) after amplification with the primer AcantF900 (5'-CCCA-GATCGTTTACCGTGAA-3′) to confirm that the product that was amplified is *Acanthamoeba* 18S rRNA. Low quality nucleotide sequences were manually checked and trimmed with Chromas (2.6.6) software (Technelysium Pty Ltd., Brisbane, Australia) (Stucky, 2012) then aligned using the MUSCLE algorithm and a phylogenetic tree was constructed using the neighbour-joining method and Bayesian approach with Kimura-2 parameters by 1,000 bootstraps in MEGA-X (Kumar et al., 2018). A phylogenetic tree was visualised using the interactive tree of life (iTOLv6) (Letunic and Bork, 2007). Publicly available nucleotide sequences of *Acanthamoeba* genotypes were retrieved from the NCBI as reference strains for phylogenetic analysis (Table 1, supplementary material). The trimmed sequence reads were blast in the NCBI nucleotide sequences database (BLASTn) to identify the *Acanthamoeba* genotypes with the highest percentage homology.

2.5. 16S sequencing and analysis

Acanthamoeba trophozoites graze different bacteria in aquatic environment, so bacterial community composition was characterised to examine the association of *Acanthamoeba* spp. incidence with bacterial

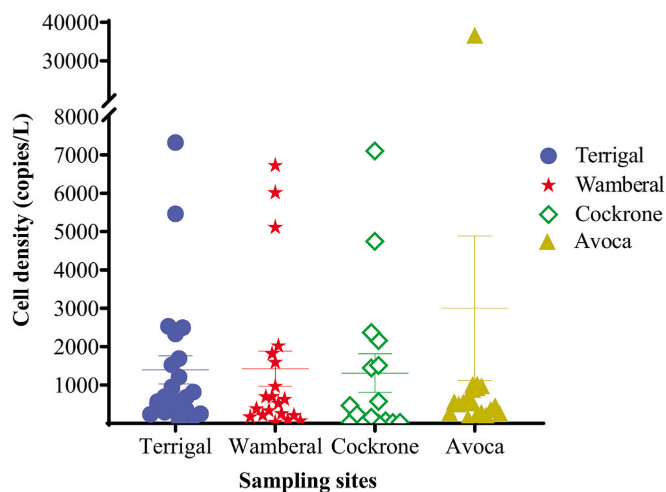

Fig. 6. Scatter plot of *Acanthamoeba* cells density (copies/litre) across four sampling lagoons among positive samples of each site, all values are mean ± SEM, *Acanthamoeba* cell density was not significantly ($p > 0.05$) different with sampling sites.

species. To identify the bacterial community composition in water samples, bacterial 16S rRNA (V3-V4) gene was amplified using the primers 341Fw (5'-CCTACGGGNGGCWGCAG-3′) and 805Rv (5'-GACTACHVGGGTATCTAATCC-3′) under the following PCR conditions: 95 °C for 3 mins followed by 25 cycles of amplification (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s) and final elongation at 72 °C for 5 mins (Illumina, 2013; Suzuki et al., 2000). The PCR amplicons were then sequenced using the Illumina MiSeq platform with 300 bp paired-end analysis (Ramaciotti Centre for Genomics, University of New South Wales). Paired-end reads (R1 and R2) were analysed using the DADA2 pipeline, where reads with any 'N' bases and V3-V4 primers were trimmed using the cutadapt plugin (Kechin et al., 2017). Both reads were truncated to eliminate low-quality terminal sides (R1 = 280 and R2 = 250). Filtered and truncated ASVs were annotated against the SILVA v138 small subunit rRNA database (SSU) with a 50% probability cut-off. The ASV feature table was further filtered to remove any ASVs not assigned as bacteria or classified as mitochondria or chloroplast and the dataset was rarefied to 30,000 counts using the vegan package (Dixon, 2003). Alpha diversity within samples was evaluated using observed ASVs (richness) and Shannon entropy index. The beta diversity between samples was compared using non-phylogenetic based Bray-Curtis dissimilarity index. Permutational multivariate analysis of variance (PERMANOVA), implemented as 'adonis2' function in the vegan (v2.6–4) R-package (Oksanen et al., 2013), was used to assess the bacterial diversity among and within groups. When multiple comparison testing was carried out, the Benjamini-Hochberg (BH), a post-hoc correction was applied to control the false-discovery rate (FDR). Statistical significance was established at $p < 0.05$ for adjusted p-values. The alpha and beta diversity of bacteria in each lagoon across four seasons (summer, autumn, winter, and spring) of sampling was compared with incidence of *Acanthamoeba* spp. Furthermore, we used 90% of top ASVs to investigate potential correlations between the incidence of *Acanthamoeba* and the relative abundance of specific bacterial species. The sequencing data was rarefied to 30,000 reads, ensuring that each sample had a consistent number of 30,000 reads for downstream analysis (supplementary material-S1). All the raw sequence files of 16S rRNA have been deposited to the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA884124.

The class 1 Integron-integrase gene (*intl1*) has been established as an effective indicator of anthropogenic contamination in aquatic habitats (Gillings et al., 2015). Therefore, *intl1* gene was assessed in all water samples processed in this study, as previously described (Williams et al., 2022a), and the *intl1* gene qPCR data was used to test for correlation with *Acanthamoeba* incidence in lagoons water.

2.6. Statistical analysis

The Kaiser-Meyer-Olkin (KMO) and Bartlett tests were carried out to ensure sampling adequacy. Principal component analysis (PCA) and Spearman rank-order correlation coefficient were evaluated to examine the impact of water physiochemical parameters and incidence of *Acanthamoeba* (Kao et al., 2014). Physicochemical parameters of water along with quantification of *Acanthamoeba* cells, were normalised to carry out PCA in an attempt to identify any positive or negative correlations. A MICtools analysis was also run using Cytoscape version 3.9.1 with the purpose of determining the Pearson's r correlation between the *Acanthamoeba* 18S rRNA measured in qPCR assay, *intl1* gene, and the bacterial communities on the ASVs level. Normally distributed data was compared using one-way ANOVA, followed by Tukey pairwise comparison to identify significant differences between groups and a p value of <0.05 was considered significant in Prism (V8, GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Optimization of qPCR assay

The qPCR assay with primer pair AcantF900 and AcantR1100 at 56 °C annealing temperature gave a linear relationship between the Cq value and *Acanthamoeba* number (Fig. 2). The lowest detection limit of the optimised qPCR was equivalent to one *Acanthamoeba* cell per sample (equivalent to $\log_{10}10$). The slope of −3.36 from the standard curve was equivalent to an amplification efficiency of 96%. The number of *Acanthamoeba* spp. detected in each sample from qPCR positive samples was in the range of 4.2 to 3.7×10^4 cells/litre, corresponding to Cq values 34.1 and 27.2, respectively.

Table 2
Rns genotypes identification of *Acanthamoeba* sequences recovered from coastal lagoons ($n = 9$) and rivers ($n = 2$).

