Climate Change Cluster, Faculty of Science,
University of Technology Sydney

Microbial source-tracking to assess the spatial extent and temporal persistence of water quality issues at Terrigal Beach

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

FOR THE ATTENTION OF:

Dr Peter Scanes, Dr Jaimie Potts & Dr Colin Johnson
NSW Department of Planning, Industry and Environment (DPIE)
Dock 4, 480 Weeroona Road
Lidcombe NSW 2141

Vanessa McCann & Dr Melanie James
Estuary Management, Central Coast Council
P.O. Box 20 Wyong NSW 2259

REPORT PREPARED BY:

Justin Seymour, Nathan Williams & Nachshon Siboni
Climate Change Cluster
University of Technology Sydney
Tel: 9514 1776
E-mail. Justin.seymour@uts.edu.au

EXECUTIVE SUMMARY

The research presented in this report was commissioned by the NSW Department [redacted] Planning, Industry and Environment (DPIE) and the Central Coast Council, and ! performed by the Ocean Microbiology Group at the University of Technology Sydney ! (UTS). Most of the funding for the research was provided by the Central Coast Council. ! Both DPIE and Central Coast Council Scientists collaborated with UTS in developing and ! executing the sampling design. The principal goal of the research was to apply molecular ! microbiological approaches to assist efforts in defining the causes and associated spatial ! and temporal dynamics of poor water quality at Terrigal Beach (NSW). Samples from ! stormwater drains and seawater samples were analysed using a suite of assays ! targeting microbial indicators of human, bird and dog faecal material. Sampling was ! conducted before, during and after a significant rainfall event (44 mm over 3 days), with ! the goals of understanding: (i) to what extent sewage *vs* animal sources of faecal ! contamination influence water quality; (ii) the principal point-sources of contamination; and (iii) the spatial extent and temporal persistence of water contamination at Terrigal ! Beach during a rainfall event. !

Under dry weather conditions, water quality within Terrigal Beach was generally very good, with Enterococci levels remaining within the lowest health risk level in the NHMRC Microbial Assessment Categories and molecular microbiological markers for human and animal faecal material generally occurring at very low or undetectable levels. However, after 20.4 mm of rain there was a substantial increase in human faecal (sewage) markers and other indicators of waste-water infrastructure, with highest levels of these contamination markers observed within stormwater drains and their adjacent seawater samples, and within Terrigal Lagoon. Increased levels of the marker for dog faeces also followed rainfall, but were generally associated with stormwater drain samples, indicating potential flushing of dog faeces from the catchment or its presence within sewage. Across the suite of sewage and wastewater markers applied here, highest levels were generally observed within, and adjacent to, the outlet of stormwater Drain 4 and within Terrigal Lagoon. Unfortunately, rough ocean conditions precluded a detailed examination of the off-shore impact of contamination from the Terrigal Beach stormwater drain network during the peak of the rainfall event, but an along-beach assessment of near-shore samples indicated that contamination was relatively restricted to the Terrigal Haven region, with little to no signature of contamination observed at Forresters Beach, Wamberal Beach or North Avoca Beach. However, the opening of the entrance to Terrigal lagoon following 48 hours of rainfall had a significant, and spatially extensive, impact on water quality at Terrigal Beach, with samples collected up to 300m from the shoreline displaying significant levels of sewage and wastewater infrastructure indicators, indicating that opening of the entrance to Terrigal Lagoon (either natural or mechanical) can impact the surrounding environment. Five days after the rainfall event, extremely high levels of the sewage markers, the markers for waste-water infrastructure and the dog faeces marker were all observed in Drain 4 and the Lagoon 5 (L5) samples, pinpointing these as potential points of persistent sewage contamination.

In conclusion, during significant rainfall events, recreational water quality at Terrigal Beach is primarily influenced by sewage contamination, rather than animal faecal material, from a network of stormwater drains (in particular Drain 4) and Terrigal Lagoon. Terrigal Lagoon, when open to the ocean has a significant impact on water quality in the adjacent coastal environment.

1.0 BACKGROUND

The Central Coast (NSW) comprises more than 40 beaches and 4 Intermittently Closed and Opened Lakes and Lagoons (ICOLLs), spanning 87km of coastline, which deliver substantial economic and social value to the region[1]. Central Coast beaches are a principal drawcard for the local tourism and hospitality markets that are worth almost $2 billion yr^{-1}, while recreational beach use delivers substantial inherent value to the Central Coast population[2], which exceeds 330,000 people. However, like many urbanised coastal environments[3], some Central Coast beaches are regularly impacted by compromised water quality[4], resulting in potential human health implications[5].

Within NSW, a state-wide recreational water-quality monitoring program is conducted by a number of Councils in partnership with the NSW Government's *Beachwatch Program*, with *Beachwatch* rating swimming beaches according to safety for recreational use[4]. In Beachwatch's most recent *State of the Beaches* report[4], water quality at 16 of 32 monitored beaches, coastal lagoons and estuaries on the Central Coast were classified as poor, with stormwater inflows following rainfall suggested as the principal contributor to reduced water quality. The high proportion of sites with low water quality within this region is likely a consequence of the large number of estuarine and lagoon locations monitored on the Central Coast. Among the most popular beaches on the Central Coast, Terrigal Beach and nearby Terrigal Lagoon have during the last several years consistently been characterised by poor water quality, resulting in substantial community concern[5] and a large ($500,000) commitment from the NSW Government to address water quality issues at Terrigal beach and the surrounding lagoons.

In 2019, the authors of this report and scientists from DPIE and the Central Coast Council performed a microbial source-tracking study to define the causes of poor water quality at Terrigal Beach, with the results identifying sewage, rather than animal sources of faecal contamination, as the principal cause of poor water quality[6]. Furthermore, three stormwater drains on Terrigal Beach were identified as the likely points of contamination. However, important questions remaining from this previous work include:

(i) ! For what period following a rainfall event is water quality compromised by sewage contamination at Terrigal Beach?

(ii) ! If poor water quality at Terrigal Beach is driven by inputs from localised point sources (i.e. specific stormwater drains), how far from these sources is water quality negatively impacted?

In light of these questions, determining the spatial extent and temporal persistence of faecal contamination within the waters of Terrigal beach is an essential next step for understanding and subsequently managing water quality issues at this popular Central Coast beach, and is the focus of this report.

2.0 OBJECTIVES

The over-arching objective of this project was to apply molecular microbiological source tracking approaches to define the sources and the spatial and temporal dynamics of poor water quality at Terrigal beach during a wet-weather event. The specific objectives of the project were to:

1) Determine whether high Enterococci levels at Terrigal Beach, Haven and Lagoon during rainfall are primarily caused by human (i.e. sewage) or animal (dog or bird) sources of faecal contamination.
2) Identify the primary points of contamination input by sampling the network of stormwater drain outlets, lagoon and seawater sites within Terrigal Beach and Haven.
3) Understand the spatial extent and temporal persistence of water contamination at Terrigal Beach, Haven and Lagoon during a wet weather event.

3.0 METHODOLOGY

3.1 Sampling Design

Water samples were collected from stormwater drain outlets and from a series of onshore-offshore transects at Terrigal Beach before, during and after a wet weather event during late May – early June 2019. The sampling design was developed in close consultation with DPIE and Central Coast Council, with the goal of identifying sources of contamination at Terrigal, as well as the spatial extent and temporal persistence of contamination during a rainfall event.

Samples were collected from 35 locations chosen according to proximity to potential points of contamination (Figure 1). Samples were collected from the outlets of 3 stormwater drains (Drains 1, 2 and 4 in Figure 1), chosen according to a previous source-tracking study performed at Terrigal Beach[6], which indicated they contribute to poor water quality at Terrigal. Drain 1 is located in the south-eastern corner of Terrigal Haven, and is believed to collect water from the Broken Head dog park. Drain 2 collects water from the region surrounding Terrigal Haven playing field, while Drain 4 represents the output from a junction of drains that are exposed to run-off from Terrigal's urban centre and potential wet weather overflow points.

Samples were also taken from 5 zones within Terrigal Lagoon (Figure 1) and from seawater directly adjacent to the mouth of the lagoon. Terrigal Lagoon is an Intermittently Closed and Opened Lakes and Lagoons (ICOLLs). While the entrance to the lagoon can intermittently open to the ocean naturally, during rainfall, very high tides or storm surges, the lagoon entrance is also periodically mechanically opened by Central Coast Council to mitigate the effects of low land flooding on public and private property. On average, natural and mechanical openings of Terrigal Lagoon occur 13 times per year, with the lagoon entrance generally remaining open for a period of 8 days.

