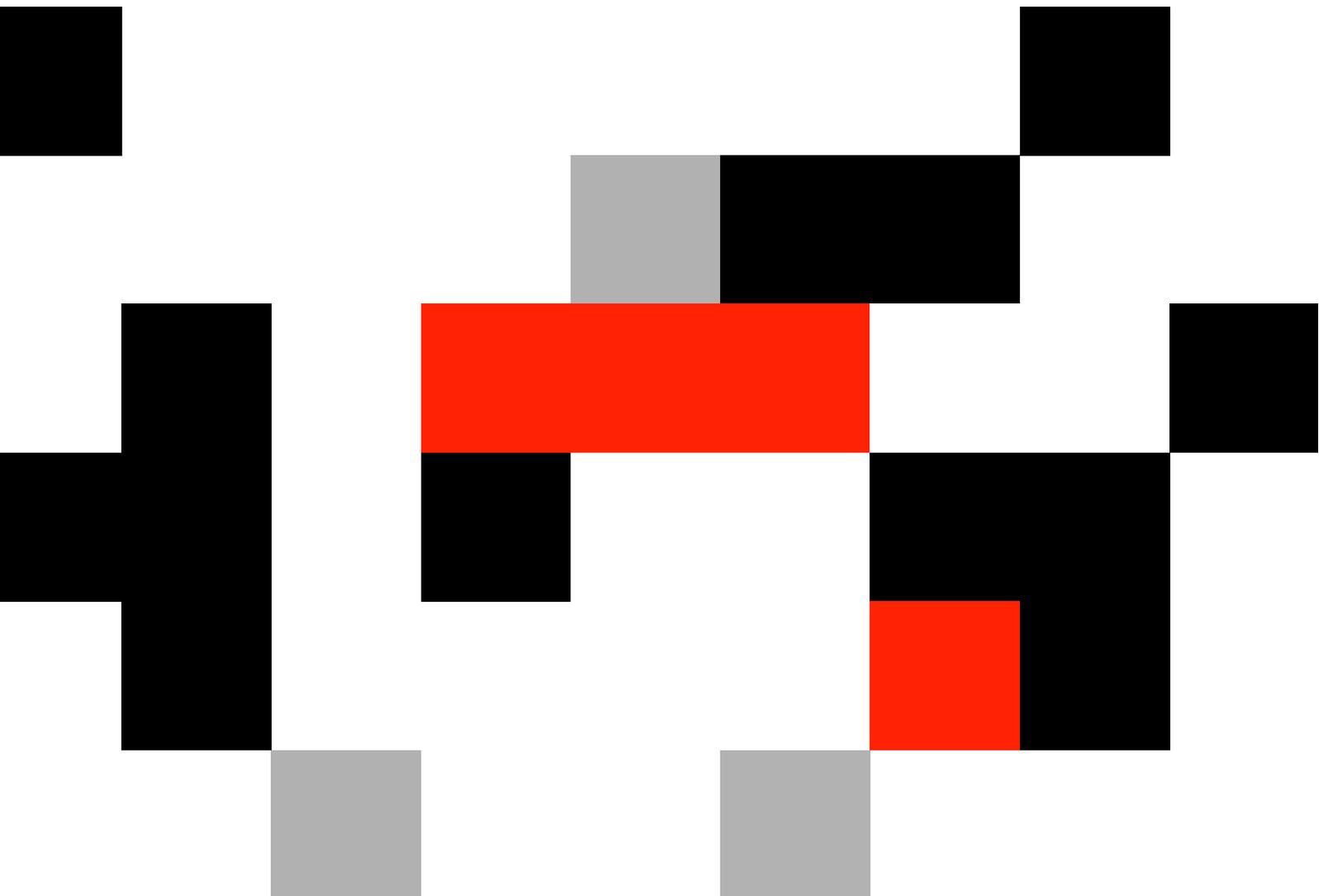
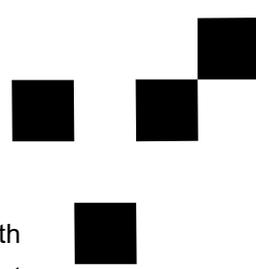
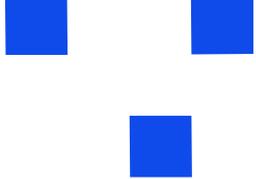




Climate Change Cluster, Faculty of Science,  
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# Microbial source-tracking to assess water quality in Central Coast Lagoons





This Report has been prepared by the University of Technology Sydney (“UTS”) in good faith as part of a collaboration with the NSW Department of Planning, Industry and Environment (DPIE) and the Central Coast Council.



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## EXECUTIVE SUMMARY

The research presented in this report was commissioned by the NSW Department of Planning, Industry and Environment (DPIE) and the Central Coast Council, and performed by the Ocean Microbiology Group at the University of Technology Sydney (UTS). The principal goal of the research was to use novel molecular microbiological source-tracking approaches to define the causes and spatial and temporal dynamics of poor water quality in several coastal lagoons situated on the Central Coast (NSW).

Water quality surveys within four Central Coast lagoons (Cockrone, Wamberal, Terrigal and Avoca Lagoons) were performed approximately monthly between August 2019 and July 2020. The sampling regime captured periods of both dry and wet-weather conditions, as well as seasonal shifts in environmental conditions.

During the study period, water quality within the surveyed lagoons was highly variable in space and time, with sewage and animal faecal material having a heterogeneous influence across the lagoons. Except for periods following significant rainfall, recreational water quality in most of the lagoons was generally good, with mean Enterococci levels during dry periods  $< 40$  MPN  $100\text{ml}^{-1}$  (i.e. NHMRC Level A risk category – low health risk) in 84% of samples. However, following major rainfall events in October 2019 and February and July 2020, Enterococci levels within all lagoons increased to very high levels, often exceeding the maximum NHMRC risk threshold and indicative of a significant risk to human health. Using molecular microbial source-tracking tools, the principal causes of this faecal contamination were examined.

Within Cockrone Lagoon, peaks in Enterococci levels following rainfall appeared to be largely related to inputs of animal faecal material, with spikes in the detection of dog and bird faeces often coinciding with decreased water quality. However, these impacts were relatively restricted in space and time, with no clear evidence for a single, consistent source of input. In Wamberal Lagoon, water quality was generally good during dry weather, although there was evidence for periodic sewage contamination at the W1 site located within Forresters Creek. Dog and bird faecal material also intermittently impacted water quality at other points within Wamberal Lagoon, but similarly to Cockrone Lagoon, this impact was spatially and temporally localised.

Avoca Lagoon had the poorest recreational water quality among the studied lagoons, with a clear signal for human faecal material following rainfall events indicative of impacts from sewage, with the point of input potentially located in the southerly region of the lagoon. However, animal faeces - most notably bird faeces in the northern parts of the lagoon – also impacted water quality within Avoca Lagoon, during periods of both wet and dry weather. Within Terrigal Lagoon, significant increases in Enterococci levels associated with rainfall were coupled with high levels of human faecal markers, indicating the presence of sewage contamination. Dog faeces also periodically contributed to reduced water quality within Terrigal Lagoon, although this impact was inconsistent in space and time.

In conclusion, recreational water quality within Central Coast lagoons is generally good in dry weather conditions, except for periods following rainfall events. After rain, sewage inputs and, to a lesser extent animal faecal material, lead to reduced water quality in Avoca and Terrigal Lagoons, and less frequently Wamberal Lagoon. In contrast, within Cockrone lagoon the impact of sewage appears to be minimal.

## 1.0 BACKGROUND AND AIMS

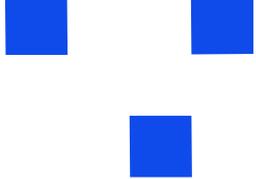
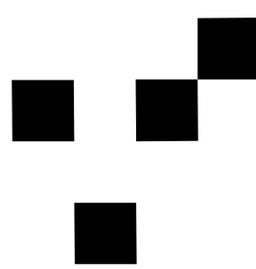
Along 87km of coastline, a substantial network of waterways including estuaries, coastal lagoons and lakes make up 13% of the Central Coast (NSW)<sup>1</sup>. These waterways deliver substantial ecosystem services and community value, representing both key habitats for aquatic flora and fauna, and recreational (e.g. swimming, fishing, kayaking) areas for the Central Coast's population<sup>1</sup>. However, due to their urbanised settings, these waterways are regularly impacted by poor water quality<sup>1,2</sup>, resulting in both compromised ecosystem health and potential human health hazards.

In 2019, Central Coast Council released a Combined Waterways Report Card<sup>1</sup>, which classified the ecological health of the Central Coast's estuaries, lakes and lagoons according to water quality metrics including turbidity, chlorophyll-a levels and seagrass depth range. Among the waterways assessed, Avoca Lagoon and Terrigal Lagoon were characterised by the lowest ecological health, with turbidity and chlorophyll-a levels often exceeding trigger values indicative of compromised water quality.

Within the state-wide recreational water-quality monitoring program conducted through the *Beachwatch Program*<sup>2</sup>, which uses measurements of the faecal indicator bacteria (FIB) Enterococci as a measure of contamination, water quality at 41% of monitored beaches and coastal lagoons on the Central Coast was categorised as "Poor", with stormwater inflows following rainfall identified as a principal contributor to reduced water quality. Notably, among the Central Coast's coastal lagoons and lakes, 90% of sites were characterised by poor water quality, indicating an increased health hazard for swimmers using these environments. Furthermore, a recent report<sup>3</sup> prepared by the University of Technology (UTS), the NSW Department of Planning, Industry and Environment (DPIE) and Central Coast Council (CCC), employed microbial source-tracking approaches to demonstrate high levels of sewage contamination within Terrigal Lagoon after rainfall. In addition, significant levels of bird and dog faecal indicator bacteria were observed within the lagoon, indicating multiple potential sources of faecal contamination within this system. Notably, this study also revealed that Terrigal Lagoon, when open to the ocean, can contribute to significantly compromised water quality in the adjacent coastal ocean waters of Terrigal Beach.

Poor water quality within the Central Coast's lagoons and estuaries has become a key area of concern for residents<sup>4</sup> and in March 2020, the Federal Government committed \$5.3 million dollars to restore coastal waterways on the Central Coast. As part of a continued collaboration between UTS, DPIE and CCC, a detailed survey of water quality within Central Coast lagoons was undertaken between August 2019 – July 2020. The principal goal of this work was to couple standard recreational water quality assessment protocols with novel molecular microbiological source tracking approaches to characterise water quality and the sources of contamination within four Central Coast lagoons. The specific objectives of the project were to:

- 1) Determine when and where poor water quality within Central Coast lagoons is primarily caused by human (i.e. sewage) or animal (dog or bird) sources of faecal contamination.

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- 2) Understand the spatial and temporal dynamics of water contamination in Central Coast lagoons over the course of a year.

## 2.0 METHODOLOGY



### 2.1 Sampling Design

Four Central Coast lagoons were selected for assessment according to prior evidence of poor water quality and level of use for recreation by the Central Coast community. These included Cockrone, Avoca, Terrigal and Wamberal Lagoons (Figure 1).

The northern-most of the four lagoons, Wamberal Lagoon, is surrounded by a predominantly natural, non-urbanised area within the Wamberal Lagoon Nature Reserve. Riparian vegetation bordering the lagoon is thought to provide a barrier from stormwater runoff, with water quality generally graded as good in the Central Coast Combined Waterways Report Card<sup>1</sup>.

Terrigal Lagoon is highly urbanised and used extensively for recreation, particularly during the summer months. However, it often experiences poor water quality<sup>1</sup>, with impacts from both the infiltration of sewage-impacted stormwater and animal faeces<sup>3</sup>.

Avoca Lagoon has the longest shoreline of the lagoons in this region of the Central Coast and is surrounded by a combination of urban and rural catchment areas. Within the Central Coast Combined Waterways Report Card<sup>1</sup>, water quality within Avoca Lagoon was graded as very poor, with turbidity and chlorophyll-a levels both exceeding trigger values throughout the sampling period.