S. N.	Strain ID	Sample site	NCBI BLASTn result for *Rns* genotype	GenBank accession no	Sequence identity of isolates to published strains (% identity, highest homology), accession number
1.	A7A1	Avoca lagoon	*Acanthamoeba* T4	OQ158988	*Acanthamoeba* spp. T4 (99%, 395/399 bp), MN70029
2.	A7F1		*Acanthamoeba* T4	OQ158981	*Acanthamoeba* spp. T4 (98%, 423/432 bp), KT897266
3.	A7O1		*Acanthamoeba* T4	OQ158982	*Acanthamoeba* spp. T4 (100%, 381/382 bp), MT378247
4.	A7O2		*Acanthamoeba* T2	OQ158984	*Acanthamoeba* spp. T2 (79%, 169/249 bp), JQ669661
5.	P3–34	Cockrone lagoon	*Acanthamoeba* T4	OQ158987	*Acanthamoeba* spp. T4 (99%, 400/403 bp), MN700295
6.	P5–7		*Acanthamoeba* T4	OQ158986	*Acanthamoeba* spp. T4 (95%, 324/341 bp), KP233869
7.	T2–42	Terrigal lagoon	*Acanthamoeba* T5	OQ158980	*Acanthamoeba* spp. T5 (97%, 368/378 bp), KF733227
8.	T2–47		*Acanthamoeba* T4	OQ158985	*Acanthamoeba* spp. T4 (100%, 375/375 bp), MT378224
9.	W2–53	Wamberal lagoon	*Acanthamoeba* T4	OQ158983	*Acanthamoeba* spp. T4 (98.1%, 370/377 bp), LC093126
10.	Para	Parramatta River	*Acanthamoeba* T4	OQ158990	*Acanthamoeba* spp. T4 (98%, 307/313 bp), KT892764
11.	R3	Hawks Nest Camping River	*Acanthamoeba* T4	OQ158989	*Acanthamoeba* spp. T4 (97%, 382/394 bp), MG945010

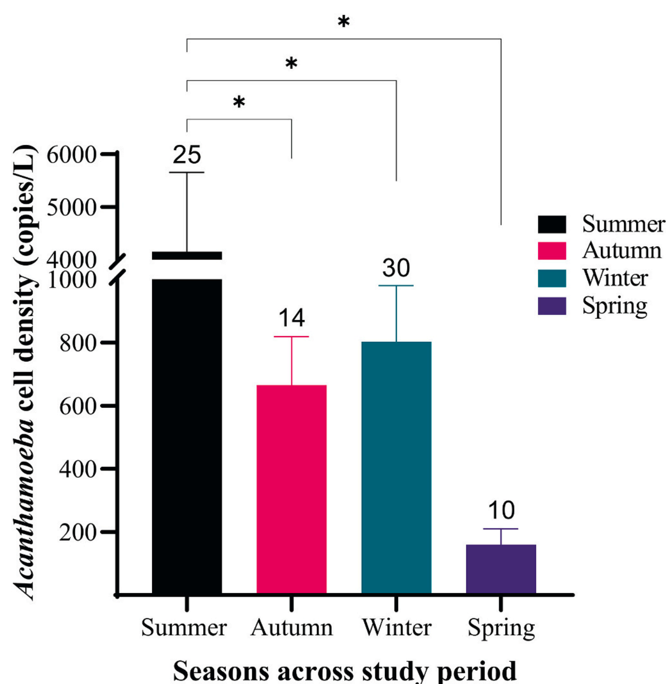

Fig. 7. *Acanthamoeba* concentrations measured by qPCR among positive water samples across four seasons of the study period, all values are mean ± SEM, $*p < 0.05$, one-way ANOVA, followed by the Tukey's multiple comparison test for between group comparisons, post hoc test, was performed.

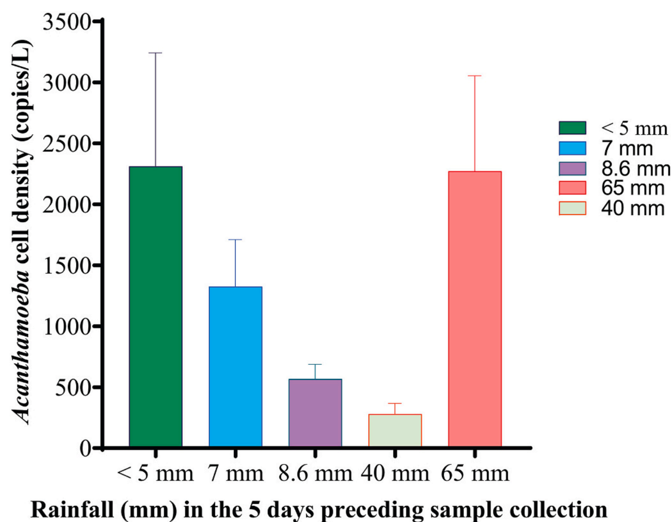

Fig. 8. *Acanthamoeba* levels determined by qPCR with respect to rainfall (mm) events recorded five days priors to samples collection, all values are mean ± SEM, quantity of rainfall (mm) was not significantly ($p > 0.05$) associated with *Acanthamoeba* concentrations. A one-way ANOVA was conducted, followed by the Tukey's multiple comparison test for between-group post hoc analysis.

3.2. Detection and quantification of Acanthamoeba from lagoon water

Among 206 water samples analysed across four lagoons of Central Coast, 38% (79/206) were positive for *Acanthamoeba* 18S rRNA in qPCR (Fig. 3). The highest number of *Acanthamoeba* positive samples was observed in January (65%, 13/20) while the lowest positive rate was in September (5%, 1/20) of 2019 (Fig. 4). More than 50% (23/45) of water samples from the highly urbanised Terrigal lagoon were positive for *Acanthamoeba,* with an average abundance of 1.4×10^3/litre. In contrast, within Cockrone Lagoon, the lagoon least impacted by urban development consisting of 70% forested area, 32% (16/49) of samples were positive for *Acanthamoeba*, although the average count of 1.3×10^3/litre was similar to the average count from Terrigal lagoon. Just under half of samples (42%; 20/48) from Wamberal and 32% (20/64) from Avoca lagoons were positive for *Acanthamoeba* spp. (Figs. 3 and 5), where the average counts among positive samples were 1.43×10^3/litre for Wamberal and slightly higher at 3.0×10^3/litre for Avoca (Fig. 6). However, the *Acanthamoeba* density (copies/litre) across the four sampling lagoons was not statistically different ($p = 0.59$, Fig. 6). The water quality of the Avoca lagoon was marked as very poor with high levels of chlorophyll-a and turbidity across the sampling months (Seymour and Williams, 2021). For a detailed description of sampling sites (longitude and latitude), qPCR detection and quantification of *Acanthamoeba* across four lagoons refer to supplementary material, Table 2.