To examine the spatial extent of dispersal of contamination from drains and the lagoon into Terrigal bay, seawater samples were also collected from points located along a series of shore-to-sea transects (Figure 1). Nine transect samples were taken along the coast from North Avoca Beach in the south to Forresters Beach in the north, with a transect located directly in front of each of Drains 1, 2 and 4. Two transect sites represented reference locations, including the routine Beachwatch sampling site (50cm depth water immediately in front of the Terrigal Surf Club) and a relatively un-impacted 'Control' site at Forresters Beach. The Control site is on the same stretch of beach as the Terrigal sampling points, but is approximately 9 km north of the Haven and is surrounded by a relatively un-developed area of bushland with little exposure to urban runoff. Samples from this point are anticipated to represent baseline levels of microbial contaminants sourced from non-drain urban infrastructure. The transects incorporated surface seawater samples collected from immediately adjacent to drains at the shoreline (where present) in water of 50cm depth, then at off-shore points in water of 5m depth and 10m depth.

Sampling was conducted before, during and after a significant rainfall event which resulted in a total of 44 mm of rain over the course of 3 days. Samples were collected from all of the locations described above on six occasions. Sampling times corresponded to: (i) May 20, 2019, which had been preceded by 6 mm of rain in the previous two weeks; (ii) May 31, 2019, which had been preceded by only 2 mm of rain during the previous 2 weeks; (iii) June 4, 2019, which had been preceded by 20.4 mm of rain during the previous 3 days, including 12 mm of rain during the preceding 24 hours; (iv) the morning of June 6, 2019, which had been preceded by 40.8 mm of rain in the previous 48 hours, including 3.6 mm that day; (v) the afternoon of June 6, after the mouth of Terrigal Lagoon had been opened to the ocean; and (vi) June 11, 2019, which was 5 days after any rain.

3.2 Sample Processing and Analyses

At each sampling site, triplicate 2 L water samples were collected using 10 L plastic containers, from which triplicate samples were filtered. 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 in liquid nitrogen, prior to being stored at -80 °C for DNA extraction, which was performed within two weeks of collection.

3.3 Microbiological Analysis

Enterococci levels were quantified using Enterolert, a Defined Substrate Technology, used to test aquatic environments for faecal indicator organisms (warm blooded animals e.g. birds, dogs, humans etc). A 10 ml ocean or pipe sample was diluted with 100 ml of sterile deionized water (1:10 dilution) in a sterile polystyrene vessel, powdered Enterolert reagent was added and mixed with the sample. The sample and reagents were then poured into a Quanti-Tray, a sterile panel with 51 wells containing the indicator substrate 4-methylumbelliferone-b-D-glucoside, which 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 were used to relate Enterococci levels to degree of potential human health risk (Table 1).

Figure 1

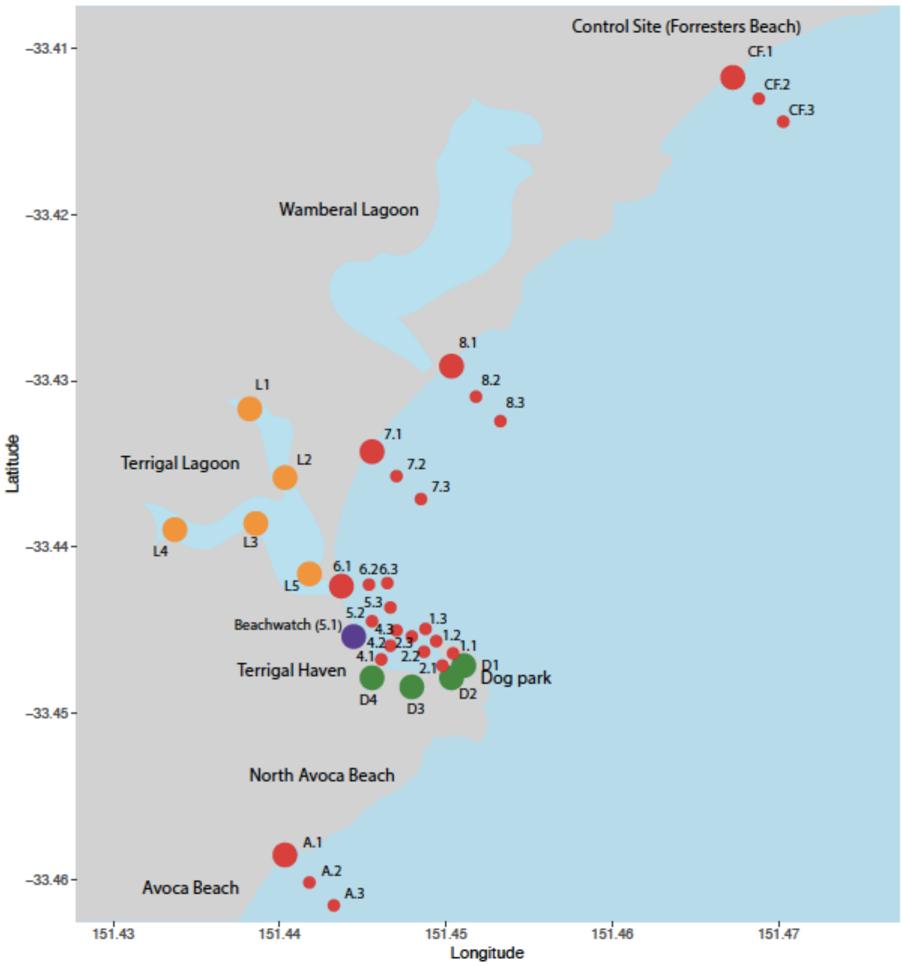

Figure 1. Map of microbial source tracking sampling design for Terrigal Bay, Terrigal Lagoon and control sites. Seawater transect samples were collected from surface seawater from the shoreline (_.1), and from points offshore in water of 5m (_.2) and 10m depth (_.3).

Table 1: Microbial Assessment Categories (NHMRC 2008)[7]

Category	95th 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

3.4 Microbial Source Tracking

Standard recreational water-quality monitoring programs, including Beachwatch, generally use global benchmarks for water quality assessment, which involve enumeration of faecal indicator bacteria (FIB), such as Enterococci. This type of analysis is employed as a proxy measure for sewage pollution in natural aquatic environments and is implemented according to standardised international guidelines[8]. However, FIB approaches cannot precisely discriminate the origin of the target bacteria between human (i.e. sewage) and animal sources, often leading to ambiguity about the true cause of elevated Enterococci counts within an environment[9]. Recently, more sophisticated ‘microbial source tracking’ approaches that can precisely identify specific indicator organisms or microbiological features (e.g. toxin genes) based on DNA signatures have shown great utility in identifying the causes and sources of aquatic pollution[10].

For molecular microbiological analysis, DNA was extracted from filters using a bead beating and chemical lysis kit (DNeasy PowerWater Kit, QIAGEN). Quantitative PCR (qPCR) was then used as the principal analytical technique. This molecular biological approach delivers precise quantification of a specific target DNA sequence that can be selected as a marker for microbial phylogenetic identity or a functional gene. We assembled a set of qPCR primers designed to target several bacterial groups that provide unambiguous discrimination of potential human and animal sources of faecal material (Table 2). All assays were prepared with an epMotion 5075l 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.

Table 2: Quantitative PCR assays used in this study

Target Organism or Gene	qPCR Primers Used	Rationale	Ref
Bacteroides 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.	11
Lachnospiraceae 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.	12
Bacteroides (Dog)	DG3	A dog faeces specific marker targeting *Bacteroides* bacteria dominating the dog faecal microbiome	13
Enterococci (Bird)	GFD	A 100% avian specific bacterial marker, which targets bird-specific Enterococci present in the faeces of gulls, geese, chickens, and ducks.	14
Integron-integrase gene (Intl1)	intl1	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.	15
Arcobacter 23S rRNA	ARCO1	Bacterial genus containing emerging enteric pathogens and species believed to inhabit waste-water infrastructure (i.e. the pipe environment). Indicative of input from stormwater/sewage pipes.	16

3.7 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 Spearmans correlation by permutation.