The smallest of the four lagoons, Cockrone Lagoon is the least impacted by urban development, with its catchment incorporating 70% forested area. Water quality within Cockrone Lagoon was rated as excellent in the Central Coast Combined Waterways Report Card<sup>1</sup>, with both turbidity and chlorophyll-a levels always below trigger values during the sampling period.

Within each lagoon, discrete water samples were collected from 5 – 7 locations to deliver an assessment of the spatial extent of water quality issues. Water samples were collected approximately monthly from August 2019 until July 2020 (except for March and April 2019 due to COVID-19 field work restrictions). This sampling program allowed for assessment of lagoon water quality across seasonal changes in environmental conditions and during two significant wet-weather events, in October 2019 and February 2020, when 40 and 65 mm of rainfall occurred in the 5 days preceding sample collection, respectively.

## 2.2 Sample Processing and Analyses

At each sampling location, triplicate 2 L water samples were collected using an integrated pole sampler that collected surface water to 1 m depth. Within 2 hours, samples were transported to a portable laboratory and filtered through 0.22 µm pore-size membrane filters (Merk-Millipore) using a peristaltic pump (100 rpm). Filtered samples were transported to UTS on dry ice, prior to being stored at -80 °C for DNA extraction, which was performed within two weeks of collection.

## 2.3 Characterisation of Environmental Conditions

Physico-chemical parameters were measured using a Xylem EXO-2 multiparameter water quality sonde. Data was recorded at approximately 0.5 m depth at one second intervals for a total of 3 minutes at each site.

A bucket of water was filled using an integrated sampler, which collects water from the top 1 m of the water column. The bucket was subsampled in triplicate for chlorophyll-a, and total suspended solids, in addition to subsamples of total nitrogen and total phosphorous.

Chlorophyll-a samples were filtered through a 0.45 µm glass fibre filter under vacuum, with the filter subsequently frozen prior to analysis. Concentrations were determined by fluorometry following extraction with 90% acetone solution, in accordance with standard methods (APHA 10200H) (APHA, 2012).

Figure 1



**Figure 1.** Map of the Central Coast lagoons examined in this study. Sites labelled C1-C5 represent sites within Cockrone Lagoon, sites labelled A1-A7 represent sites within Avoca Lagoon, sites labelled T1-T5 represent sites within Terrigal Lagoon and sites labelled W1-W5 represent sites within Wamberal Lagoon.

## 2.4 Faecal Indicator Bacteria Analysis

Enterococci levels were derived using Enterolert<sup>®</sup>, a Defined Substrate method, used to test aquatic environments for faecal indicator organisms (from warm blooded animals e.g. birds, dogs, humans etc). Lagoon water samples (10 ml) were diluted with 100 ml of sterile deionized water (1:10 dilution) in a sterile polystyrene vessel and powdered Enterolert<sup>®</sup> reagent mixed, and the sample-reagent combination added and mixed. The sample and reagents were then poured into a Quanti-Tray, a sterile panel with 51 wells containing indicator substrate, 4-methylumbelliferone-b-D-glucoside, that fluoresces when metabolized by Enterococci. Quanti-Trays were then sealed and incubated for 24 hrs at 41°C ± 0.5°C. The count of total fluorescent wells after 24 hrs (using a 365-nm-wavelength UV light with a 6-W bulb) was taken and then referred to a most probable number (MPN) table. The NHMRC Microbial Assessment Categories<sup>5</sup> use Colony Forming Units (CFU/100 mL) of Enterococci to relate Enterococci levels to degree of potential human health risk (Table 1). While the MPN approach estimates the number of viable microbes in a liquid suspension and the CFU approach quantifies the number of bacterial colonies that grow on a defined solid media, they are widely considered as equivalent (i.e. 1 CFU = 1 MPN) and for the purposes of this report, particularly where references to the NHMRC Microbial Assessment categories are made, this assumption will be applied.

**Table 1: Microbial Assessment Categories (NHMRC 2008)<sup>5</sup>**

Category	95 <sup>th</sup> percentile of enterococci (CFU/100 mL)	Basis of derivation	Estimation of probability
A	< 40	No illness seen in most epidemiological studies	GI illness risk < 1% AFRI risk < 0.3%
B	41- 200	Upper limit is above the threshold of illness transmission reported in most studies	GI illness risk < 1-5% AFRI risk < 0.3 – 1.9%
C	201- 500	Represents a substantial elevation in the probability of adverse health outcomes	GI illness risk >5 – 10% AFRI risk < 1.9-3.9%
D	> 500	Above this level there may be a significant risk of high levels of illness transmission	GI illness risk > 10% AFRI risk > 3.9%

GI = gastrointestinal

AFRI = acute febrile respiratory illness

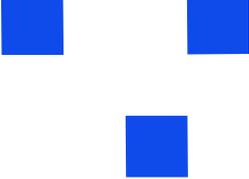
## 2.5 Microbial Source Tracking

Standard recreational water-quality monitoring approaches, using faecal indicator bacteria (FIB), such as Enterococci, cannot precisely discriminate the origin of the target bacteria between human (i.e. sewage) and animal sources, often leading to ambiguity about the cause of compromised water quality within an environment<sup>6</sup>. This is significant within the context of water quality assessment of Central Coast lagoons, which are potentially subject to sewage contamination, but also inhabited by significant bird-life and frequented by dog-walkers, creating significant uncertainty around the causes of reduced water quality.

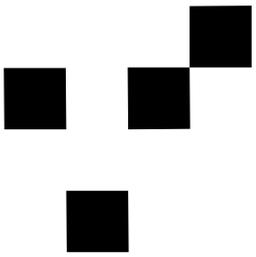
Molecular microbial source tracking approaches, can precisely identify specific indicator organisms or microbiological features based on DNA signatures, providing unambiguous discrimination of potential human and animal sources of faecal material. To assess the impact of different sources of water quality within Central Coast coastal lagoons, we employed a microbial source tracking approach, which involved the application of a set of quantitative Polymerase Chain Reaction (qPCR) primers targeting highly specific microbial indicators of human (sewage) and animal sources of faecal material (Table 2).

**Table 2:** Quantitative PCR assays to be applied in this study

Target Organism or Gene	qPCR Primers Used	Rationale	Ref
<i>Bacteroides</i> 16S rRNA (human)	HF183	A major component of the human gut microbiome and an excellent discriminator of human faecal material. Indicative of human sewage, allowing discrimination from animal faecal material signals.	7
<i>Lachnospiraceae</i> 16S rRNA	Lachno3	A major component of the human gut microbiome and a highly specific marker for human faecal contamination. Indicative of human sewage, allowing discrimination from animal faecal material signals.	8
<i>Bacteroides</i> (Dog)	DG3	A dog faeces specific marker targeting <i>Bacteroides</i> bacteria dominating the dog faecal microbiome	9
<i>Helicobacter</i> (Bird)	GFD	A 100% avian specific bacterial marker, which targets bird-specific <i>Helicobacter</i> present in the faeces of gulls, geese, chickens, and ducks.	10
Integron-integrase gene (Int1)	int1	Bacterial gene shown to be an excellent proxy for anthropogenic pollution, due to its links to antibiotic and heavy metal resistance genes. Indicative of human contamination.	11



For microbial source tracking, microbial DNA was extracted from filters using a bead beating and chemical lysis kit (DNeasy PowerWater Kit, QIAGEN), prior to qPCR analysis. All assays were prepared with an epMotion 5075I Automated Liquid Handling System and performed on a Bio-Rad CFX384 Touch Real-Time PCR Detection System with three technical replicates, a standard curve, and negative controls.



## 2.6 Statistical Analysis

To test for differences in levels of qPCR markers between sites and time points, the nonparametric Kruskal-Wallis test was used in conjunction with Mann-Whitney pairwise comparisons, whereby Bonferroni corrected p values were used. One-way ANOVAs and Tukey's Pairwise tests were used where data was normally distributed. In order to test correlations between Enterococci plate counts (single replicate) and the qPCR samples (three biological replicates) average values for qPCR data were used. For correlations between Enterococci counts and data derived from qPCR assays, data was transformed  $\log(x+1)$ , with samples that had either 0 Enterococci or a qPCR result below detection limit being removed in order to capture correlations within samples that had contamination. Correlations were determined using Spearman's correlation by permutation.



## 3.0 Results

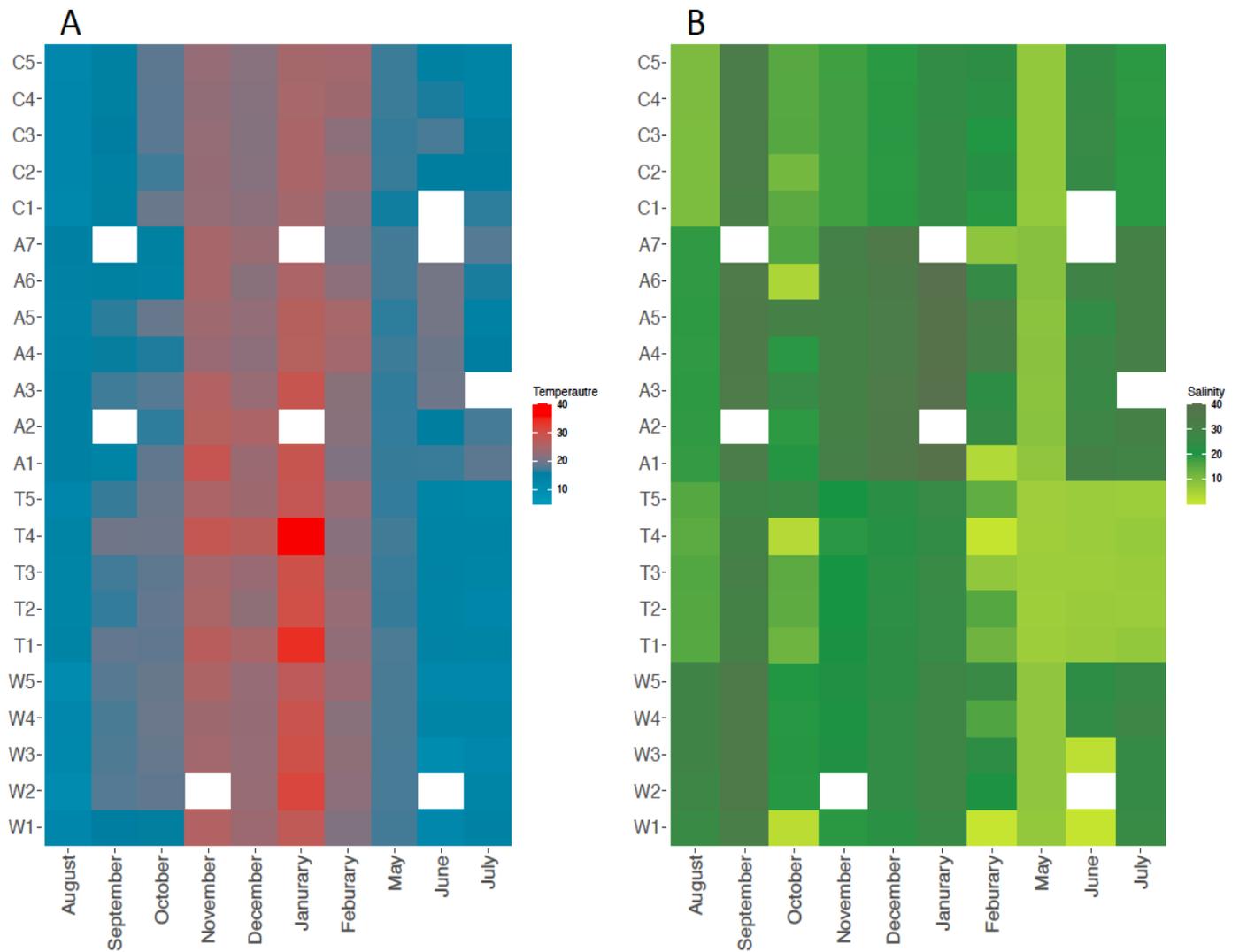
### 3.1 Environmental Conditions

Over the 11-month study period, physical and chemical conditions varied substantially between lagoons and over time. Water temperature exhibited predictable seasonal variability, ranging between 10.2°C in June 2020 (W1 sample) to 36.1°C in January 2020 (T4 sample). While some inter- and intra-lagoon variability in water temperature was apparent, all lagoons displayed the same seasonal temperature trends (Figure 2A).