Acanthamoeba levels showed a significant difference ($p = 0.008$) across sampling seasons, with high levels coinciding with the summer months of the study period (Fig. 7). The mean *Acanthamoeba* cell density among positive samples in summer months (December, January, and February) was 4.2×10^3/litre followed by autumn 8.1×10^2/litre (only May, as samples were not collected in March and April - 2020 due to COVID-19 restrictions), winter 8×10^2/litre (June, July, and August) and spring (September, October, and November) 1.9×10^2/litre, respectively. In February 2020, water sampling was preceded by 65 mm of rain in the five days. At that month, the average *Acanthamoeba* level across all sites reached 2.1×10^3/litre while the level was 2.8×10^2/litre in October 2019 when 40 mm rainfall was recorded five days prior samples collection (Table 1). However, the concentration of *Acanthamoeba* in water samples was not different when collected after high rainfall (40–65 mm) compared to low rainfall (<5 mm) ($p = 0.72$, Fig. 8). The rainfall events of all sampling sites across ten months study period are provided in supplementary material, Fig. 1.

3.3. Identification of Acanthamoeba genotypes from qPCR positive water samples

The partial nucleotide sequence of *Acanthamoeba* 18S rDNA DF3 region (18S rRNA gene; *Rns*) was aligned using MUSCLE multiple sequence algorithm and showed inter-strain/genotype nucleotide sequence variation (Fig. 2, supplementary material). The low quality trimmed nucleotide sequence producing the highest alignment (% identity) with sequences in NCBI of existing genotypes of *Acanthamoeba* was assigned to each strain (Table 2). The neighbour-joining phylogenetic analysis of *Acanthamoeba* amplicons from nine lagoons and two *Acanthamoeba* isolates recovered from river water in our another study (Parramatta River and Hawks Nest Camping River, NSW, Australia) formed three major clades, with most isolates (9/11, 69%) belonging to T4 genotype, one amplicon from Avoca (A7O2) being assigned to T2 and another amplicon from Terrigal (T2–42) being assigned to genotype T5 (Fig. 9). The two sequences from river isolates were included to gauge the phylogenetic relatedness with isolates from lagoons strains. All eleven nucleotide sequences have been deposited at GenBank under accession numbers OQ158980 to OQ158990 (Table 2).

3.4. Association of Acanthamoeba incidence and abiotic factors in water

Statistical analyses were carried out to evaluate whether there were associations between the detection of *Acanthamoeba* and water physicochemical parameters within each sampling site. Through Spearman rank-order correlation coefficient (Table 3), *Acanthamoeba* 18S rRNA detection was significantly positively correlated with dissolved O_2 ($p = 0.023$, $r_s = 0.43$) and water turbidity ($p = 0.001$, $r_s = 0.042$). But the correlation coefficient between water turbidity and *Acanthamoeba* incidence was found to be only 0.042, indicating a very weak correlation. The levels of dissolved O_2 (%) and water turbidity of each sampling site across study period are presented in supplementary material, Figs. 3

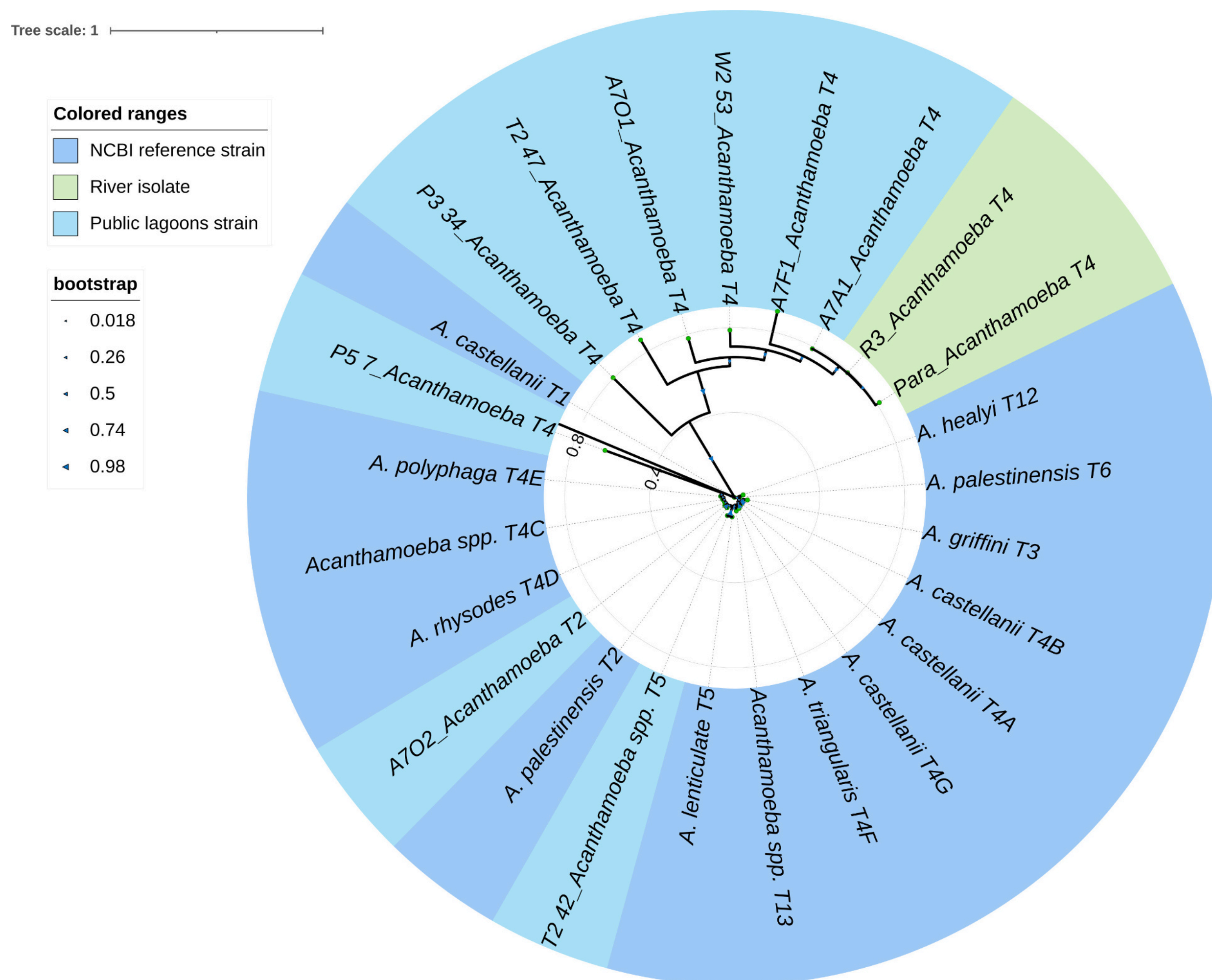

Fig. 9. The 18S rRNA nucleotide sequences of published *Acanthamoeba* genotypes were retrieved from the NCBI GenBank database, aligned with lagoon and river strains sequences using MUSCLE and the phylogenetic tree was reconstructed using Kimura parameter with 1000 bootstraps using MEGAX and visualised using iTOLv6.

Table 3
Association between incidence of *Acanthamoeba* and abiotic water factors, *correlation is significant at the 0.05 level (2-tailed).