4.0 Results

SUMMARY OF RESULTS

A detailed description of the results for this project are provided below, but a brief synopsis of the major findings is presented here:

Prior to rainfall, water quality at Terrigal Beach was very good, with little to no influence of sewage contamination or animal faecal material. However, after 20.4 mm of rain there was a substantial increase in human faecal (sewage) markers (HF183 and Lachno3) and other indicators of waste-water infrastructure (*Arcobacter* and *IntI1*), primarily within stormwater drain and adjacent seawater samples, and Terrigal Lagoon samples. These patterns generally reflected measurements of Enterococci levels, with significant correlations observed between each of these markers (HF183, Lachno3, *Arcobacter* and *IntI1*) and Enterococci levels. Among these genetic markers, highest levels were generally observed within, and adjacent to, the outlet of stormwater Drain 4 and within Terrigal Lagoon. Patterns within shore-to-sea transects indicate that these impacts were relatively restricted to near the shore-line, but it should be noted that very rough ocean conditions during the time of this study may have influenced the extent of off-shore dispersal of contamination. However, after the entrance to Terrigal Lagoon was opened, there was substantial evidence for both along-shore and off-shore contamination (HF183, Lachno3, *Arcobacter* and *IntI1*) impacts on the surrounding coastal environment. Increased levels of the marker for dog faeces following rainfall were often associated with stormwater infrastructure, indicating potential flushing of dog faeces from the catchment or its presence within sewage, while elevated levels of the bird Enterococci marker within lagoon samples following rainfall, may suggest flushing of bird faeces into Terrigal Lagoon. Five days after the rainfall event, extremely high levels of the sewage markers (HF183, Lachno3), the markers for waste-water infrastructure (*Arcobacter*, *IntI1*) and the dog *Bacteroides* marker were all observed in Drain 4 and the Lagoon 5 (L5) samples, pinpointing these as points for persistent sewage contamination up to several days following rainfall.

4.1 Enterococci Analysis

Following a period where only 6 mm of rain had occurred in the preceding two weeks (20/5/19), Enterococci levels were very low (mean: 10 CFU 100 mL^{-1}) within all seawater samples collected from Terrigal Beach and Lagoon, and with the exception of one sample from Terrigal Lagoon (TL4: 63 CFU 100 mL^{-1}) were well below the lowest health risk level (Category A [< 40 CFU 100 mL^{-1}]) in the NHMRC Microbial Assessment Categories[7] (Table 1) [NB: during this period Drains 1, 2 and 4 could not be sampled due to insufficient water flow]. This pattern was repeated on 31/5/19, after only a further 2 mm had occurred in the preceding 2 weeks, when Enterococci levels within all samples remained < 30 CFU 100 mL^{-1}, with 94 % of samples at or below the lowest limit of detection (Figure 2).

On 4/6/19, following 20.4 mm of rain in the preceding 3 days, Enterococci levels within Drains 1, 2 and 4 were extremely high, reaching the maximum limit for detection (24,196 CFU 100 mL^{-1}) (Figure 2). Due to very rough ocean conditions, which precluded vessel-based sampling, it was only possible to acquire samples from the nearest shore sampling points in the onshore-offshore spatial transects on this date. Within these near shore water samples, Enterococci levels increased significantly at several points along Terrigal Beach (Figures 2, 3). Highest levels occurred within the near shore samples adjacent to Drains 2 (9,804 CFU 100 mL^{-1}) and 4 (17,329 CFU 100 mL^{-1}), where levels exceeded the NHMRC maximum threshold for significant risk of illness (Category D; Table 1). High Enterococci levels were also observed in nearshore samples associated with transects 5 (1,726 CFU 100 mL^{-1}), 6 (428 CFU 100 mL^{-1}) and CF (1,515 CFU 100 mL^{-1}) at this time.

Two days later, on 6/6/19, after a further 20.4 mm of rain (40.8 mm total in the preceding 48 hours), Enterococci levels within seawater samples generally decreased dramatically. All near shore samples, with the exception of the sample adjacent to Drain 2 (51 CFU 100 mL^{-1}) were at or below the lowest limit of detection (10 CFU 100 mL^{-1}). Within one of the two drains that could be sampled (Drain 2), Enterococci levels occurred in high levels (626 CFU 100 mL^{-1}), but were substantially lower than those observed on 4/6/19. In contrast to the Terrigal Beach seawater samples, Enterococci levels within Terrigal Lagoon were very high on 6/6/19, with levels exceeding the NHMRC maximum threshold for significant risk of illness (Category D; Table 1) in all samples and exceeding 10,000 CFU 100 mL^{-1} in one case (L2).

On the afternoon of 6/6/19, the entrance to Terrigal Lagoon was mechanically opened to the ocean, so an additional set of samples was collected. Following the lagoon entrance being opened, Enterococci levels remained low (10-20 CFU 100 mL^{-1}) within most Terrigal Bay seawater samples, with the exception of samples from transect 6, located immediately adjacent to the mouth of the lagoon. Within these samples, Enterococci levels increased from 10 CFU 100 mL^{-1} in the morning samples, to 262 CFU 100 mL^{-1} (NHMRC risk category C) in the nearshore sample and 41 CFU 100 mL^{-1} in the sample collected in 5 m depth seawater, approximately 100 m from shore (Figure 3).

Five days after the rainfall event (11/6/19), Enterococci levels within all Terrigal Beach seawater samples had decreased to the lower limit of detection (10 CFU 100 mL^{-1}). Within Terrigal Lagoon, Enterococci levels remained slightly elevated within some samples reaching 120 CFU 100 mL^{-1} in L1 and 41 CFU 100 mL^{-1} in L2 and 5.

Figure 2

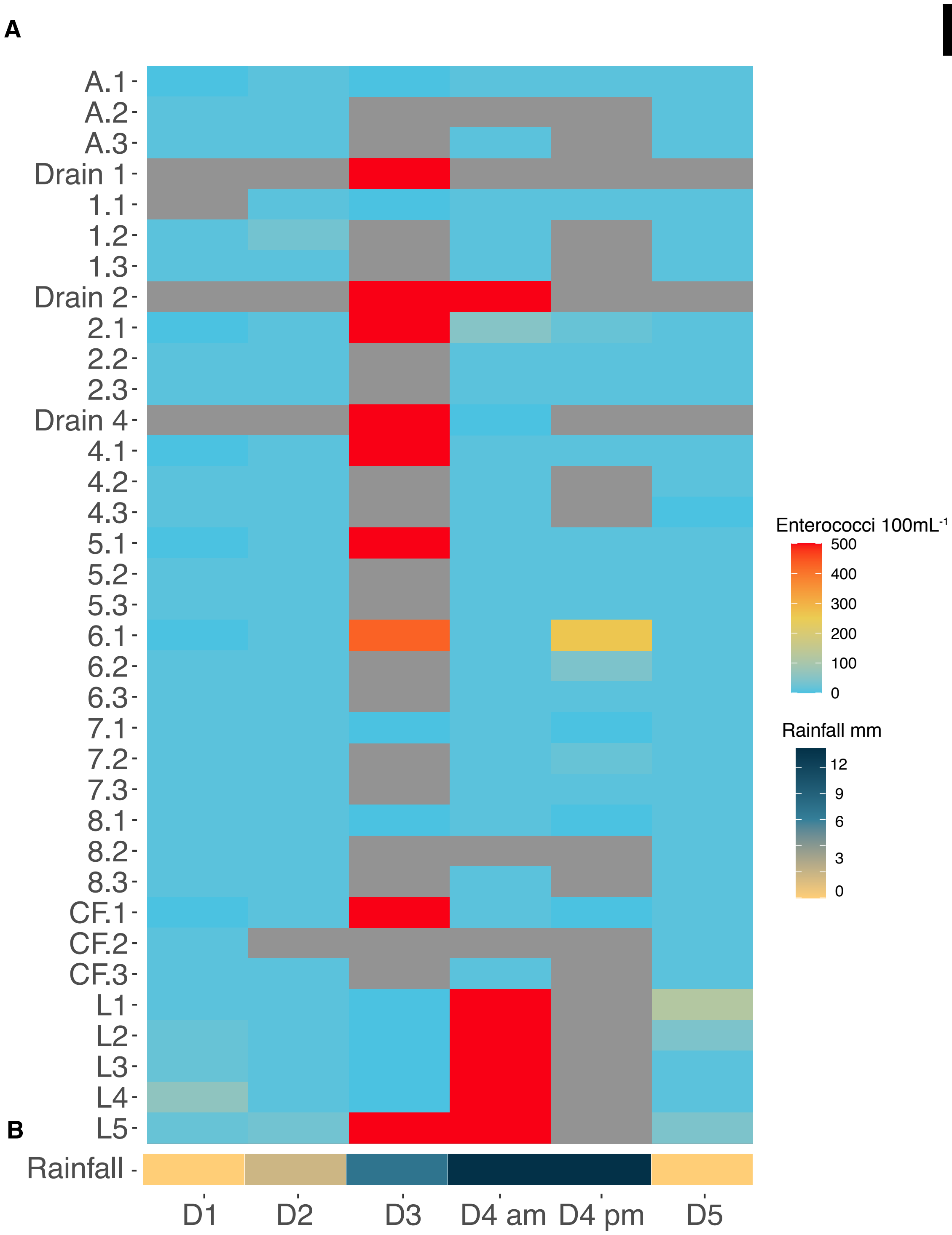

Figure 2: (A) Heatmap displaying the distribution of Enterococci levels determined using standard membrane filtration techniques (AS/NZS 4276.9:2007) across sampling locations (Y axis) and days (X axis). The colour scale corresponds to Enterococci count data. Grey cells represent samples not collected either due to lack of water flow in drains or rough ocean conditions and poor safety levels during the rainfall event. (B) Heatmap displaying intensity of rainfall on each sampling day.