Salinity displayed more pronounced inter-lagoon differences, with Avoca Lagoon characterised by highest salinity levels, particularly during the warmer summer months, presumably as a consequence of elevated evaporation levels (Figure 2B). In addition to seasonal influences, shorter-term variability in salinity was driven by rainfall events, with decreased salinity observed across all lagoons in October 2019 and February 2020 associated with significant rainfall events (Figure 3), whereby sampling was preceded by 40 mm and more than 65 mm of rain respectively. Except for June and July 2020, where sampling was preceded by 7 and 8.6 mm of rain, respectively, less than 5 mm of rain occurred in the 5 days preceding all other sampling times.

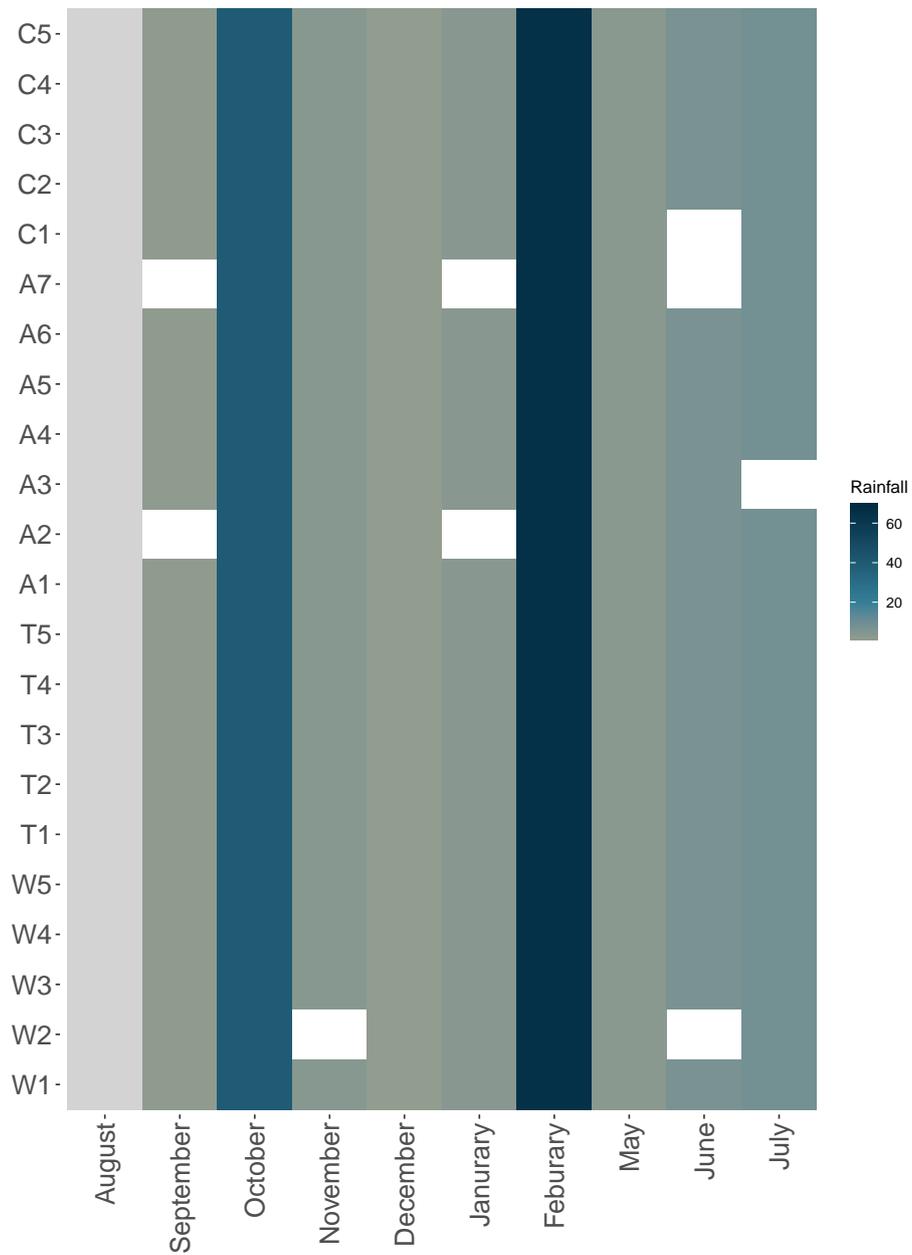
Evidence for differences in the ecological status of the four lagoons is provided by patterns in chlorophyll-*a* and turbidity (Figures 4a,b) and nutrient concentrations (total N and P) (Figures 5a,b), whereby elevated levels of these parameters were apparent within Avoca Lagoon relative to the other lagoons. These patterns were particularly evident during the summer months, when chlorophyll-*a* and turbidity levels became extremely high within Avoca Lagoon, exceeding 40  $\mu\text{g L}^{-1}$  and 40 NTU, respectively, in some samples. These chlorophyll-*a* and turbidity levels significantly exceed trigger values for these measures of water quality<sup>12</sup>.

**Figure 2**



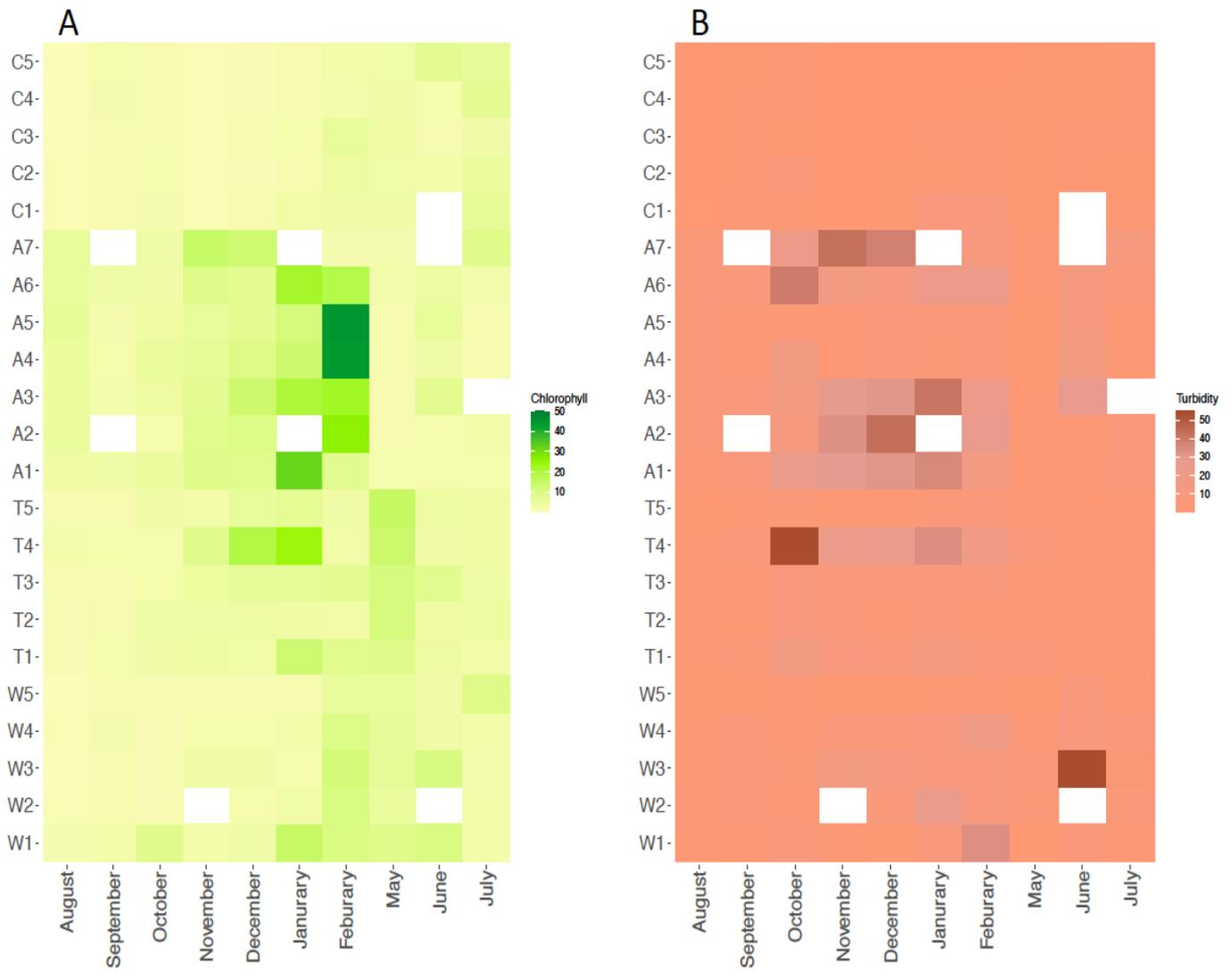
**Figure 2:** Water (a) temperature and (b) conductivity across the four lagoons [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] (Y-axis) and months (X-axis). White cells represent samples not collected.

**Figure 3**



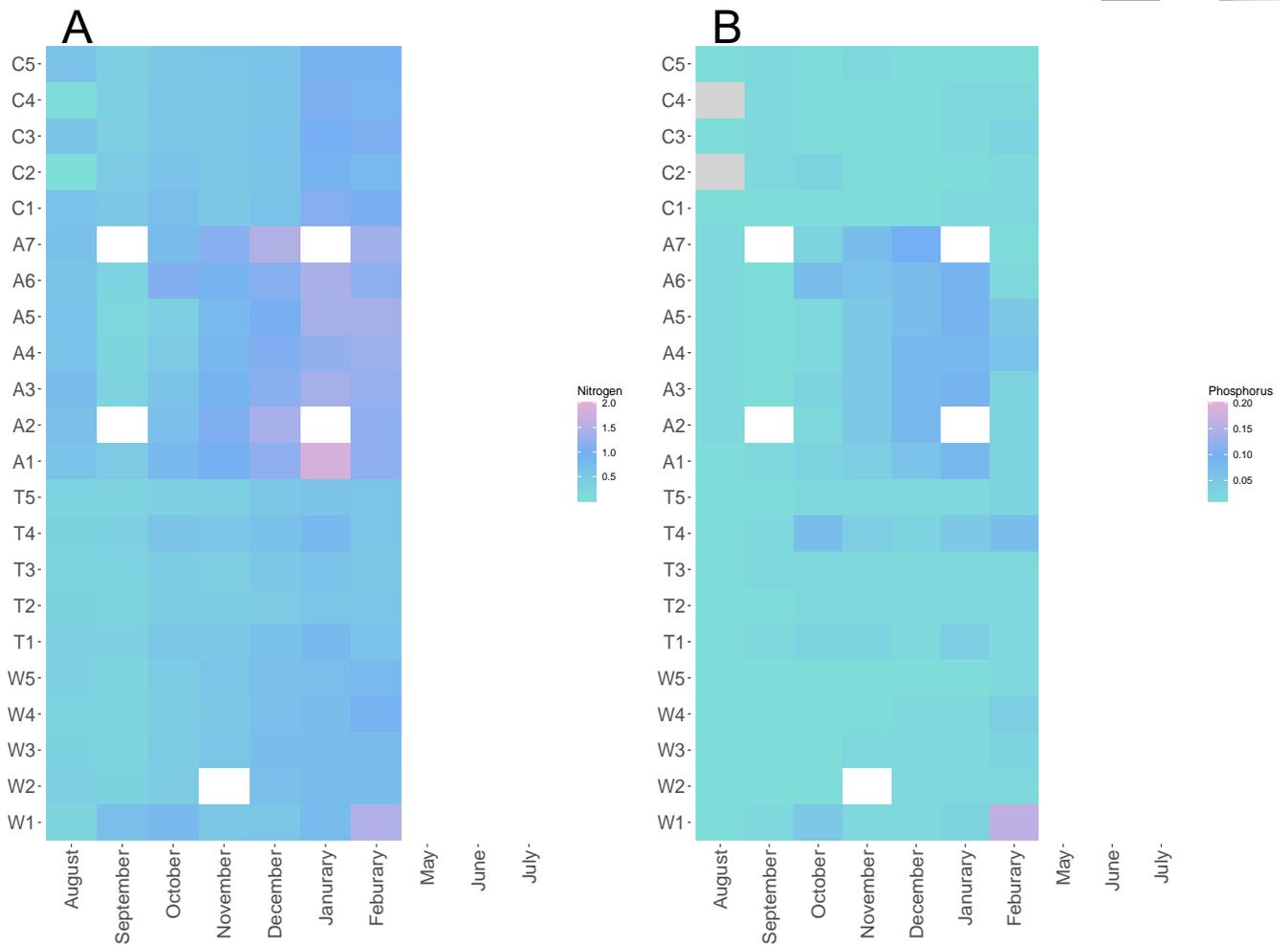
**Figure 3:** Rainfall (mm) in the 5 days preceding sample collection [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] (Y-axis) and months (X-axis). White cells represent samples not collected.