Abiotic water factors	Spearman rank correlation test (ρ)		*Acanthamoeba* positive samples		*Acanthamoeba* negative samples	
	p-value	R	Range	Mean+/-SD	Range	Mean+/-SD
ODO (mg/litre)	0.05*	0.18	4.92–12.09	7.95 ± 1.32	3.25–12.65	7.8 ± 1.55
ODO (%)	0.023*	0.43	60.02–195.28	94.5 ± 20.13	42.82–210.39	96.5 ± 18.91
Turbidity (NTU)	0.001*	0.042	0.32–53.56	7.20 ± 8.81	0.29–43.44	8.5 ± 10.11
Chlorophyll (μg/litre)	0	0	0.58–26.05	4.98 ± 4.70	0.56–44.34	5.81 ± 6.84
Chlorophyll RFU	0	0	0.18–6.43	1.24 ± 1.16	0.17–10.93	1.46 ± 1.7
Conductivity (μS/cm)	0.95	0.004	510.6–59,073.9	25,091.9 ± 14,248.5	3319.9–61,818.2	32,401.0 ± 12,852.4
Temperature (°C)	0.019	0.15	10.1–33.6	18.8 ± 5.1	10.6–36.1	16.44 ± 4.95
Salinity (ppt)	0.56	0.065	0.27–39.22	17.46 ± 9.67	2.17–38.55	22.87 ± 8.97
pH	0.48	−0.08	4.14–9.21	7.66 ± 0.69	5.86–9.22	7.75 ± 0.43
Total Phosphorus	0.10	0.18	0.01–0.16	0.02 ± 0.01	0.00–0.10	0.02 ± 0.02
Total Nitrogen	0.38	−0.19	0.29–1.46	0.63 ± 0.28	0.08–1.82	0.64 ± 0.32
Ammonia	0.56	0.065	0.003–0.52	0.07 ± 0.098	0.004–0.75	0.06 ± 0.08

ODO; optical dissolved oxygen, RFU; relative fluorescence units, NTU; nephelometric turbidity unit.

and 4. With the use of Bonferroni testing, it became apparent that the presence of *Acanthamoeba* alone was not correlated with the percentage of optical dissolved oxygen (ODO, $p = 0.365$). However, a significant correlation was observed between amoebal cell density and ODO ($\tau b = 0.942$) in Kendall's tau-b (τb) correlation. This was also the case with turbidity ($\tau b = 0.905$) (supplementary material-S1). Similarly, the PCA analysis showed a correlation of 0.6 between water turbidity and *Acanthamoeba* cell density. The water temperature (°C) was found to have a weak positive linear relationship with the detection of *Acanthamoeba* in water samples from the lagoons ($r_s = 0.15$, $p < 0.05$) (Table 3 and Fig. 11). No significant positive correlations were observed between the presence of *Acanthamoeba* and water conductivity, total phosphorus, total nitrogen levels, pH, salinity, chlorophyll, or ammonia. In the PCA, the first three components accounted for 63.13% of the variation among

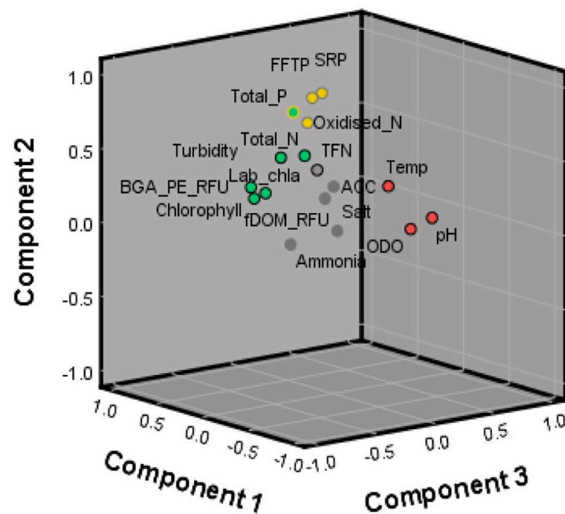

Fig. 10. Component plot in rotated space, PC1 explaining just 33.7% of the variation, which was predominantly contributed by turbidity, chlorophyll, blue-green algae phycoerythrin (BGA-PE), total phosphorous and nitrogen (shown in green), PC2 (18.74%) consists of oxidised N_2, soluble reactive phosphorus and field filtered total phosphorus (highlighted yellow) and PC3 (10.69%) correlates temperature, O_2 percentage and pH (indicated in red). "Acc" denotes *Acanthamoeba* and "BGA" represents cyanobacteria.

sampling sites based on their measured water quality parameters and the presence of *Acanthamoeba* (Fig. 10).

3.5. Correlations between Acanthamoeba and bacteria

To determine whether specific ASVs detected in the 16S rRNA amplicon sequencing data were indicative of *Acanthamoeba* 18S rRNA in all positive water ($n = 79$) samples across four lagoons, network analyses were performed. The average number of unique ASVs detected among *Acanthamoeba* positive water samples was 627 (SD ± 633; 95% CI 545 to 710). The level of cyanobacteria ASVs (*Scytonema* spp. and *Cyanobium* spp.) and *Acanthamoeba* 18S rRNA genes were positively correlated (Pearson's $r > 0.15$) (Fig. 11). Additionally, other bacterial ASVs of phyla Proteobacteria (*Pseudomonas* spp., *Vibrio pacinii*, *Pantoea dispersa*, *Methylomonas* spp., *Aquicoccus porphyridii),* Verrucomicrobiota (*Verrucomicrobia* spp.), Bacteroidota (*Flavobacterium* spp., *Fluviicola* spp., *Pseudarcicella* spp.), marine bacteria of the Actinobacteria (*Mycobacterium* spp., *Microcella putealis*, *Candidatus* Planktoluna spp., *Curvibacter* spp.) and Campylobacterota (*Sulfuricurvum kujiense*) were positively associated showing weak to moderate correlation with the presence of *Acanthamoeba* 18S rRNA genes among *Acanthamoeba* qPCR positive water samples (Pearson's $r > 0.14$, supplementary material-S1). The incidence of *Acanthamoeba* in lagoons water was significantly and positively correlated with the presence of the *intl1* gene (Pearson's $r = 0.32$, $p < 0.05$), indicating possible sewage contamination (Fig. 5, supplementary material). This finding suggests that the detection of *intl1* gene can serve as a reliable molecular indicator of anthropogenically impacted water, which may support the proliferation of *Acanthamoeba* spp. in aquatic habitats.

3.6. Bacterial diversity and incidence of Acanthamoeba in lagoons across four seasons

The average ASVs count was 617 (SD ± 606; 95% CI 568 to 666) across all water samples processed in the study. Among the *Acanthamoeba* contained water samples, the highest number of unique ASVs was identified in Avoca lagoon (mean = 740; SD ± 499; 95% CI 607 to 872) followed by Terrigal (mean = 705; SD ± 824; 95% CI 507 to 904), Wamberal (mean = 663, SD ± 684; 95% CI 478 to 848), and Cockrone (mean = 347; SD ± 170; 95% CI 298 to 395). The observed ASVs and Shannon entropy index across the four seasons of the study were found to be significantly different ($p < 0.05$) between the four lagoons, except for the diversity between Terrigal and Wamberal (Fig. 12). The detection rate of *Acanthamoeba* spp. was relatively high in summer, therefore, bacterial alpha and beta diversity were assessed across four seasons between *Acanthamoeba* positive and negative water samples. We observed significant differences ($p < 0.05$) in bacterial richness (number of observed ASVs) or alpha diversity (Shannon index) between the four seasons, except between Autumn and Summer in Avoca lagoon. However, there was no significant association between the presence of *Acanthamoeba* and bacterial alpha diversity in Avoca water samples ($p > 0.05$) (Fig. 13). Similarly, the presence of *Acanthamoeba* spp. in Wamberal and Cockrone lagoons did not result in a significant difference ($p > 0.05$) in bacterial alpha diversity across all four sampling occasions (Figs. 6 and 7, supplementary material). This study found that alpha diversity in Terrigal lagoon was significantly higher in summer than in winter and spring ($p < 0.05$). Moreover, the presence of *Acanthamoeba* spp. in Terrigal lagoon showed higher bacterial diversity, as measured by the richness ($F = 13.6$, $p < 0.001$) and Shannon index ($F = 7.3$, $p < 0.05$), across all four sampling seasons (Fig. 14).