Figure 3

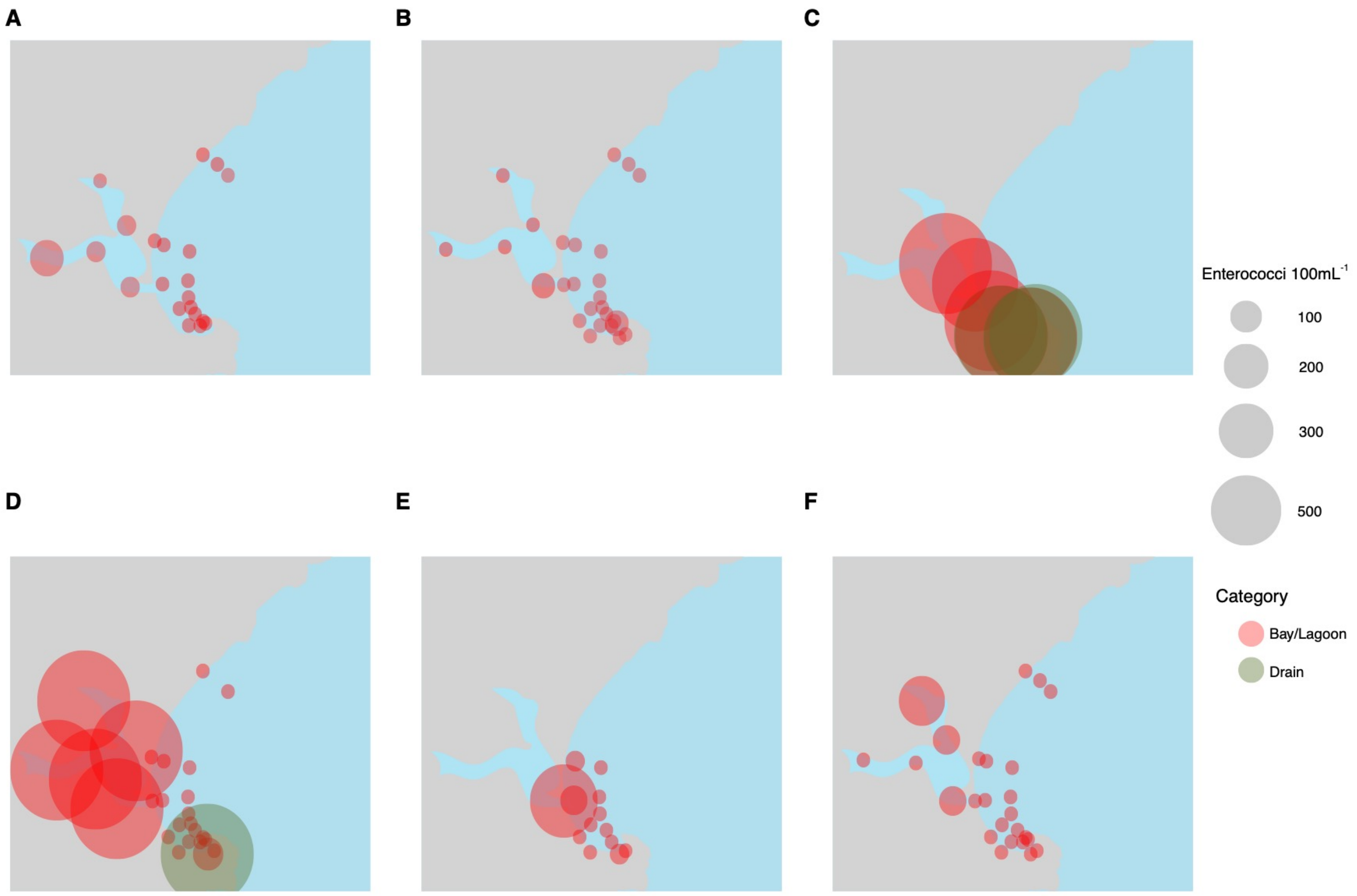

Figure 3: Distribution of Enterococci counts determined using standard membrane filtration techniques (AS/NZS 4276.9:2007) across sampling locations within Terrigal Beach on **(A)** 20/5/19 – which had been preceded by 6 mm of rain in the previous 2 weeks; **(B)** 31/5/19 – which had been preceded by 2 mm of rain in the previous 2 weeks; **(C)** 4/6/19 - which had been preceded by 20.4 mm of rain in the previous 3 days; **(D)** Morning of 6/6/19 - which had been preceded by 40.8 mm of rain in the previous 48 hours; **(E)** Afternoon of 6/6/19 – after the mouth of Terrigal Lagoon had been opened; **(F)** 11/6/19 – after 5 days without rain. Bubble size scales to data magnitude bins (refer to side scale). Red bubbles correspond to seawater and lagoon samples, while green bubbles correspond to samples collected in Drains.

4.3 Animal Faecal Markers

4.3.1 Dog Bacteroides

Across the entire dataset, the DG3 marker for dog-faeces associated *Bacteroides* was detectable in only 18% of samples. Detection of the DG3 marker here is, however, in contrast to the previous microbial source tracking study conducted at Terrigal Beach and Haven, where this dog faeces marker was not detected in any samples[7]. Following a period of relatively dry weather conditions on 20/5/19, the DG3 marker was detected in low to moderate levels, but only within 16% of samples, with all detections in nearshore samples, while on the 31/5/19 this dog faeces marker was not detected in any samples (Figures 4, 5). However, a significant correlation ($r = 0.824$; $p < 0.001$) was observed between the DG3 marker and Enterococci levels across the entire data-set.

On 4/6/19, following 20.4 mm of rain in the preceding 3 days, the DG3 marker was detected in significantly ($p < 0.001$) higher concentrations within all lagoon samples, in Drains 1 and 4, and the adjacent seawater samples (Figures 4, 5). Highest concentrations were observed in Drain 1. Similar patterns were observed on 6/6/19, with significant levels of the DG3 marker observed in all Terrigal Lagoon samples, but in only two nearshore seawater samples located immediately adjacent to stormwater drain outlets. Following the opening of the entrance to Terrigal Lagoon on the afternoon of 6/6/19, the DG3 marker was observed only in the nearshore sample of Transect 6, immediately adjacent to the mouth of Terrigal Lagoon. On 11/6/19, five days since any rain, the DG3 marker was detected in very high levels within Drain 4 and one lagoon sample (L5), with moderate levels observed within the nearshore seawater sample adjacent to Drain 4 and lagoon sample L3 (Figures 4, 5). It was below detection limit in all other samples.