**Figure 4**



**Figure 4:** (a) Chlorophyll-a ( $\mu\text{g L}^{-1}$ ) and (b) turbidity (NTU) levels across the four lagoons [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] (Y axis) and months (x axis). White cells represent samples not collected.

**Figure 5**



**Figure 5:** (a) Total Nitrogen (mg/L) and (b) Total Phosphorous (mg/L) levels across the four lagoons [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] (Y axis) and months (x axis). White cells represent samples not collected. At the time of this report being prepared, nutrient levels for May-July 2020 were not available.

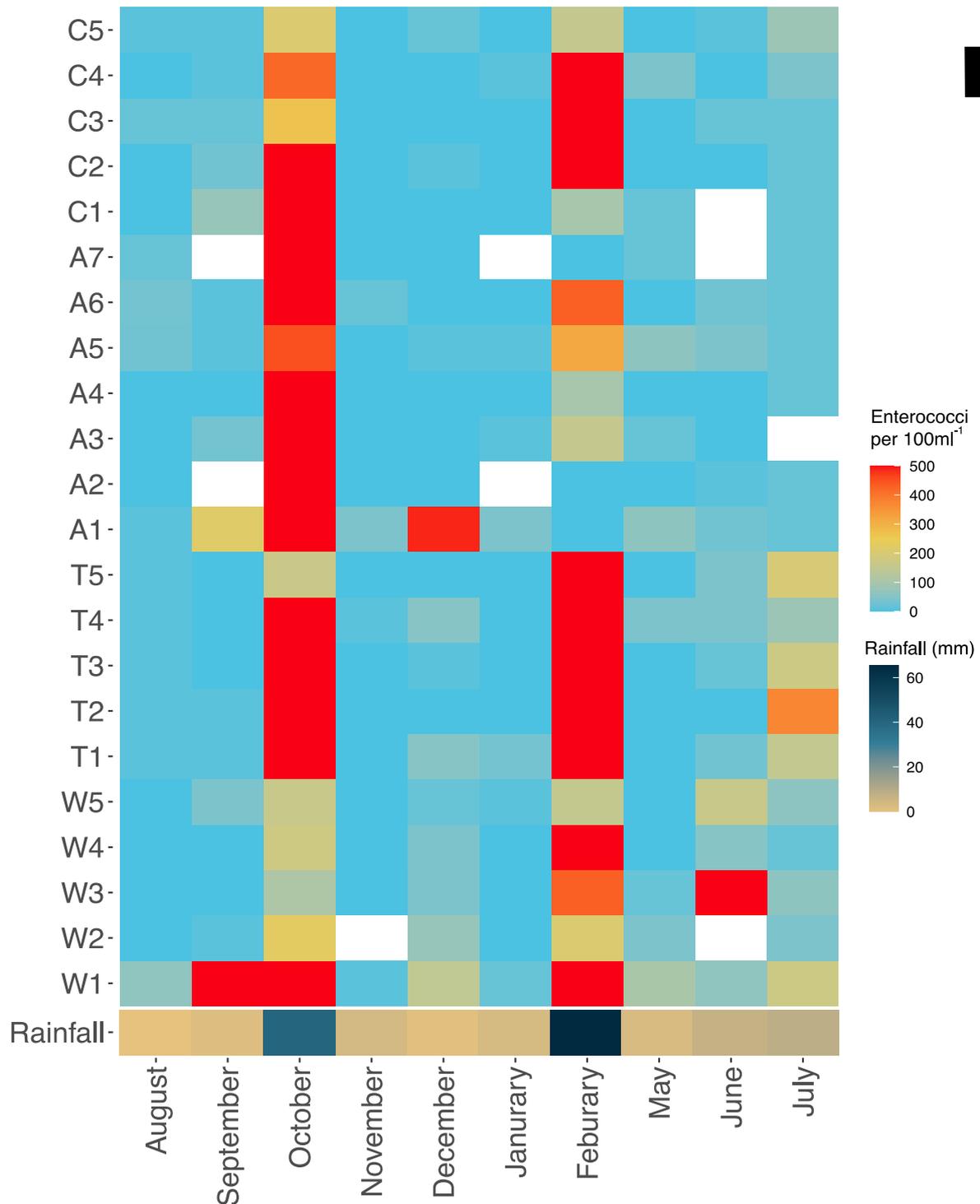
### 3.2 Enterococci Analysis

Across the ten months that samples were collected, Enterococci levels in the four lagoons reached or exceeded NHMRC Microbial Assessment Category C<sup>5</sup>, which is indicative of a substantial elevation in the probability of adverse health outcomes, in 17.5% of samples. Enterococci levels were significantly correlated ( $r = 0.51$ ,  $p < 0.001$ ) with rainfall throughout the study period, with highest levels coinciding with the rainfall events occurring in October 2019 and February 2020, where levels often substantially exceeded the maximum NHMRC risk threshold (500 CFU 100ml<sup>-1</sup>), reaching over 20,000 MPN 100ml<sup>-1</sup> in several samples. During dry weather periods (<5 mm of rain in the 5 days preceding sampling), however, Enterococci levels were undetectable to very low (< 40 MPN 100ml<sup>-1</sup>, i.e. NHMRC risk Category A: < 1% health risk<sup>5</sup>) in 84% of lagoon samples, with average levels < 22 MPN 100ml<sup>-1</sup>.

In October 2019, lagoon water sampling was preceded by 40 mm of rain in the 5 days prior. At this time, average Enterococci levels across all sites reached 7,348 MPN 100ml<sup>-1</sup>, which is indicative of very high levels of faecal contamination and significant risk of illness following human exposure<sup>5</sup> (Figure 6). Highest mean Enterococci levels at this time were observed in Avoca Lagoon (20,804 MPN 100ml<sup>-1</sup>), while lowest levels were observed within Terrigal Lagoon (432 MPN 100ml<sup>-1</sup>). In Wamberal Lagoon, significant spatial heterogeneity in Enterococci abundance was apparent, with levels within the W1 (Forresters Creek) sample exceeding 9,800 MPN 100ml<sup>-1</sup>, while average levels across all other samples were < 170 MPN 100ml<sup>-1</sup>. Within Cockrone Lagoon, the C2 sample displayed the highest Enterococci levels (1,435 MPN 100ml<sup>-1</sup>).

In February 2020, more than 65 mm of rain occurred in the 5 days preceding sampling and Enterococci levels again became very high (mean: 4,236 MPN 100ml<sup>-1</sup>). Unlike October 2019, Avoca Lagoon only exhibited moderate increases in Enterococci levels at this time (mean levels: 143 MPN 100ml<sup>-1</sup>), while highest levels were observed in Wamberal Lagoon (mean levels: 9,637 MPN 100ml<sup>-1</sup>), indicating a heterogeneous impact of rainfall on lagoon water quality across different rainfall events (Figure 6). The low Enterococci levels within Avoca Lagoon during the February rainfall event may be linked to the lagoon being open to the ocean at this time, leading to flushing of contaminated water out of the lagoon. Within Wamberal Lagoon, the W1 water sample again displayed markedly higher Enterococci levels (34,333 MPN 100ml<sup>-1</sup>) than other sampling locations, indicating an ongoing impact from the Forresters Creek catchment on Wamberal Lagoon. Relative to October 2019, Enterococci levels (mean: 7,792 MPN 100ml<sup>-1</sup>) indicated a substantially higher degree of contamination within Terrigal Lagoon, with extremely high levels (25,860 MPN 100ml<sup>-1</sup>) occurring within the T4 sample. Within Cockrone Lagoon, the C2 sampled again displayed the highest Enterococci levels (2,441 MPN 100ml<sup>-1</sup>).

Figure 6



**Figure 6:** Enterococci levels determined using Enterolert®, a Defined Substrate method across the four lagoons [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] (Y axis) and months (x axis). Colour scale corresponds to Enterococci most probable number (per 100ml<sup>-1</sup>). White cells represent samples not collected. Bottom bar displays intensity of rainfall in the 5 days preceding each sampling date.

While Enterococci levels were generally undetectable to very low in all lagoons during periods of dry weather, a spatially and temporally sporadic sub-set of samples was characterised by high Enterococci levels. These included the A1 sample in Avoca Lagoon, where Enterococci levels reached 487 MPN 100ml<sup>-1</sup> in December 2019, and the W1 and W3 samples within Wamberal Lagoon, where elevated Enterococci levels occurred on several occasions. During July 2019, following 8.6 mm of rain in the preceding 5 days, all samples within Terrigal Lagoon and the W1 sample within Wamberal Lagoon exhibited moderate to high levels (82-374 MPN 100ml<sup>-1</sup>) of Enterococci (Figure 6).

### 3.3 Human Faecal Markers

The two markers for human faecal material, Lachno3 and HF183, which are indicative of human gut microbiome associated *Lachnospiraceae* and *Bacteriodes* bacteria<sup>7,8</sup> exhibited weak, but significant correlations (Lachno3:  $r = 0.32$ ,  $p < 0.001$ ; HF183:  $r = 0.25$ ,  $p < 0.001$ ) to Enterococci levels. However, over the entire 10 months of sampling, these two markers for sewage contamination were detectable in only 35 and 38% of samples, respectively (Figures 7 & 8). Highest levels of these markers typically occurred after rainfall events, as reflected by positive correlations with rainfall levels (Lachno3:  $r = 0.27$ ,  $p < 0.001$ ; HF183:  $r = 0.18$ ,  $p < 0.05$ ).

Within Cockrone Lagoon, low levels of HF183 and Lachno3 were detectable in only 24 and 31% of samples, respectively. Where detected, average levels of these markers were significantly lower in Cockrone Lagoon than all other lagoons [HF183, Kruskal-Wallis,  $\text{Chi}^2 = 7.987$ ,  $p < 0.05$ , Lachno3, Kruskal-Wallis,  $\text{Chi}^2 = 3.062$ ,  $p < 0.05$ ], with average HF183 and Lachno3 levels in this lagoon 21- and 9-times lower than the average across all lagoons, indicating a relatively small impact of sewage within Cockrone Lagoon. Highest levels of HF183 and Lachno3 occurred during October 2019 and February and July 2020, and February 2020, respectively, which coincided with the most significant rainfall events and highest Enterococci levels observed at this site during the study period (Figure 6).