Two-dimensional nMDS (non-metric multidimensional scaling) plots of Bray-Curtis dissimilarly index revealed significant differences ($F = 13.9$ to 59.7, $p < 0.001$) in the composition of bacterial communities (beta diversity) inhabiting in four different lagoons (Fig. 15). Similarly, bacterial distribution and diversity were different ($p < 0.001$) as per sampling season in each lagoon, except between Spring and Autumn in Cockrone ($p > 0.05$) (Fig. 16). The beta diversity of bacterial communities differed significantly ($F = 3.2$ to 5.5, $p < 0.05$) in the presence of *Acanthamoeba* in all lagoons but was not different ($p = 0.39$) in Wamberal (Fig. 8, supplementary material).

4. Discussion

The presence of *Acanthamoeba* spp. is emerging as potentially significant pathogen, causing severe and sometimes fatal infections in humans, often following contact with contaminated water (Khan, 2006). Exposure to *Acanthamoeba* spp. can occur during recreational use of natural aquatic environments and swimming pools (Mohd Hussain et al., 2022). The main aim of this study was to identify and quantify *Acanthamoeba* spp. within seawater in intermittently closed and open lagoons and examine the contribution of factors that may support the growth of *Acanthamoeba*. This study focused on four coastal lagoons in south-eastern Australia. These lagoons are highly valuable natural resources, delivering ecosystem services and supporting recreational activities such as swimming, stand up paddle boarding, kayaking, and board paddling. However, ecological health and recreational water quality within these lagoons are routinely rated as poor due to high chlorophyll and turbidity levels, faecal contamination, especially following rainfall in summer due to stormwater runoff (Seymour and Williams, 2021).

During this study from August 2019 to July 2020, *Acanthamoeba* was found in 38% of water samples assessed from the four lagoons, with samples from the Terrigal lagoon, which is one of the most urbanised lagoons that has previously been characterised as periodically having poor water quality (Williams et al., 2022a), exhibiting the highest *Acanthamoeba* prevalence (51%). These levels of occurrence are slightly higher than was previously observed in domestic tap water in the greater Sydney region (Australia), where 29% of bathroom sinks were colonised by *Acanthamoeba* spp. (Carnt et al., 2020). Other studies have shown various levels of prevalence of *Acanthamoeba* spp. in natural aquatic ecosystems globally, including 61% occurrence in river water in Japan (Kawaguchi et al., 2009), 51% in seawater in Turkey (Koyun et al.,

Fig. 11. Correlations with a Pearson's $r > 0.14$ between *Acanthamoeba* 18S rRNA and bacterial ASVs detected via qPCR and sequencing, respectively within the same water sample. The line thickness (coefficient of determination) indicates the strength of the correlation, and circle size represents bacterial relative abundance (ASVs) within the processed data set. Water salinity, temperature, and *intl1* gene were also included in the network analysis. Dark green circle at the circle represents *Acanthamoeba* spp. and bacteria phyla/group are indicated in colour legend. Please refer to the PDF of the figure and zoom in to identify bacteria ASVs label and names (genus level) of all bacteria are listed in supplementary material-S1.

2020), 50% in seawater in West Indies (Lorenzo-Morales et al., 2005), 33% in environmental water in Uganda (Sente et al., 2016), and 21% in pond water of parks in Iran (Ghaderifar et al., 2018). To the best of the authors' knowledge, this is the first study to gauge *Acanthamoeba* incidence in coastal seawater in Australia.

The high level of occurrence of *Acanthamoeba in* Terrigal lagoon is notable, given that this lagoon is in a highly residential area and is used extensively for recreational activities, especially during the summer season. In addition, poor water quality is frequently reported due to impact of animal faeces and the infiltration of sewage-affected stormwater (Seymour et al., 2020). Contamination of lagoon water with runoff of animal excreta and sewage can impact proliferation and occurrence of *Acanthamoeba* since it actively grazes on small organic particles and microorganisms (Huws et al., 2008). The present study found a significant and moderate positive correlation between the detection of *Acanthamoeba* across four lagoons and an elevated level of the *intl1* gene. This result suggests that sewage contamination of lagoon water may favour the proliferation of *Acanthamoeba* strains, as *intl1* can serve as a proxy for anthropogenic water contamination. Previous studies have reported that *Acanthamoeba* spp. are more prevalent in contaminated, bacteria-rich water, irrespective of water abiotic parameters (Bonilla-Lemus et al., 2014).

Contaminated recreational or domestic water serve as a source of *Acanthamoeba* spp., which is associated with contact lens related *Acanthamoeba* keratitis (Kaji et al., 2005; Kilvington et al., 2004). A recent retrospective study among 52 AK patients treated at a referral hospital in Sydney between January 2002 to December 2016 showed that 17.6% of patients developed AK infection after swimming with their soft contact lenses in fresh or seawater (Höllhumer et al., 2020); which is possibly attributed to the coastal area of city. Similarly, 19.4% (6/31) of contact lens wearers from Melbourne, Australia, reported swimming in lakes/rivers/sea or chlorinated water as a risk factor of AK (Lee et al., 2018).

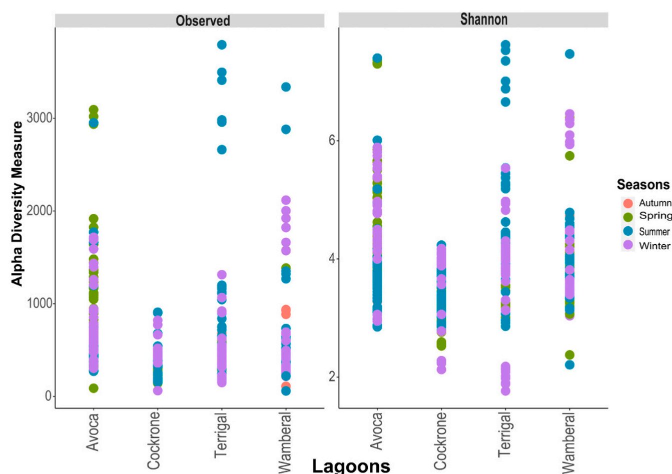

Fig. 12. Alpha diversity (both observed ASVs and Shannon) of bacteria was significant when compared across four lagoons by seasons of sampling, with the exception of the comparison between Terrigal and Wamberal.