Figure 4

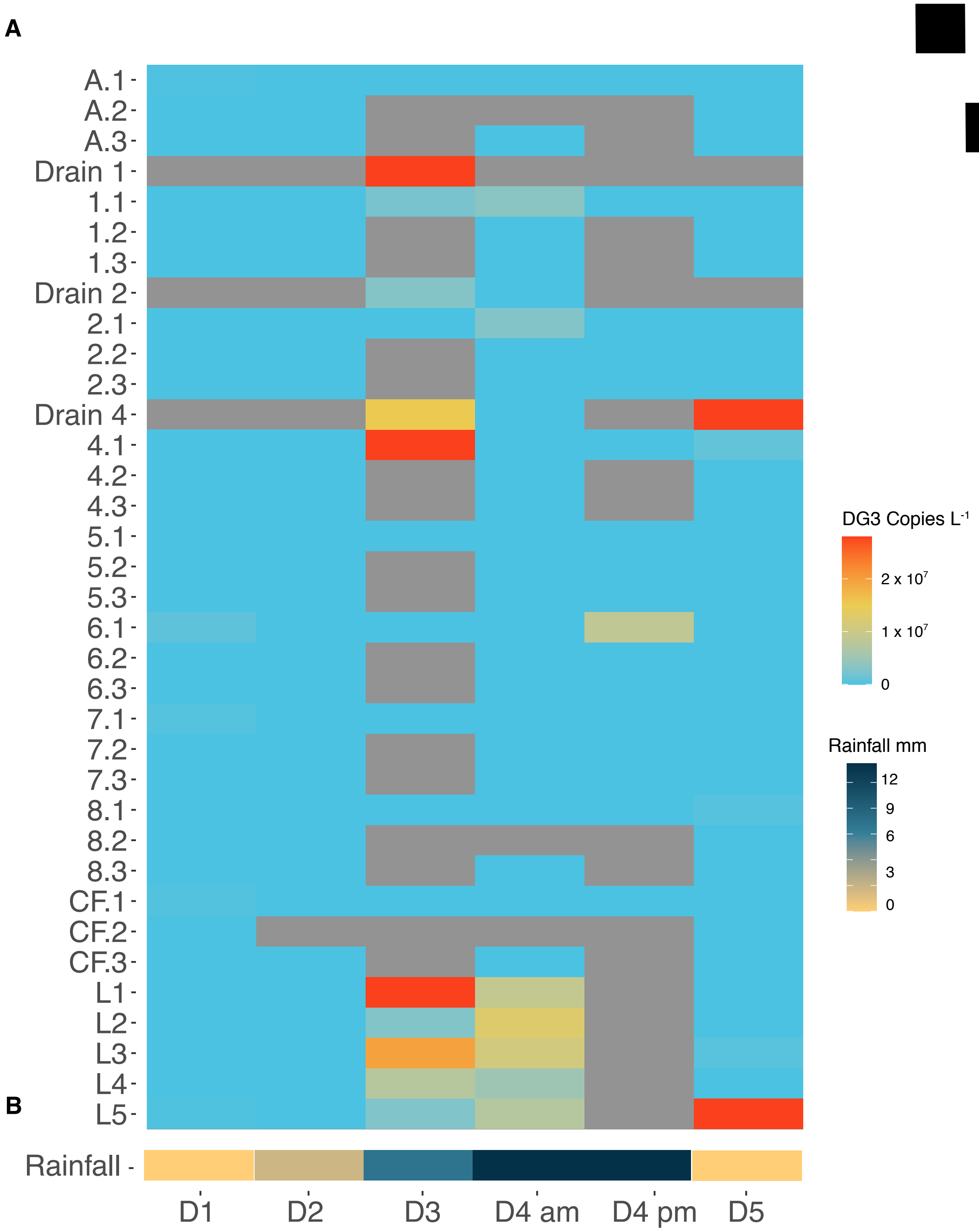

Figure 4: (A) Heatmap displaying distribution of DG3 marker for canine *Bacteroides* (dog ! faeces) across sampling locations (Y axis) and days (X axis). Colour scale corresponds to ! copy numbers L^{-1} defined using qPCR. Grey cells represent samples not collected either ! due to lack of water flow in drains or rough ocean conditions and low safety levels during the ! rainfall event. **(B)** Heatmap displaying intensity of rainfall on each sampling day. !

Figure 5

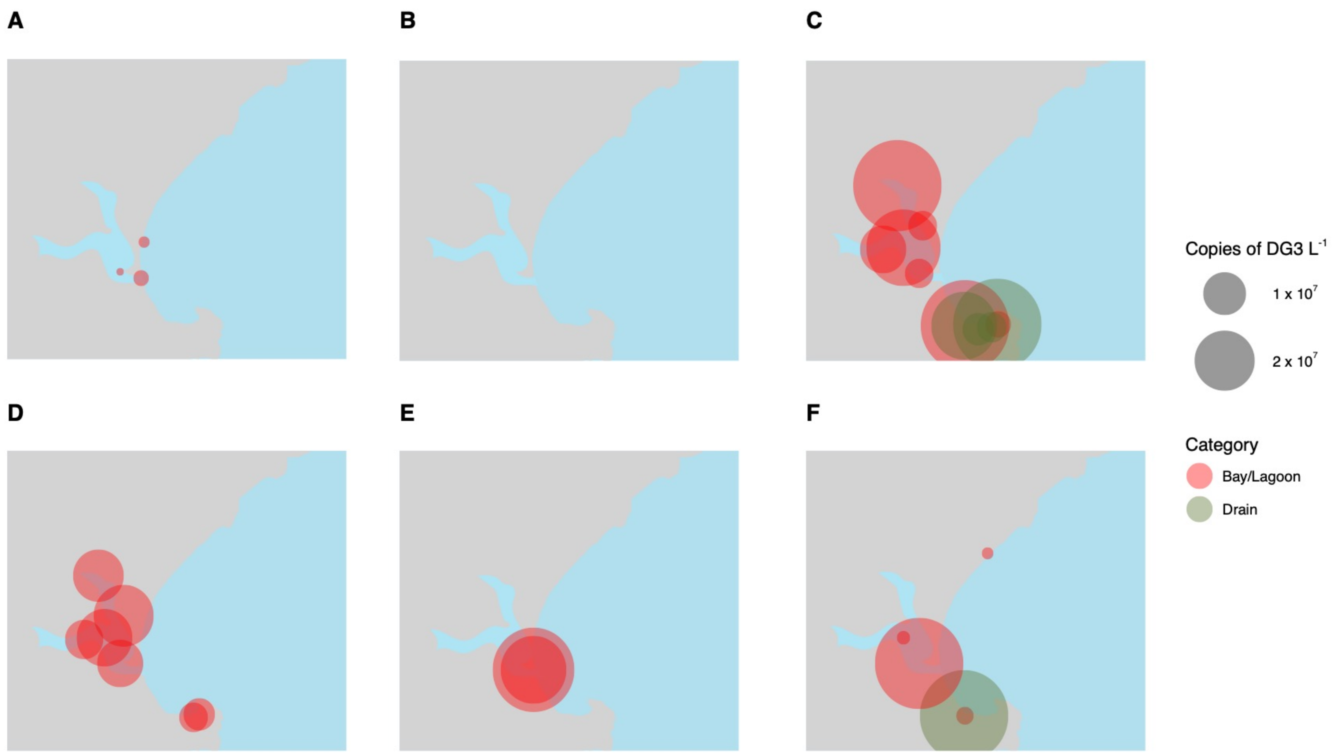

Figure 5: Distribution of DG3 marker for canine *Bacteroides* (dog faeces) across sampling locations on Terrigal Beach on **(A)** 20/5/19 – which had been preceded by 6 mm of rain in the previous 2 weeks; **(B)** 31/5/19 – which had been preceded by 2 mm of rain in the previous 2 weeks; **(C)** 4/6/19 - which had been preceded by 20.4 mm of rain in the previous 3 days; **(D)** Morning of 6/6/19 - which had been preceded by 40.8 mm of rain in the previous 48 hours; **(E)** Afternoon of 6/6/19 – after the mouth of Terrigal Lagoon had been opened; **(F)** 11/6/19 – after 5 days without rain. Bubble size scales to data magnitude bins (refer to side scale). Red bubbles correspond to seawater and lagoon samples, while green bubbles correspond to samples collected in Drains.

4.3.2 Bird Enterococci

The GFD Avian Enterococci marker was observed in 82% of samples, but levels of this marker were not significantly elevated in Terrigal bay seawater samples relative to the 'pristine' control site at Forrester's Beach, during either dry ($p > 0.3$) or wet ($p > 0.08$) conditions. Concentrations of this marker did, however, display a weak, but statistically significant correlation to Enterococci levels ($r = 0.31$, $p < 0.01$). Despite this, there was no clear trend of increasing levels of the GFD marker in Terrigal seawater samples following rainfall, with concentrations of this marker in fact decreasing following rainfall in many samples (Figures 6, 7). However, within Terrigal Lagoon, rainfall led to both a marked increase in the proportion of samples that the GFD marker was detected in and a significant ($p < 0.001$) increase in concentrations of this marker. Indeed, on both 4/6/19 and 6/6/19, highest levels of the GFD marker were observed in the Terrigal Lagoon samples. Five days after rainfall, concentrations of the GFD marker in most samples were comparable to those observed during the rainfall event, with the one notable exception of the Terrigal Lagoon L1 sample, where concentrations of this bird Enterococci marker were 3 times higher than the highest concentrations observed during the rainfall event, and over 70 times higher than the average GFD levels observed across the study. Notably, these very high bird Enterococci levels coincided with elevated (120 CFU 100 mL^{-1}) total Enterococci counts observed in this sample. It is important to note that simultaneous increases in GFD and Enterococci do not imply that one caused the other, both may have been caused by rainfall independently.