Within Wamberal Lagoon, the human faecal markers HF183 and Lachno3 were detectable in only 15 and 19% of samples. Highest levels of these markers were detected within the W1 sample in February 2020 (both HF183 and Lachno3) and July 2020 (HF183 only), coinciding with rainfall events and highly elevated Enterococci levels (Figure 6) in each of these months.

Both Terrigal and Avoca lagoons were characterised by periodically very high levels of both human faecal markers, indicative of a more pronounced impact of sewage on water quality in these systems. Within Avoca Lagoon, HF183 and Lachno3 were detectable in 55 and 50% of samples across the data set, the highest proportion observed in any lagoon in this study. Both Lachno3 and HF183 occurred at very high levels in several Avoca Lagoon samples in October 2019 (Figures 7 and 8), which coincided with the highest average levels of *Enterococci* observed in any lagoon, at any time, throughout the study period (Figure 6). At this time, highest levels of these human faecal markers were observed in the A5-A7 samples. These patterns indicate that sewage was a principal contributor to poor water quality in Avoca Lagoon at this time. During the other major rainfall event in February 2020, both HF183 and

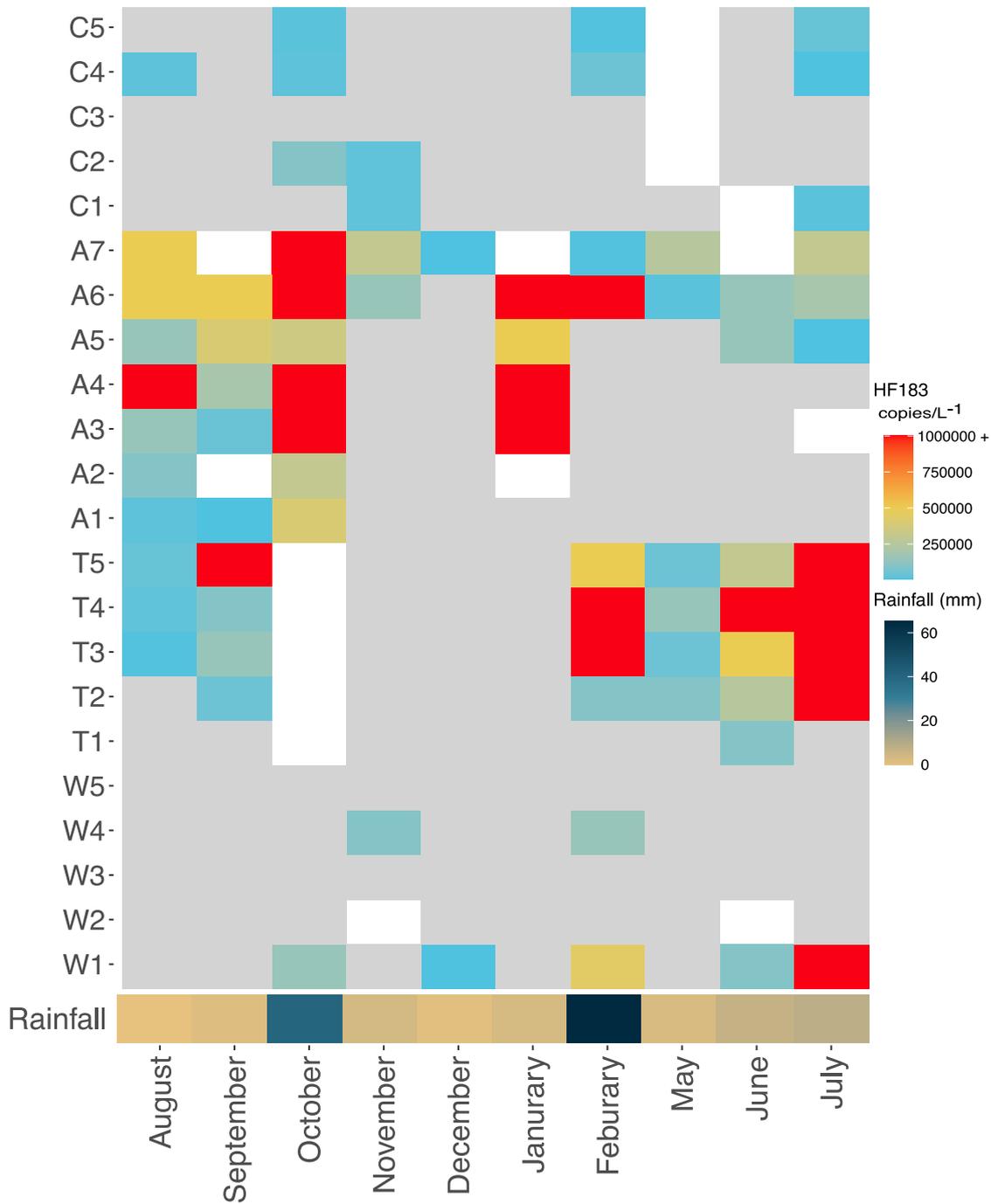


Lachno3 reached moderately high levels within the A6 sample, but these were 3- and 10-times, respectively, lower than the peak levels observed in October 2019. This trend is, however, highly consistent with Enterococci levels, which were also two orders of magnitude lower within this lagoon in February 2020 than October 2019 and highest in the A6 sample. On the other hand, a de-coupling of Enterococci levels and human faecal markers occurred within Avoca Lagoon during August and September 2019 and January 2020, when concentrations of both Lachno3 and HF183 were elevated within several samples (Figures 7 and 8) while only low to moderate Enterococci levels were recorded (Figure 6).

Within Terrigal Lagoon, microbial source-tracking samples were unfortunately not collected during October 2019, when high Enterococci levels coincided with a significant (40 mm) rain event. However, in February 2020, when the highest Enterococci levels were recorded in Terrigal Lagoon during another major rainfall (65 mm) event, high levels of both human faecal markers occurred within several samples (particularly T3-T5), indicating a significant impact of sewage contamination. During July 2020, when 8.6 mm of rain led to moderately high levels of Enterococci in some samples within Terrigal Lagoon (Figure 6), high levels of both the Lachno3 and HF183 markers also occurred across the lagoon (Figures 7 and 8), indicating that sewage also led to the elevated Enterococci counts at this time.

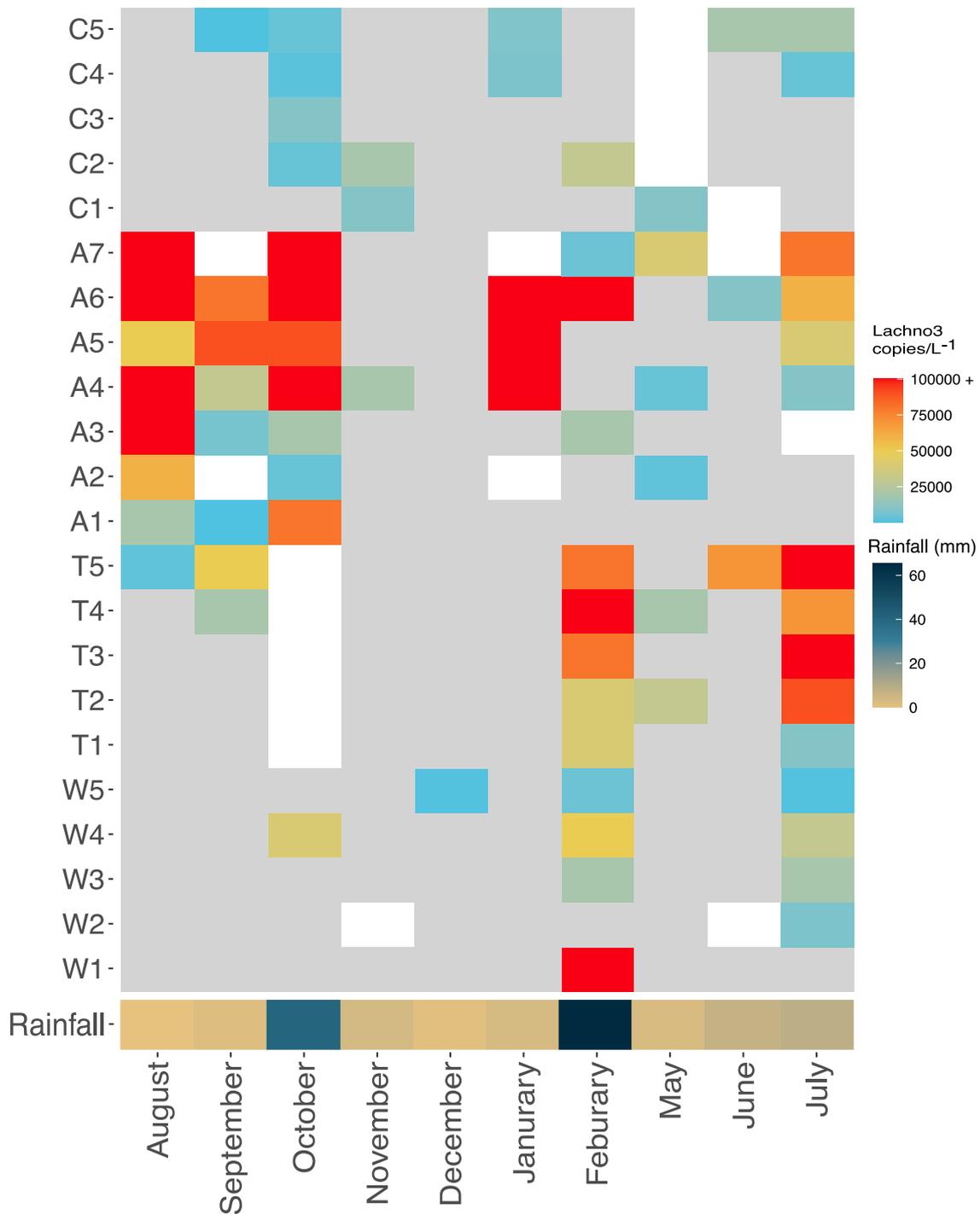


**Figure 7**



**Figure 7:** Heatmap displaying the distribution of the HF183 marker for the human faecal bacteria *Bacteroides* (sewage marker) across sampling locations (Y axis) [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] and sampling times (x axis). Colour scale corresponds to copy numbers L<sup>-1</sup> defined using qPCR. Grey cells denote samples below detection limit. White cells represent samples not collected. Bottom bar displays intensity of rainfall (mm) in the 5 days preceding each sampling date.

**Figure 8**



**Figure 8:** Heatmap displaying the distribution of the Lachno3 marker for the human faecal bacteria *Lachnospiraceae* (sewage marker) across sampling locations (Y axis) [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] and sampling times (x axis). Colour scale corresponds to copy numbers L<sup>-1</sup> defined using qPCR. Grey cells denote samples below detection limit. White cells represent samples not collected. Bottom bar displays intensity of rainfall (mm) in the 5 days preceding each sampling date.