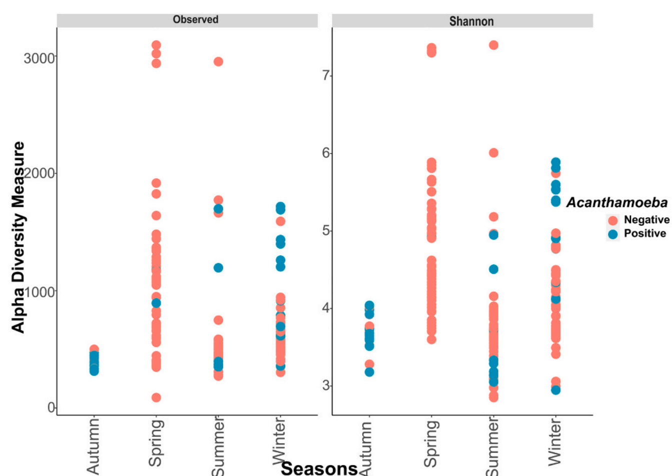

Fig. 13. Bacterial alpha diversity of Avoca water samples was not significantly corrected with incidence of *Acanthamoeba* spp. across sampling seasons.

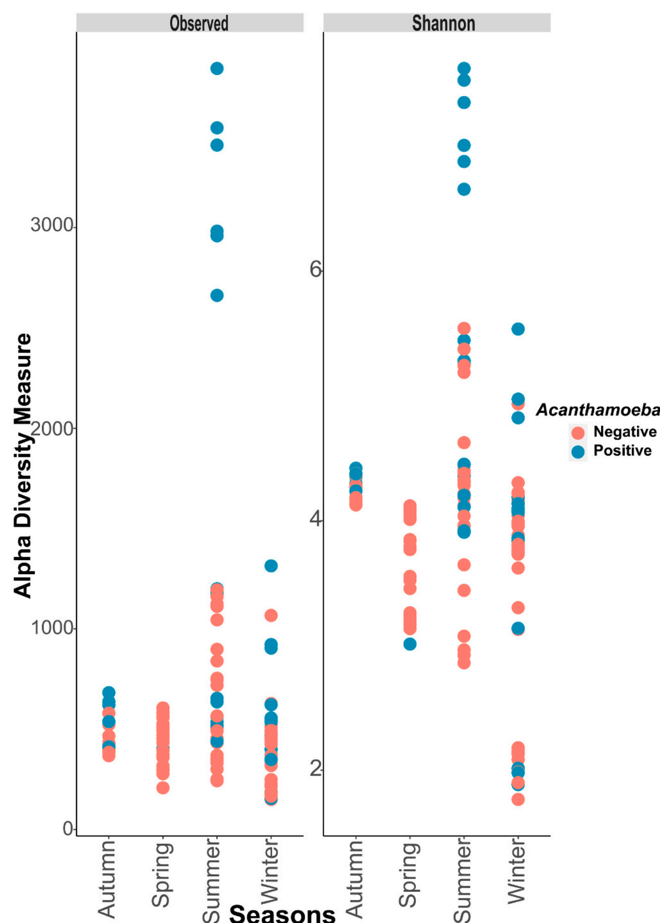

Fig. 14. Bacterial alpha diversity (observed ASVs and Shannon index) of Terrigal lagoon was statistically significant with incidence of *Acanthamoeba* spp. across four sampling seasons.

Similar to these findings, rivers/lakes and seawater exposure was observed as a significant soft contact lens related AK risk factor for 21.8% (12/55) of AK patients in England and Wales (Radford et al., 2002). A case-control study from UK reported that water activity in ocean/sea/river/lake or public/private pools and hot tubs was a potential AK risk factor (63.5%) for AK patients using reusable daily wear contact lens (Carnt et al., 2018b). In the present study, the numbers of *Acanthamoeba* varied by season ($p = 0.008$) with the highest level in summer. A number of findings have demonstrated a seasonal variation in the incidence and concentration of *Acanthamoeba* in water bodies with higher rates during summer/warmer months along with increased AK cases (Kao et al., 2013; List et al., 2021; Randag et al., 2019). The higher numbers of *Acanthamoeba* in summer may be attributed to increased organic matter or bacterial biomass following heavy rainfall in February, which corresponds to the summer season in Sydney. The urban stormwater washed into coastal aquatic ecosystems in summer months during rain can contain a plethora of microorganisms from numerous sources such as soil, buildings, animal, and bird faeces (Williams et al., 2022b).

The predominant keratitis causing *Acanthamoeba* genotype T4 was prevalent in lagoon waters; among nine sequence reads of qPCR positive samples seven (78%) were of genotype T4. A previous study has also reported genotype T4 as the most common type (75%) in domestic shower settings and tap water in the greater Sydney (Carnt et al., 2020). Two sequences from lagoon waters, one from Avoca and the other from Terrigal, were identified as genotypes T2 and T5, respectively. Genotypes T2 and T5 have also been sporadically isolated from patients with AK (Diehl et al., 2021). *A. lenticulate* T5 has been isolated from a contact lens wearer AK patient in South Australia (van Zyl et al., 2013). No studies have reported genotype T2 from Australia, although it has been reported in other countries from clinical and pool or marine water samples (Jercic et al., 2019; Maghsood et al., 2005; Mohd Hussain et al., 2022). Colonization of pathogenic *Acanthamoeba* genotypes in lagoons highlights the notion that users of these waters should remain vigilant to prevent contamination of contact lenses with water, especially during recreational activities in lagoons or beaches.

The impacts of water abiotic factors on the distribution and abundance of *Acanthamoeba* spp. remain poorly investigated. *In vitro* studies have shown that *Acanthamoeba* isolates able to grow at high osmolarity and temperature correlate with their higher pathogenicity which may indicate pathogenic potential of a particular isolate (Booton et al., 2004; Khan, 2006). But our study identified a weak positive linear correlation between the detection rate of *Acanthamoeba* spp. and the temperature of lagoon water (List et al., 2021; McAllum et al., 2009). *Acanthamoeba* strains isolated from beach sand and tap water can withstand elevated osmolarity by triggering encystment and a much faster rate of mature cyst formation (Booton et al., 2004; Cordingley et al., 1996). Although in the current study, there were no significant associations between *Acanthamoeba* incidence and salinity of water, a wide range of salinity