Figure 6

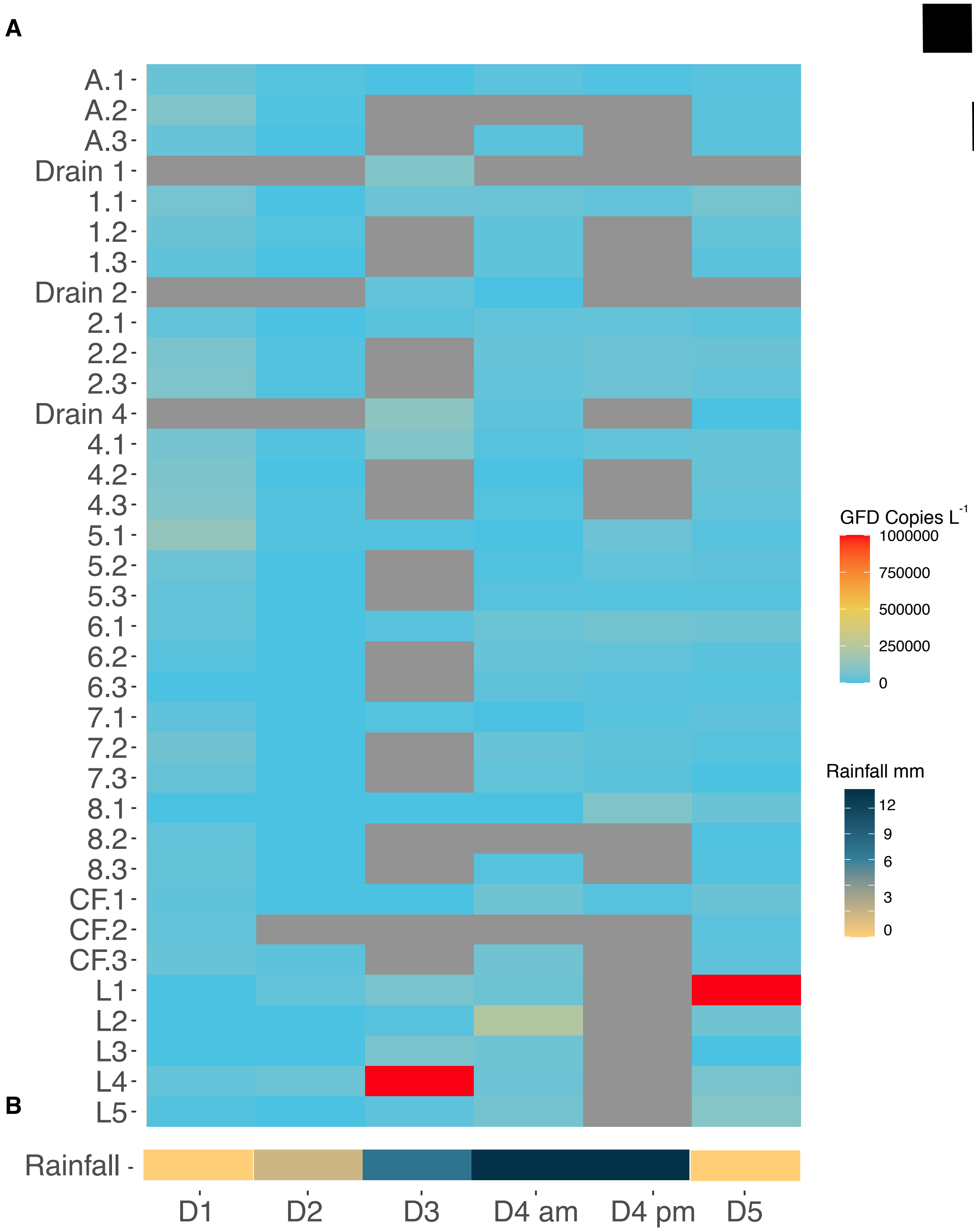

Figure 6: (A) Heatmap displaying distribution of GFD marker for the avian *Enterococci* (bird faeces) across sampling locations (Y axis) and days (X axis). Colour scale corresponds to copy numbers L^{-1} defined using qPCR. Grey cells represent samples not collected either due to lack of water flow in drains or rough ocean conditions and low safety levels during the rainfall event. **(B)** Heatmap displaying intensity of rainfall on each sampling day.

Figure 7

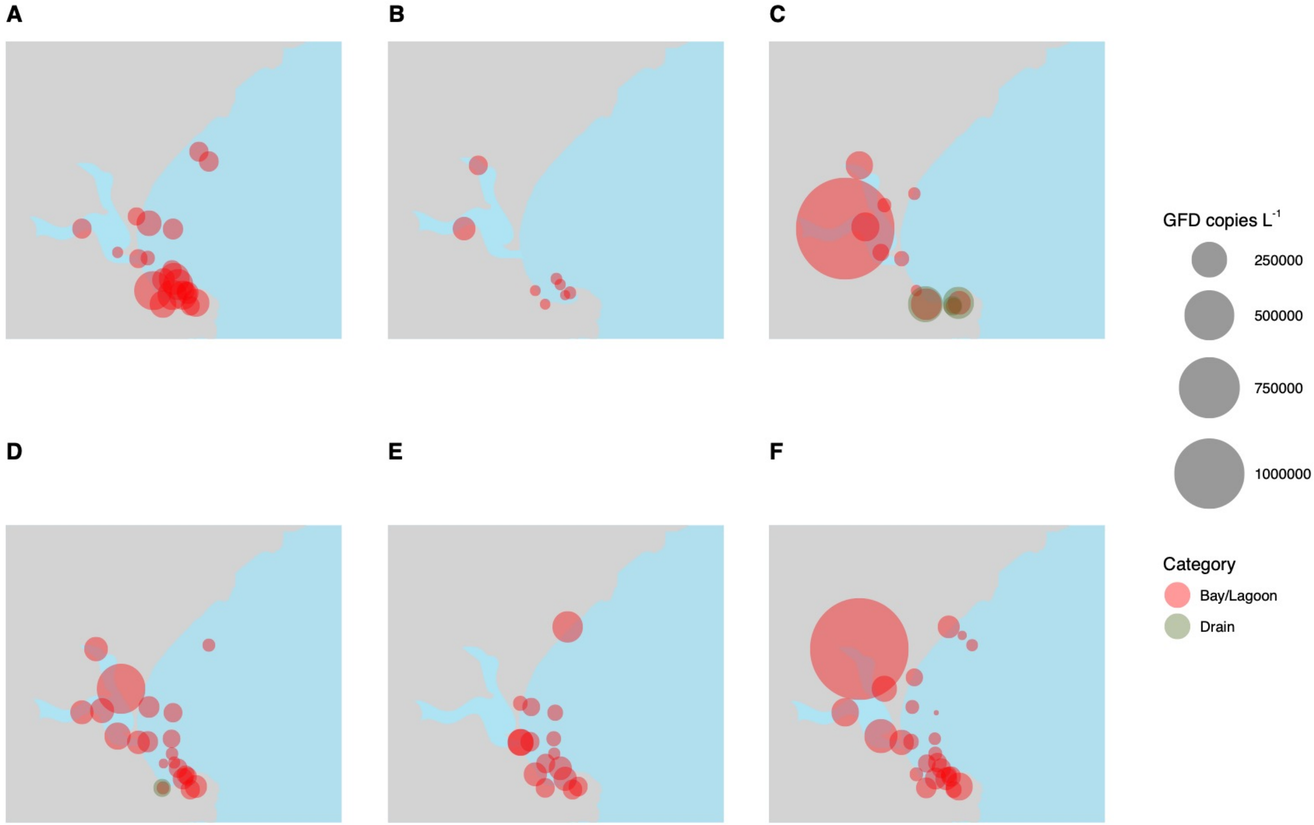

Figure 7: Distribution of GFD marker for the avian *Enterococci* (bird faeces) across sampling locations within Terrigal Beach on: **(A)** 20/5/19 – which had been preceded by 6 mm of rain in the previous 2 weeks; **(B)** 31/5/19 – which had been preceded by 2 mm of rain in the previous 2 weeks; **(C)** 4/6/19 - which had been preceded by 20.4 mm of rain in the previous 3 days; **(D)** Morning of 6/6/19 - which had been preceded by 40.8 mm of rain in the previous 48 hours; **(E)** Afternoon of 6/6/19 – after the mouth of Terrigal Lagoon had been opened; **(F)** 11/6/19 – after 5 days without rain. Bubble size scales to data magnitude bins (refer to side scale). Red bubbles correspond to seawater and lagoon samples, while green bubbles correspond to samples collected in Drains.