### 3.4 Animal Faecal Markers

#### 3.4.1 Dog *Bacteroides*

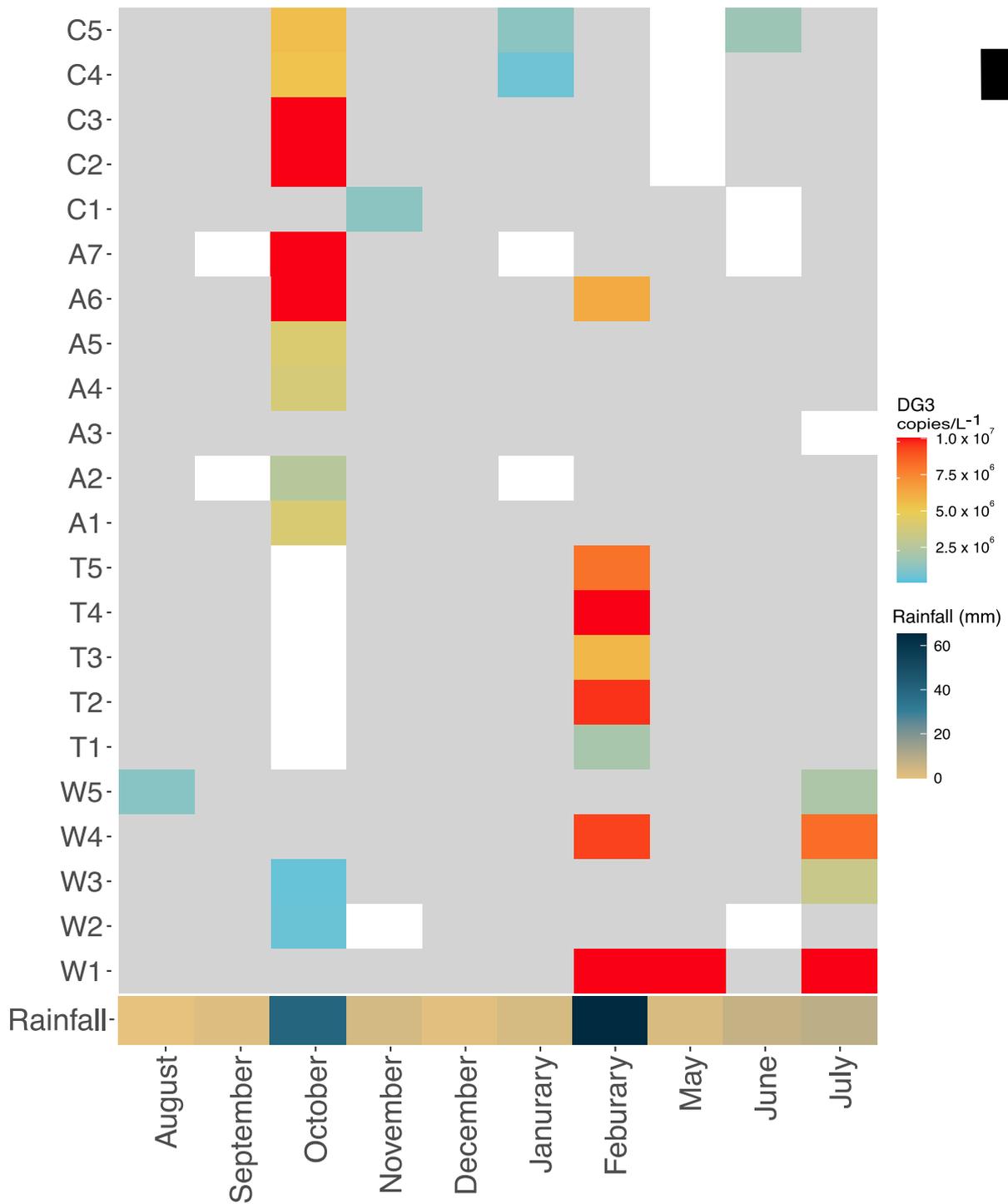
Across the entire dataset, the DG3 marker for dog-faeces associated *Bacteroides*<sup>9</sup> was detectable in only 15% of samples, but exhibited a significant correlation with Enterococci levels ( $r = 0.43$ ;  $p < 0.0001$ ). DG3 levels also exhibited a significant correlation with rainfall ( $r = 0.41$ ;  $p < 0.0001$ ).

Within Cockrone Lagoon, levels of DG3 were generally undetectable to low, with the exception of the October 2019 rainfall event, when some of the highest levels recorded throughout the entire study occurred in the C2 and C3 samples (Figure 9). Similarly, within Avoca Lagoon, the DG3 marker was below detection limits during dry periods but was recorded in moderate to very high levels within October 2019 rainfall event samples, and in one sample (A6) during the February 2020 rain event.

Although Terrigal Lagoon was not sampled during the October 2019 rain event, very high levels of the DG3 dog faeces marker were observed at this site during the February 2020 rain event. However, the DG3 marker was undetectable within Terrigal Lagoon at all other times.

Within Wamberal Lagoon, high levels of the DG3 dog faeces marker occurred within two samples (W1 and W4) during the February 2020 rainfall event, while moderate to high levels of this marker also occurred during a smaller (8.6 mm) rainfall event in July 2020 (Figure 9). During May 2020, high levels of the DG3 marker were observed in the W1 sample, in the absence of any significant rainfall.

**Figure 9**



**Figure 9:** Heatmap displaying distribution of the DG3 marker for canine *Bacteroides* (dog faeces) across sampling locations (Y axis) [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] and sampling times (x axis). Colour scale corresponds to copy numbers L<sup>-1</sup> defined using qPCR. Grey cells denote samples below detection limit. White cells represent samples not collected. Bottom bar displays intensity of rainfall (mm) in the 5 days preceding each sampling date.

### 3.4.2 Bird *Helicobacter*

The GFD Avian *Helicobacter* marker was observed in 98% of samples and was significantly correlated to total Enterococci levels ( $r = 0.26$ ;  $p < 0.001$ ) and rainfall ( $r = 0.27$ ;  $p < 0.001$ ). Relative to the other source-tracking markers used in this study, inter-lagoon differences in the abundance of the GFD marker were more apparent (Figure 6), with significantly higher levels [Kruskal-Wallis,  $\text{Chi}^2 = 5.417$ ,  $p < 0.001$ ] of this marker for bird faeces present within Avoca Lagoon than all other lagoons.

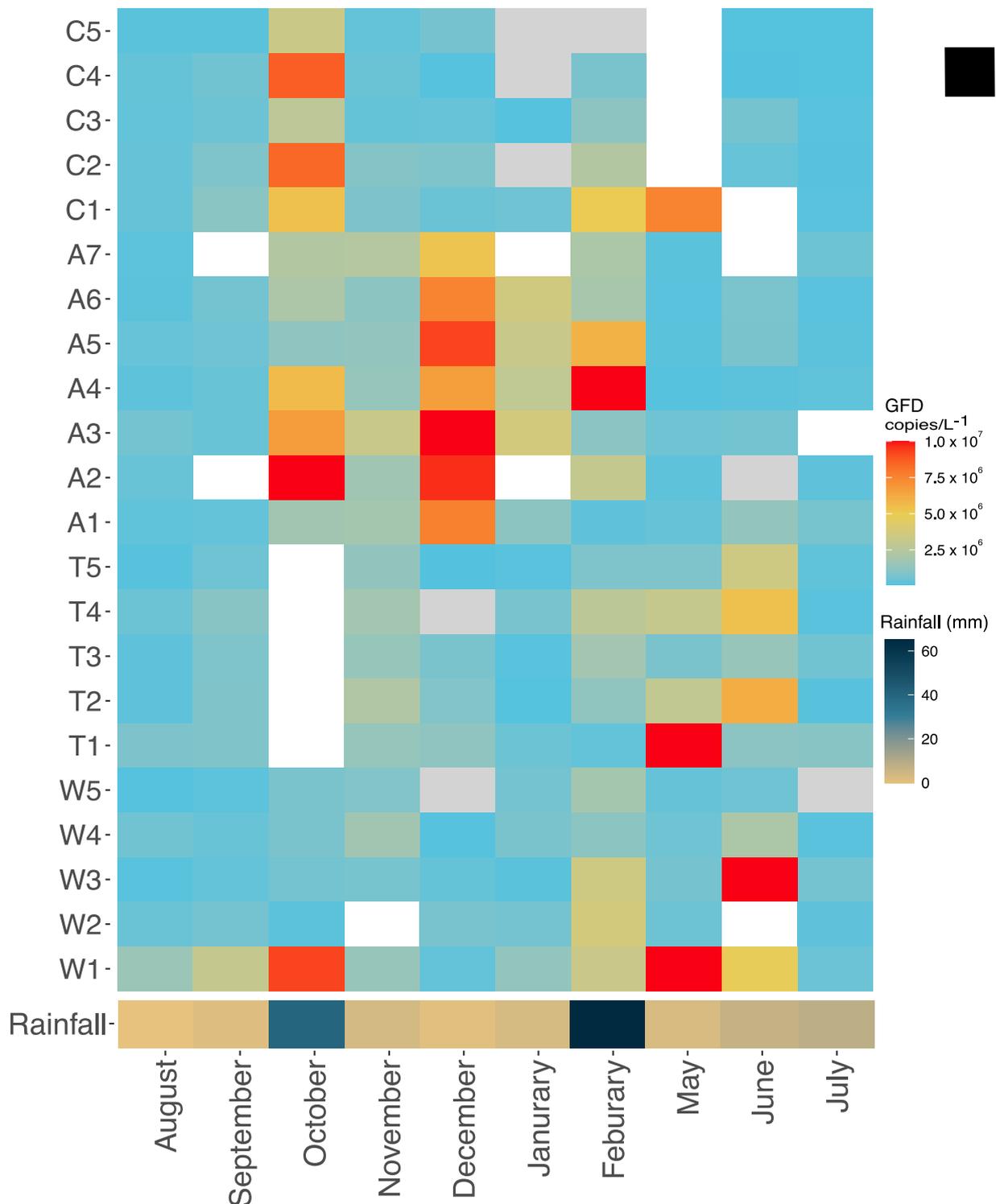
Within Avoca Lagoon, seasonal variability in the occurrence of the GFD marker was also apparent, with significantly higher levels [Kruskal-Wallis,  $\text{Chi}^2 = 27.2$ ,  $p < 0.001$ ] observed during the summer months (November-February) than other periods of the year (Figure 10). Indeed, within Avoca Lagoon, highest levels of the GFD marker occurred across all samples during December, when only 1 mm of rainfall occurred, indicating a level of de-coupling of GFD occurrence and rainfall within this lagoon. It is perhaps noteworthy that during December, the Avoca Lagoon A1 sample exhibited elevated Enterococci levels (487 MPN 100ml<sup>-1</sup>) that could not be explained by any other marker used in this study. Other incidences of high levels of GFD within Avoca Lagoon corresponded with the major rainfall events in October 2019 and February 2020.

Within Cockrone Lagoon, highest levels of the GFD marker were observed during the October 2019 rainfall event. Given the relatively low levels of the human faecal markers (Lachno3 and HF183) within this lagoon, we posit that the combined effects of bird and dog faecal material contributed to the elevated Enterococci levels within Cockrone Lagoon observed in October 2019.

Average levels of the GFD Avian *Helicobacter* marker within Terrigal Lagoon were significantly lower than those observed in Avoca and Cockrone lagoons, with levels remaining relatively low even during significant rainfall periods (i.e. February and July 2020). One significant peak in GFD levels occurred within the T1 sample in May 2020, but notably this did not coincide with a significant peak in total Enterococci counts.

Within Wamberal Lagoon, low to moderate levels of the GFD Avian *Helicobacter* marker were observed in all samples, with the exception of a handful of sporadically distributed samples where high levels were observed, including the W1 sample in September and October 2019 and May 2020 and the W3 sample in June 2020. Notably, while elevated *Enterococci* levels were observed in all of these samples (Figure 6), no other human or animal faecal markers were elevated within the September 2019 W1 or June 2020 W3 samples, implying that bird faecal material may have been the primary contributor to high Enterococci levels within these samples.

**Figure 10**



**Figure 10:** Heatmap displaying distribution of GFD marker for avian *Enterococci* (bird faeces) across sampling locations (Y axis) [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] and sampling times (x axis). Colour scale corresponds to copy numbers L<sup>-1</sup> defined using qPCR. Grey cells denote samples below detection limit. White cells represent samples not collected. Bottom bar displays intensity of rainfall (mm) in the 5 days preceding each sampling date.