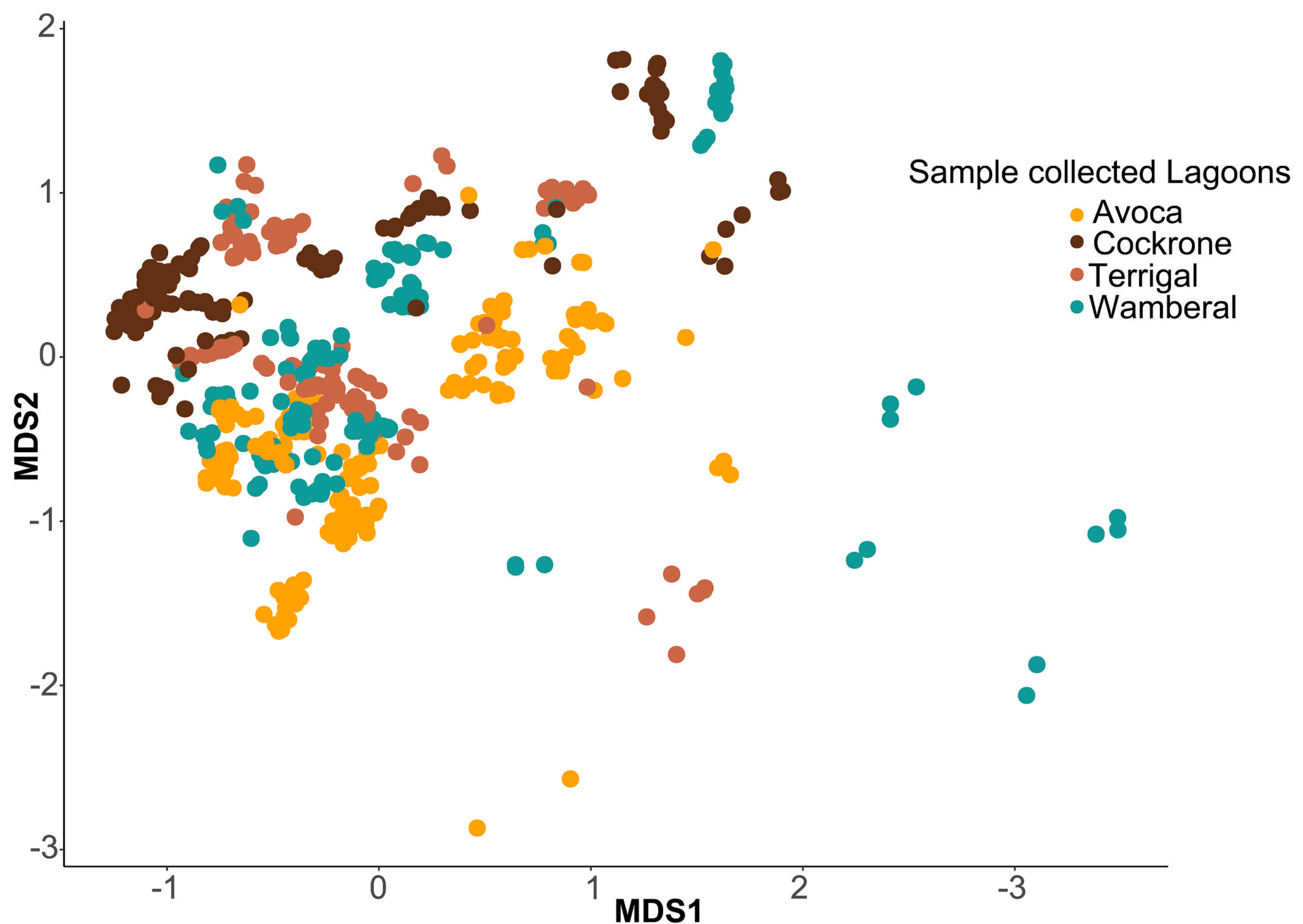

Fig. 15. Beta diversity of bacteria in four different coastal lagoons by sampling seasons. Two-dimensional nMDS plot of Bray-Curtis dissimilarly index compared between samples collected from Avoca, Cockrone, Terrigal, and Cockrone lagoon. Axes represent the first two dimensions of the multidimensional scaling (MDS) plot, and each point on the plot represents the bacterial diversity of an individual water sample (orange = samples collected from Avoca, blue = Wamberal, light brown = Terrigal, and dark brown = Cockrone).

(0.27–39.2 ppt, 17.5 ± 9.7) was observed in *Acanthamoeba*-positive water samples. Similarly, a wide range of pH (4.1–9.2, 7.66 ± 0.69) was found in *Acanthamoeba*-positive water samples. It is therefore likely that *Acanthamoeba* can adapt to different chemical and environmental changes in the environment. The incidence of *Acanthamoeba* was positively correlated ($p < 0.05$) with turbidity and dissolved O_2 of water samples. The mean turbidity in water containing *Acanthamoeba* was lower (7.2 NTU, ± 8.8) compared with the mean (8.5 NTU, ± 10.1) in *Acanthamoeba* negative water samples. This finding is supported by previous studies from Taiwan and China where lower turbidity water from reservoirs and taps were associated with the incidence of *Acanthamoeba* (Kao et al., 2014; Liu et al., 2019), indicating that low turbidity water may be preferred condition for *Acanthamoeba* spp. Dissolved O_2 (DO %, 94.5 ± 20.1) was also slightly lower in water containing *Acanthamoeba* compared with *Acanthamoeba*-negative samples (DO %, 96.5 ± 18.9). A previous laboratory study in a closed system suggested that *Acanthamoeba* cells may deplete the dissolved O_2 concentration in aquatic niches supporting survival and replication of microaerophilic bacteria (Bui et al., 2012). Although, in another laboratory study in a closed system, the presence of *Acanthamoeba* spp. was associated with increase dissolved O_2 and decrease the numbers of aerobic bacteria such as cyanobacteria and Firmicutes (Tsai et al., 2020).

Lagoon water can host a plethora of microorganisms ranging from aerobes to obligate anaerobes. In the current study, a weak (Pearson's $r > 0.15$) positive correlation was observed between the occurrence of *Acanthamoeba* spp. and the presence of cyanobacteria. *Acanthamoeba* can graze on unicellular and filamentous cyanobacteria (Wright et al., 1981). The incidence of FLA in the water column has been associated with cyanobacterial blooms in a predator/prey relationship (Urrutia-Cordero et al., 2013). The level of bacteria from the phyla Proteobacteria (*Pseudomonas* spp., *Vibrio pacinii, Methylomonas* spp., *Aquicoccus porphyridii*), Verrucomicrobiota (*Verrucomicrobia* spp.), Bacteroidota (*Flavobacterium* spp., *Fluviicola* spp., *Pseudarcicella* spp.) and Actinobacteria (*Candidatus* Planktoluna spp., *Curvibacter* spp., *Mycobacterium* spp.) were also positively correlated with the numbers of *Acanthamoeba*, indicating higher incidence of *Acanthamoeba* spp. is associated with presence of different bacteria in aquatic environment. The current study found that bacterial alpha diversity was not affected by the presence of *Acanthamoeba* in Avoca, Wamberal, and Cockrone lagoons. However, it was significantly associated with higher bacterial diversity in Terrigal lagoon over the study period. This increase in bacterial diversity in Terrigal lagoon may be attributed to a heavy rainfall event during sampling, which resulted in 40 mm of rain. Conversely, the presence of *Acanthamoeba* in all lagoons, except Wamberal, was associated with significant differences in the composition of bacterial communities (beta diversity). The natural water environment harbors diverse amoebae possessing broad-spectrum bactericidal properties, which can profoundly influence bacterial microbiomes in water resources (Rayamajhee et al., 2022b). The voracious feeding feature of *Acanthamoeba* spp. leads to the coexistence of sympatric bacteria within the same isolate, creating a sort of ‘microbial village’. Given its role as a phagocytic predator of bacteria and an environmental host for other microbes,