4.4 Human Faecal Markers

The two human faecal marker genes employed here, Lachno3 and HF183 (which are indicative of human gut microbiome associated *Lachnospiraceae* and *Bacteriodes* bacteria and sewage contamination[11,12]) were detected in 49 and 50% of samples respectively. Both markers displayed statistically significant correlations to total Enterococci counts (Lachno3: r = 0.67, p =0.001; HF183: r =,0.69, p =0.001).

During relatively dry weather conditions on 20/5/19 and 31/5/19 (6mm and 2mm of rain in the preceding 2 weeks, respectively), concentrations of Lachno3 and HF183 were detected within only 6 and 8% of samples respectively, which is consistent with the low Enterococci levels observed during this period and indicative of a low to negligible influence of sewage contamination. However, following 20.4 mm of rain on 4/6/19, both human faecal markers were recorded in high concentrations in Drains 1, 2 and 4 and near-shore seawater samples immediately adjacent to these stormwater drains (Figures 8, 9). Concentrations of both markers also increased considerably within all Terrigal Lagoon samples, with highest levels observed in the L4 sample. The maximum concentrations of both markers for human faeces occurred in Drain 4, where concentrations were between 2 to 54 times higher than in Drains 1 and 2. This pattern is consistent with observations in a previous microbial source tracking study conducted at Terrigal Beach, which also revealed that Drain 4 is the most significant source of sewage contamination at this site[6]. Also in accordance with this pattern, the highest levels of the human faecal markers present in Terrigal Beach seawater samples occurred in the sample immediately adjacent to Drain 4 (Figures 8, 9, 10, 11). It is notable that in other nearshore seawater samples that were not located immediately adjacent to stormwater drains, concentrations of both sewage markers generally remained low or often undetectable. This was particularly true for transects outside of the Terrigal Haven region (i.e. at North Avoca [Transect A.1-A.3], Wamberal Beach [Transect 8] and Forresters beach [Transect CF1]). Unfortunately, due to rough ocean conditions, it was not possible to acquire offshore transect samples on this date, precluding an assessment of the seaward spatial extent of contamination from stormwater drains.

On 6/6/19, after 40.8 mm of rain in the preceding 48 hours, mean levels of both human faecal markers within Drains 2 and 4 and adjacent seawater samples (T4.1) decreased significantly ($p < 0.001$), directly mirroring the patterns seen in Enterococci levels. However, in contrast to the Enterococci measurements, near shore concentrations of both human faecal markers remained slightly elevated at other points along Terrigal Beach, in particular adjacent to Drains 1 and 2. Although, significant concentrations of these markers did not extend beyond the shore-line samples in any of the off-shore transects (Figures 10, 11).

Mean levels of both human faecal markers ($p < 0.001$) were significantly higher within Terrigal Lagoon than in Terrigal Beach seawater samples on both 4/6/19 and the morning 6/6/19, with these markers occurring in significant levels within all lagoon samples, indicative of sewage contamination within Terrigal Lagoon. Following the opening of the lagoon entrance on the afternoon of 6/6/19, a significant ($p < 0.01$) increase in the concentrations of both human faecal markers was

Figure 8

A

B

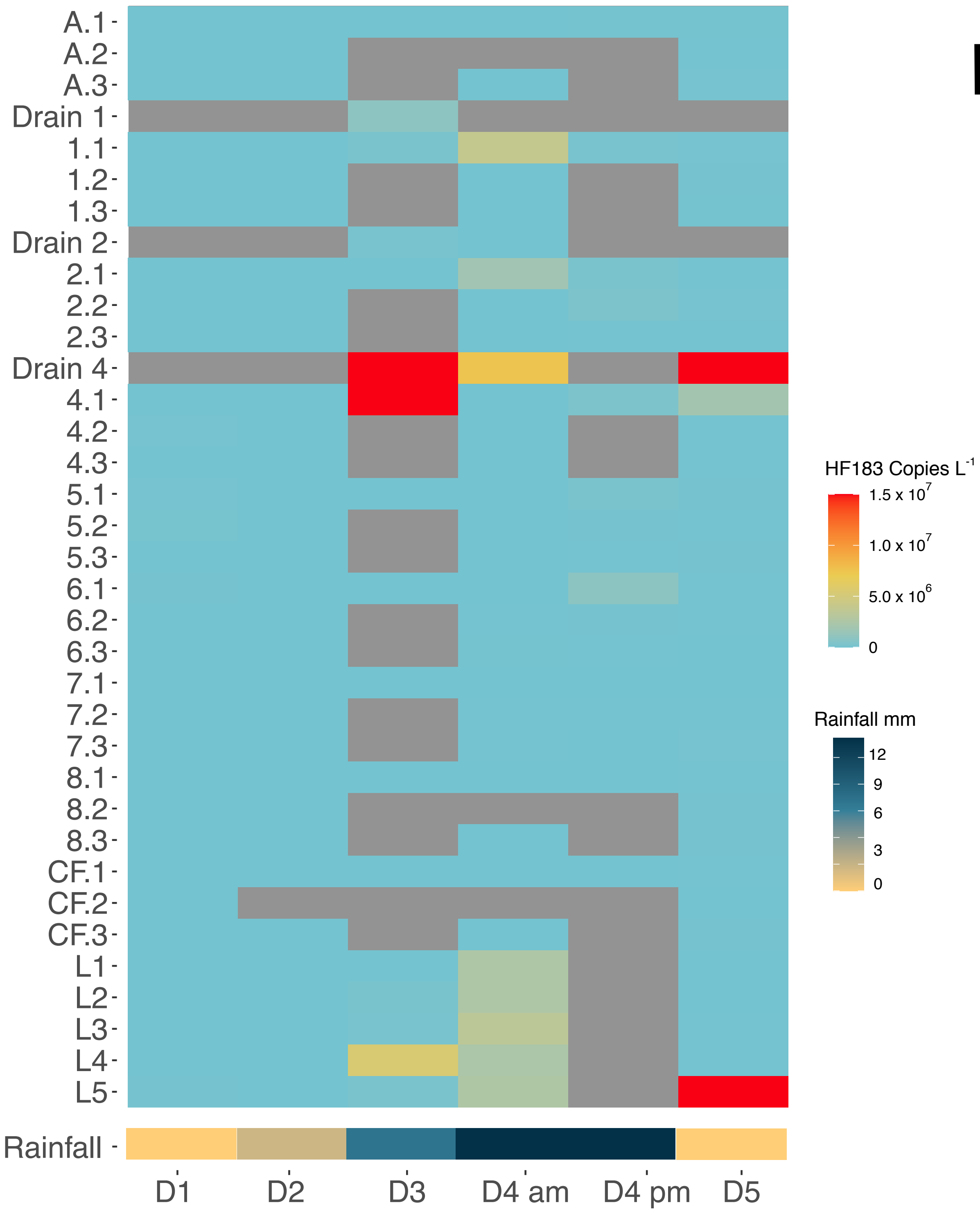

Figure 8: (A) Heatmap displaying distribution of HF183 marker for the human faecal bacteria *Bacteroides* (sewage marker) across sampling locations (Y axis) and days (X axis). Colour scale corresponds to copy numbers L^{-1} defined using qPCR. Grey cells represent samples not collected either due to lack of water flow in drains or rough ocean conditions and low safety levels during the rainfall event. **(B)** Heatmap displaying intensity of rainfall on each sampling day.