### 3.5 Indicators of Anthropogenic Impact

#### 3.5.1 Class 1 Integron-integrase gene

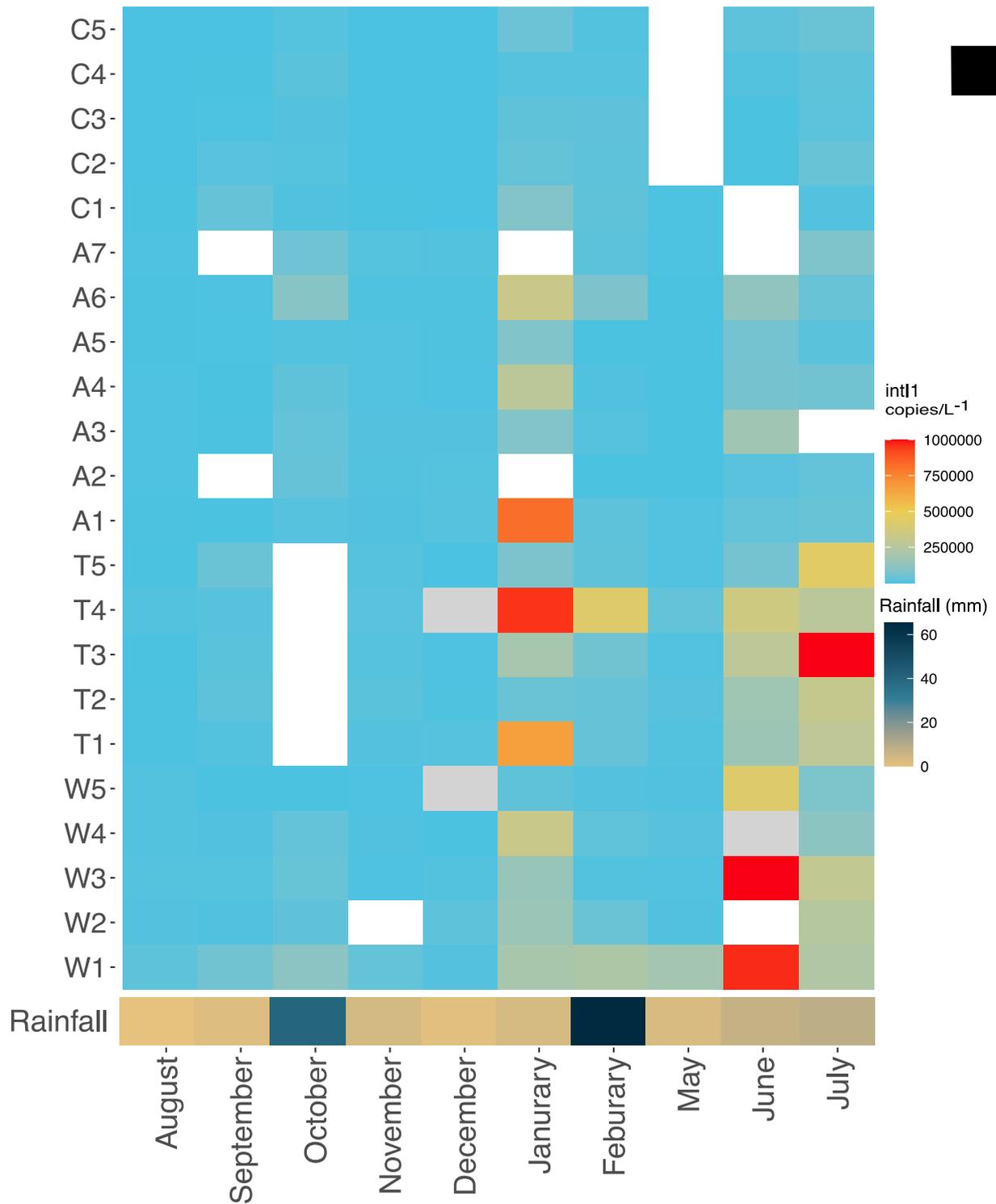
The Class 1 Integron-integrase gene (*intl1*) has been identified as an excellent microbial measure of anthropogenic contamination in aquatic habitats<sup>13</sup>. The *intl1* marker was detected in 97 % of samples across the data-set and displayed a significant correlation with Enterococci levels ( $r = 0.32$ ;  $p < 0.001$ ). Although detected in a large proportion of samples, levels of *intl1* varied by over 4 orders of magnitude over time and between lagoons, with highest average levels observed within Terrigal and Wamberal Lagoons (Figure 11).

Lowest levels of *intl1* occurred within Cockrone Lagoon, where this marker for anthropogenic impact remained undetectable to low throughout the entire study period (Figure 11). Similarly, despite evidence for intermittent sewage contamination (Figures 7 and 8), *intl1* levels within Avoca Lagoon were generally low, except for 3 samples during January 2020 (Figure 11).

Within Terrigal Lagoon, highest *intl1* levels occurred during January 2020 (T1 and T4 samples) and July 2020 (T3 sample). Somewhat intriguingly, the elevated *intl1* levels during January 2020 did not coincide with elevated Enterococci counts or levels of any of the human or animal faecal markers employed in this study. On the other hand, significantly [Kruskal-Wallis,  $\text{Chi}^2 = 8.986$ ;  $p < 0.001$ ] elevated average levels of *intl1* during July 2020 coincided with significant levels of both human faecal (sewage) markers.

Highest levels of the *intl1* marker occurred within Wamberal Lagoon in June 2020 (W1 and W3 samples). While high Enterococci levels ( $1,674 \text{ MPN } 100\text{ml}^{-1}$ ) occurred within the W3 sample at this time, the human faecal markers were undetectable. However, high levels of the bird *Helicobacter* GFD marker were observed within this sample, which is perhaps notable given that recent research has demonstrated substantial levels of *Intl1* in bird faeces<sup>14</sup>.

Figure 11



**Figure 11:** Heatmap displaying distribution of the *Int1* gene (marker for anthropogenic impact) across sampling locations (Y axis) [C= Cockrone, A= Avoca, T= Terrigal, W = Wamberal] and sampling times (x axis). Colour scale corresponds to copy numbers L<sup>-1</sup> defined using qPCR. Grey cells denote samples below detection limit. White cells represent samples not collected. Bottom bar displays intensity of rainfall (mm) in the 5 days preceding each sampling date.

## 4.0 Interpretation of Results & Discussion

This project applied a microbial source tracking approach to: (i) *characterise the spatial and temporal dynamics of water contamination in four Central Coast lagoons* and (ii) *determine when and where periodically poor water quality within lagoons is primarily caused by sewage or animal sources of faecal contamination*.

The Central Coast's four coastal lagoon systems (Wamberal, Terrigal, Avoca and Cockrone) are classified as true ICOLLs (Intermittently Closed and Open Lakes and Lagoons), which are significant natural ecosystems that represent important nurseries for coastal fish and crustacean species, habitats for aquatic birds, and key features within coastal nutrient and biogeochemical cycling processes<sup>1,15</sup>. Furthermore, these lagoons are used extensively by the Central Coast community for a range of recreational activities<sup>1</sup>. As a consequence of their predominantly urban settings, these ecosystems often experience poor water quality<sup>2</sup>, yet the causes and sources of contamination are generally not well resolved.

Over the course of the 11-month study period, standard metrics for water quality assessment, including chlorophyll *a*, turbidity and Enterococci levels highlighted differences, both *between* the four lagoons and over-time *within* each lagoon. In-line with previous assessments<sup>1</sup>, Terrigal and Avoca lagoons were generally characterised by poorer water quality than Cockrone and Wamberal lagoons, according to standard indices defined by both the NSW Natural Resources Monitoring, Evaluation and Reporting (MER) Program<sup>12</sup> (i.e. chlorophyll-*a* and Turbidity) and Beachwatch<sup>2</sup> (i.e. Enterococci levels). However, all four lagoons experienced periods of compromised water quality during the study period, with the molecular microbial source tracking approaches applied here revealing that the causes are often complex and heterogeneous in space and time.

**Cockrone Lagoon:** According to traditional metrics of water quality, Cockrone lagoon was characterised by the most 'pristine' conditions of all lagoons examined in this study, with the lowest average levels of chlorophyll-*a* and turbidity. Furthermore, Enterococci levels were generally lower within Cockrone Lagoon than all other lagoons, with 74% of samples below 40 MPN 100ml<sup>-1</sup> (i.e. NHMRC risk Category A– low health risk/suitable for recreation). However, on two occasions, corresponding to the rainfall affected months of October 2019 and February 2020, Enterococci levels became elevated within all samples, exceeding the maximum NHMRC risk category (Category D; significant risk of illness) in 50 % of these samples. A microbial source tracking approach was applied to examine the potential causes of these high Enterococci levels.

In October 2019, when average Enterococci levels within Cockrone Lagoon exceeded 670 MPN 100ml<sup>-1</sup>, a low-level signal for sewage contamination was present in some samples (C2, C4 and C5). However, it is notable that the levels of the HF183 and Lachno3 markers for human faeces at this site were well over an order of magnitude lower than those observed in Avoca Lagoon at the same time, implying a relatively minimal level of sewage contamination. Furthermore, both the GFD marker for avian *Helicobacter* and the DG3 marker for canine

*Bacteroides* were significantly elevated relative to other times [DG3, Kruskal-Wallis,  $\text{Chi}^2 = 7.899, p < 0.001$ ; GFD, Kruskal-Wallis,  $\text{Chi}^2 = 12.21, p < 0.001$ ], indicating a contribution of animal faecal material to the high Enterococci levels observed at this time. The fact that these two animal sources of faecal material were only elevated during significant rainfall, and were either undetectable, or at very low levels, at other times implies that bird and dog faecal material was washed into the lagoon from the surrounding environment during the rainfall event, rather than being a consistent impact within Cockrone Lagoon.

The causes of very high Enterococci levels within three Cockrone Lagoon samples (C2-C4) during the rainfall affected February 2020 sampling event are not clear. At this time, the dog *Bacteroides* marker was below detection limits in all samples, implying a negligible impact of dog faeces. Only very low levels of the human faecal markers were observed in three of these samples (Lachno 3 in C2 and HF183 in C4 and C5), again implying a very small influence of sewage contamination, while the bird *Helicobacter* marker occurred in only moderately high levels within corresponding samples. Considering the relatively low degree of urban development and given the apparently small influence of each of the faecal sources targeted here, we posit that during this period, elevated Enterococci levels within Cockrone Lagoon were potentially caused by other sources of animal faecal material not assessed in this study, such as domesticated/agricultural animals from surrounding peri-urban areas, and other wildlife.

In summary, Cockrone Lagoon is generally characterised by very good water quality in dry weather conditions, but susceptible to contamination immediately following significant rainfall, when animal (bird and dog) faecal material from the surrounding environment can lead to elevated Enterococci levels. The low levels of the two markers for human faecal material and the Class 1 Integron-integrase gene (*intl1*) indicate that impacts from sewage were relatively small within this lagoon during the study period.

**Avoca Lagoon:** Across the entire data-set, average levels of chlorophyll-a, turbidity, total nitrogen and total phosphorous were all highest within Avoca Lagoon, with chlorophyll-a and turbidity levels regularly exceeding trigger values defined by the NSW Natural Resources Monitoring, Evaluation and Reporting (MER) Program<sup>12</sup>. This pattern is consistent with observations made in the Central Coast Waterways Report Card<sup>1</sup>, which rated water quality in Avoca Lagoon as 'Very Poor', according to consistently very high chlorophyll-a and turbidity levels. Similarly, Avoca Lagoon recorded the highest average Enterococci levels across the entire data-set, as well as some of the maximum recorded Enterococci levels observed (e.g. 24,195 MPN 100ml<sup>-1</sup> during October 2019). The high levels of Enterococci found throughout the current study are consistent with the results of the State of the Beaches Report<sup>2</sup>, prepared by Beachwatch, which rated water quality in Avoca Lagoon as 'Poor'.