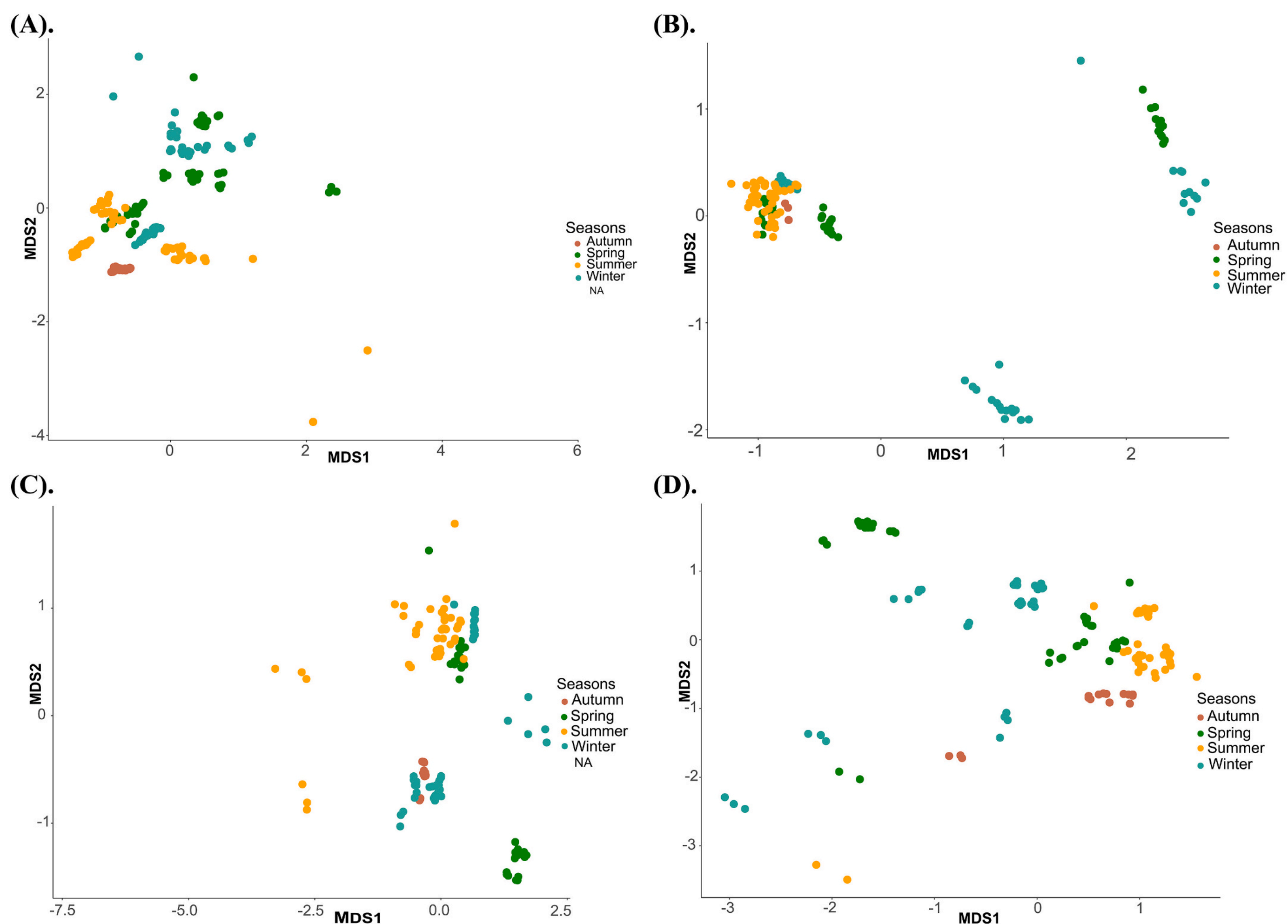

Fig. 16. Beta diversity of bacteria in four different coastal lagoons by sampling seasons. Two-dimensional nMDS plots of Bray-Curtis dissimilarly index compared between four seasons of samples collected from (A). Avoca, (B). Cockrone, (C). Terrigal, and (D). Wamberal lagoon. Axes represent the first two dimensions of the multidimensional scaling (MDS) plot, and each point on the plot represents the bacterial diversity of an individual water sample (orange = samples collected in summer, blue = winter, brown = autumn, and green = spring).

the presence of *Acanthamoeba* spp. in coastal lagoons has the potential to impact the bacterial composition and diversity within these natural ecosystems.

5. Conclusions

The increasing incidence of the potentially blinding cornea infection *Acanthamoeba* keratitis (Randag et al., 2019), which has substantial impacts on quality of life, is a global public health concern (Carnt et al., 2022). One of the major risk factors for *Acanthamoeba* keratitis is water contact during contact lens wear. This study has shown the incidence of *Acanthamoeba* in lagoons water of the Central Coast, NSW, Australia, with higher concentrations during summer, when recreational activities are likely to be at their highest. Notably the most commonly identified genotype in the lagoon water was T4, which is the most frequently associated *Acanthamoeba* genotype among keratitis patients. Furthermore, there was a higher incidence of *Acanthamoeba* in lagoons most likely impacted by urban development with associated recreational activities. Water turbidity, temperature, dissolved O_2, *intI1* gene concentration, and bacteria such as cyanobacteria, *Pseudomonas* spp., *Candidatus* spp., and marine bacteria of the Actinobacteria phylum were associated with a higher incidence of *Acanthamoeba* in lagoons, however, *Acanthamoeba* concentration was not correlated with rainfall levels during the study period. The composition of bacterial communities, as measured by beta diversity differed significantly in presence of *Acanthamoeba* spp. in lagoons water. Given that raised temperatures combined with increased stormwater runoff as a consequence of climate change support algal blooms in seawater (Gobler, 2020), hence, urbanised coastal waterways may become an ideal habitat for *Acanthamoeba,* posing an increased public health risk.

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Not applicable.

CRediT authorship contribution statement

Binod Rayamajhee: Experimentation, methodology, data analysis and interpretation, graphics, and writing – manuscript draft. Nathan L. R. Williams: Field sampling and analysis. Nachshon Siboni: Supervision, field sampling, methodology, supervision - data analysis, writing - review and editing. Kiri Rodgers: Analysis and writing - review. Mark

Willcox: Study conceptualization, supervision, analysis, writing - review and editing. Fiona L. Henriquez: Study Conceptualization, supervision, writing - review and editing. Justin R. Seymour: Conceptualization, writing - review and editing. Jaimie Potts: Field sampling, writing - review and editing. Peter Scanes: Field sampling, writing - review and editing. Colin Johnson: Field sampling, writing - review and editing. Nicole Carnt: Study conceptualization, supervision, writing - review and editing, and funding acquisition.

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.

Data availability statement

Data will be made available from the corresponding author on reasonable request. The assigned GenBank accession number of the nucleotide sequence ranged from OQ158980 to OQ158990 (https://www.ncbi.nlm.nih.gov/nuccore/?term=OQ158980:OQ158990[accn]). All the raw sequence files of 16S rRNA have been deposited to the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA884124.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165862.

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