The highest Enterococci levels observed in Avoca Lagoon occurred throughout the high rainfall event in October 2019, when average levels across the 7 discrete sampling sites within the lagoon exceeded 20,800 MPN 100ml<sup>-1</sup>. These levels are more than 40 times higher than the threshold for maximum risk to

human health in the NHMRC Microbial Assessment Categories<sup>5</sup>, and represent the highest average *Enterococci* levels observed within any lagoon at any time of this study. Samples collected in Avoca lagoon at this time were also characterised by the highest levels of the two markers for human faecal material (HF183 and Lachno3) recorded during this study. The high levels of human faecal markers within these samples indicate that sewage contamination significantly contributed to the very high *Enterococci* levels after rainfall, with some of the highest concentrations of both HF183 and Lachno3 indeed generally corresponding to samples with the highest *Enterococci* levels. At this time, the highest concentrations of both the HF183 and Lachno3 marker occurred within the A6 and A7 samples located in the southerly corner of Avoca Lagoon, potentially indicating proximity to the point source of contamination during or after rainfall.

While it appears that sewage contamination contributed to the very high *Enterococci* levels observed within Avoca Lagoon in October 2019, it is notable that both the GFD marker for avian *Helicobacter* and the DG3 marker for canine *Bacteroides* were also elevated within several Avoca Lagoon samples at this time, indicating that like Cockrone Lagoon, animal faeces is washed into Avoca Lagoon from the surrounding environment during rainfall. However, during this time there was a tight coupling in the distribution of the DG3 marker and human faecal markers (i.e. highest levels in the A6 and A7 samples), which may also indicate that dog faeces was introduced to the lagoon *with* sewage, as has been reported elsewhere in NSW coastal environments<sup>16</sup>. Highest levels of the bird *Helicobacter* marker were, on the other hand, observed in the A2 sample, which is on the other side of Avoca Lagoon to the A6 and A7 samples, implying this is an independent (from sewage) input of faecal material.

*Enterococci* levels within Avoca Lagoon only exceeded 300 MPN 100ml<sup>-1</sup> on three other occasions throughout the study period. These include the second major wet weather event (65 mm in the preceding 5 days), occurring in February 2020 (A5 and A6 samples) and a dry weather sample during December 2019 (A1 sample). In February 2020, moderate levels of both human faecal markers and the dog faeces marker occurred in A6, again indicating a coupled contribution of sewage and dog faeces to the elevated *Enterococci* counts. However, in both the February A5 sample and the December A1 sample, neither of the human faecal markers, nor the dog faecal marker were detected, while high levels of the avian *Helicobacter* GFD marker occurred, indicating that bird faeces in some instances had a spatially localised impact on water quality within Avoca Lagoon.

In summary, Avoca Lagoon exhibited some of the poorest water quality observed during this study, particularly following a major rainfall event in October 2019. During this time, a clear human faecal marker signal indicates that sewage input, potentially located in the southern region of the lagoon near to the A6 and A7 sample sites, impacted the lagoon's water quality. However, in addition to sewage inputs, there is evidence that dog and bird faecal material also contributed to some periods of elevated *Enterococci* levels within Avoca Lagoon.

**Wamberal Lagoon:** Water quality within Wamberal Lagoon was relatively good throughout the study period, except for heavy rainfall periods and a few sporadic and spatially localised events. Enterococci levels remained below 200 MPN 100ml<sup>-1</sup>, in over 83% of samples, which equates to < 5% risk of gastrointestinal illness, according to the NHMRC Microbial Assessment Categories<sup>5</sup>. However, relative to the more pristine Cockrone Lagoon, Enterococci, Chlorophyll-a, and turbidity levels were often substantially higher within Wamberal Lagoon, with significant peaks in these parameters (e.g. Enterococci: 34,333 MPN 100ml<sup>-1</sup> in W1 in February 2020; Turbidity: 53.6 NTU in W3 in June 2020) observed at certain times during the study period.

Enterococci counts reached highest levels within Wamberal Lagoon during February 2020, when sampling was preceded by > 65 mm of rainfall. During this time, average Enterococci levels across the lagoon exceeded 9,600 MPN 100ml<sup>-1</sup>, with the highest Enterococci levels recorded in any lagoon throughout the entire study period (34,333 MPN 100ml<sup>-1</sup>) observed in the W1 sample. High levels of the human faecal markers HF183 and Lachno3 both also occurred in this sample, indicating that sewage was a cause of these very high Enterococci levels. Notably, the DG3 canine *Bacteriodes* marker within this sample occurred in the highest levels recorded across all lagoons during the study period, potentially indicating the presence of dog faeces in the stormwater system alongside infiltrated sewage<sup>16</sup>.

Elevated concentrations of the DG3 marker and moderately high levels of the human faecal markers also coincided with high levels of Enterococci (13,062 MPN 100ml<sup>-1</sup>) within the W4 sample in February 2020, further indicating a combined influence of dog and human faecal material on Enterococci levels within Wamberal Lagoon. Notably, very high levels of the DG3 marker also occurred within W1 in May and July 2020, although Enterococci levels only occurred in moderate levels at these times.

High *Enterococci* levels also occurred in the W1 sample in September and October 2019 and the W3 sample during June 2020. Both human faecal markers and the DG3 dog faecal marker were undetectable within the September W1 sample and the June W3 sample, ruling out the influence of sewage contamination and dog faeces. On the other hand, some of the highest levels of the GFD bird *Helicobacter* marker recorded across the entire study (i.e. all times and all lagoons) occurred within the W1 sample in October and W3 sample in June (with moderately high levels in W1 in September), pointing to an influence of bird faeces on the high *Enterococci* levels observed in these samples. In October 2019, moderate levels of the HF183 marker point to a combined contribution of sewage and bird faeces to the high Enterococci levels observed in W1.

In summary, Wamberal Lagoon is generally characterised by good water quality, with high Enterococci levels (i.e > 500 MPN 100ml<sup>-1</sup>) observed in less than 10% of samples collected during the study period. However, in some spatially and temporally localised samples, Enterococci levels reached very high levels, indicating periodically substantial levels of faecal contamination. Extremely high Enterococci levels occurred within the major developed tributary

in Wamberal Lagoon (W1 sample) during a heavy rainfall event in February 2020, with these coinciding with high levels of human faecal markers, indicating an impact from sewage contamination within this part of Wamberal lagoon. It is noteworthy that W1 is (i) located near to Forresters Creek, which is the main tributary into the lagoon from an urbanised area and (ii) is the furthest sampling point from the lagoon opening, which potentially leads to reduced flushing/mixing levels. Furthermore, this part of Wamberal Lagoon has previously been impacted by short-term sewer main breakages. The evidence for periodically high levels of human faecal markers in W1 potentially provides grounds for stormwater sampling to determine the likely catchment source and targeted examinations of sewer infrastructure near this site as part of the Central Coast Council sewer network remediation program.

High levels of both dog and bird faecal markers coincided with high Enterococci levels in other Wamberal Lagoon samples, when the human faecal markers were undetectable or in low levels, indicating that animal faecal material can also contribute to water quality within Wamberal Lagoon. However, the impact of animal faeces was highly localised in both space and time, with only about 10% of Wamberal Lagoon samples exhibiting high levels of bird and dog faecal material, meaning it is not possible to define a consistent influence of animal faeces on water quality in Wamberal Lagoon.

**Terrigal Lagoon:** Rainfall had a clear influence on water quality in Terrigal Lagoon, with Enterococci levels significantly correlated with rainfall during the study period ( $r = 0.51$ ;  $p < 0.001$ ) and highest Enterococci levels occurring following the largest rainfall event during the study period in February 2020. During dry weather periods, Enterococci levels were undetectable to low within all samples.

During the February 2020 rainfall event (65 mm of rain in preceding 5 days), high Enterococci levels coincided with high levels of the human faecal markers HF183 and Lachno3, indicating a significant impact from sewage on water quality in the lagoon. Highest levels of these markers occurred within the T3 – T5 samples, potentially highlighting point sources of sewage contamination in the southern region of the lagoon. In addition to the high levels of human faecal markers observed in Terrigal Lagoon at this time, the DG3 dog faeces marker also occurred in high levels in corresponding samples, indicating that dog faeces may have also contributed to high Enterococci levels recorded at this time. High levels of this marker may be indicative of either storm event-driven diffuse flushing of dog faeces from the surrounding environment into the lagoon or, as has been observed elsewhere<sup>16</sup>, the presence of dog faeces within sewage. Only low to moderate levels of the bird *Helicobacter* marker GFD were observed within Terrigal Lagoon at this time, indicating a minimal impact from bird faecal material on water quality.

In July 2020, a smaller rainfall event (8.6 mm in preceding 5 days) also led to moderately elevated Enterococci levels (average: 194 MPN 100ml<sup>-1</sup>) within Terrigal Lagoon. High levels of both human faecal markers were again observed within Terrigal Lagoon at this time, with highest levels occurring within the T2-T5 samples, further pinpointing this region of the lagoon as potential receiving

waters of sewage contamination following rainfall. The DG3 dog faeces and GFD bird faeces markers were undetectable and at very low levels, respectively, at this time, indicating that sewage, rather than animal faeces may be responsible for the elevated Enterococci levels occurring during periods of low rainfall.

Unfortunately, samples for microbial source tracking were not collected from Terrigal Lagoon during the October 2019 rainfall event (40 mm in preceding 5 days), when Enterococci levels averaged 432 MPN 100ml<sup>-1</sup> across the lagoon. However, by extrapolating the patterns observed in February and July 2020, we suggest that it is likely that sewage contamination was primarily responsible for elevated Enterococci levels.

In summary, water quality in Terrigal Lagoon is generally suitable for primary contact recreation during dry weather conditions. However, rainfall leads to substantial levels of faecal contamination within the lagoon, with microbial source tracking results indicating that sewage is the predominant cause of this contamination. Highest levels of human faecal markers were generally observed in the southern part of the lagoon (i.e. T3-T5 samples), suggesting that this arm of the lagoon is a potential source of sewage contamination to downstream swim sites during rainfall.

## 5.0 Conclusions

Water quality within the four major Central Coast lagoons (Cockrone, Wamberal, Terrigal and Avoca) is highly variable in space and time, with sewage and animal faecal material having a heterogeneous influence across the lagoons. Except for periods following significant rainfall, water quality in most of the lagoons is generally suitable for primary contact recreation. Within Cockrone Lagoon, peaks in Enterococci levels following rainfall appear to be largely related to inputs of animal faecal material, with spikes in the detection of dog and bird faeces often coinciding with decreased water quality. However, these impacts are relatively rare, with no clear evidence for a consistent point of input. Within Wamberal Lagoon, water quality is generally good, but there is evidence for periodic sewage contamination at the W1 site, located within Forresters Creek, which sometimes contributes to extremely high Enterococci levels. Dog and bird faecal material also very occasionally influence water quality at other points within Wamberal lagoon, but similarly to Cockrone Lagoon, this impact is spatially and temporally intermittent. According to several water quality indices, Avoca Lagoon is characterised by the poorest water quality among the studied lagoons. A clear signal for human faecal material was present within this lagoon following rainfall events, indicating impacts from sewage, with the points of input potentially located in the southerly region of the lagoon. However, animal faeces - most notably bird faeces in the northern parts of the lagoon – also impacted water quality with Avoca Lagoon, during periods of both wet and dry weather. While water quality in Terrigal Lagoon was generally suitable for primary contact recreation during dry weather periods, rainfall caused significant increases in Enterococci levels, which were linked to high levels of human faecal markers, indicating an impact of sewage

contamination. Taken together, these findings indicate that recreational water quality within Central Coast lagoons is generally suitable for swimming in dry weather, with the exception of periods immediately following rainfall events, when the relative impacts from sewage inputs and animal faecal material vary substantially from one lagoon to another.

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