Figure 9

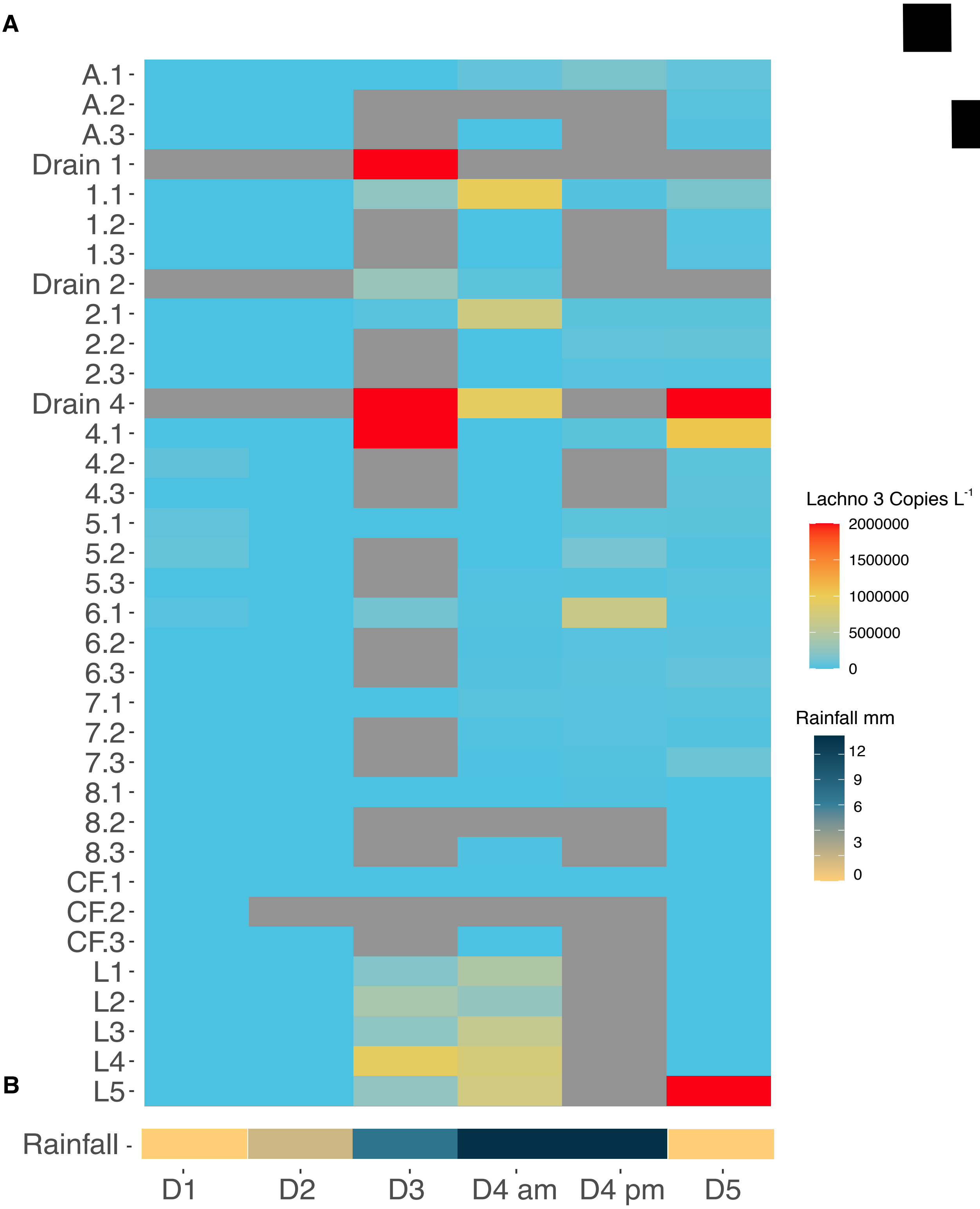

Figure 9: (A) Heatmap displaying distribution of Lachno3 marker for the human faecal *Lachnospiraceae* bacteria (sewage marker) across sampling locations (Y axis) and days (X axis). Colour scale corresponds to copy numbers L^{-1} defined using qPCR. Grey cells represent samples not collected either due to lack of water flow in drains or rough ocean conditions and low safety levels during the rainfall event. **(B)** Heatmap displaying intensity of rainfall on each sampling day.

observed in samples within Transect 6, located immediately adjacent to the mouth of the lagoon.

In the nearest-shore sample of transect 6, concentrations of the Lachno3 and HF183 markers increased by 41- and 109-times respectively following opening of the lagoon entrance. Notably, following the opening of the lagoon entrance, increased levels of the human faecal markers were also observed in Transect 5 and 6 samples located in 5 and 10m depth water, situated approximately 100 m and 300 m from the shore-line, indicating that opening Terrigal Lagoon to the ocean resulted in a measurable sewage signature that extended for a few hundred metres to the east and south of the entrance point.

Following 5 days without rain, on 11/6/19 concentrations of the human faecal markers decreased significantly from those observed during the peak rainfall events, but remained significantly ($p < 0.001$) elevated relative to the pre-wet weather event samples (i.e. 20/5/19 and 31/5/19). Most notably, very high levels of both markers occurred within Drain 4, where concentrations were higher than those observed during the actual rain-fall event. Concomitantly high levels of both markers were observed in the adjacent seawater sample (T4.1), indicating a localised impact of this drain on the surrounding environment. This pattern is consistent with results from a previous microbial source-tracking study performed at this site[6], which indicated that Drain 4 can be a significant source of sewage contamination to Terrigal Beach. Within Terrigal Lagoon, very high concentrations of both markers were also observed within the L5 sample, where concentrations at this time also significantly ($p < 0.01$) exceeded those observed in this sample during the rainfall event, yet the source of this contamination is as yet undetermined.

Figure 10

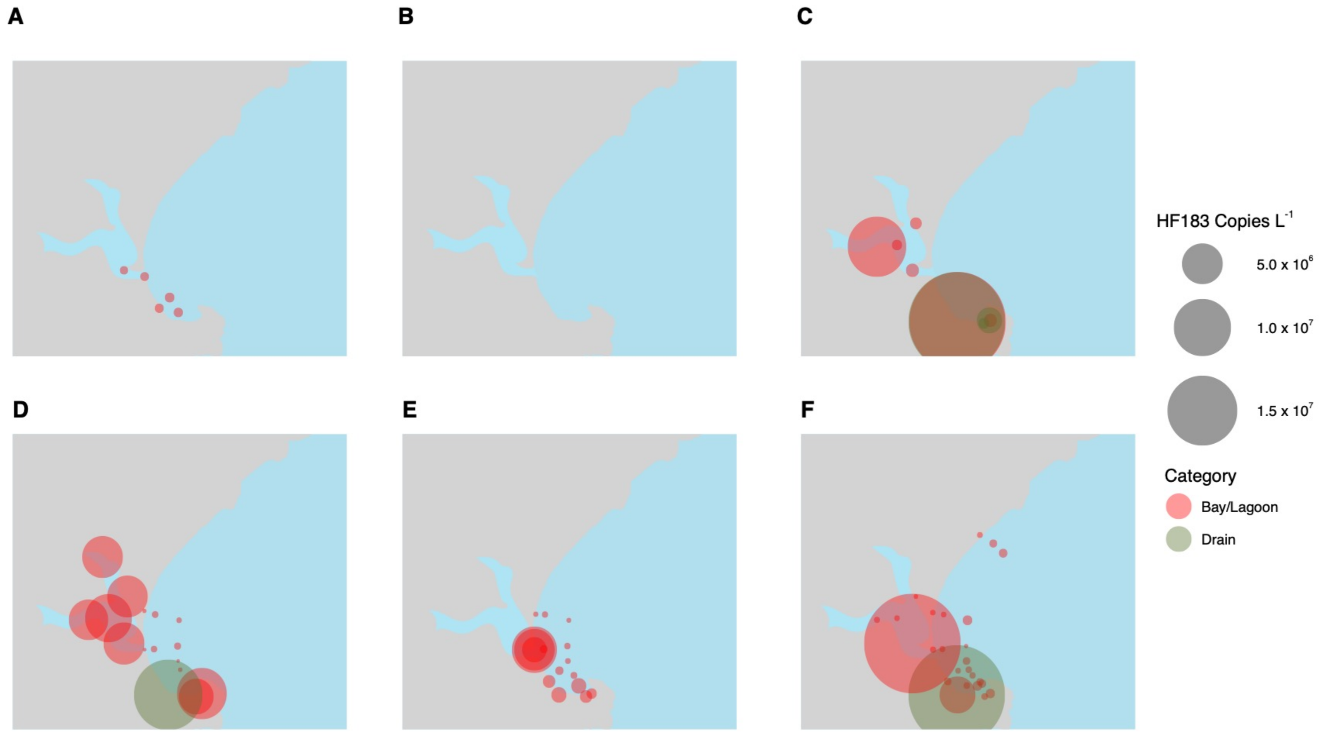

Figure 10: Distribution of HF183 marker for the human faecal bacteria *Bacteroides* (sewage marker) across sampling locations within Terrigal Beach on **(A)** 20/5/19 – which had been preceded by 6 mm of rain in the previous 2 weeks; **(B)** 31/5/19 – which had been preceded by only 2 mm of rain in the previous 2 weeks; **(C)** 4/6/19 - which had been preceded by only 20.4 mm of rain in the previous 3 days; **(D)** Morning of 6/6/19 - which had been preceded by only 40.8 mm of rain in the previous 48 hours; **(E)** Afternoon of 6/6/19 – after the mouth of Terrigal Lagoon had been opened; **(F)** 11/6/19 – after 5 days without rain. Bubble size scales to data magnitude bins (refer to side scale). Red bubbles correspond to seawater and lagoon samples, while green bubbles correspond to samples collected in Drains.

Figure 11

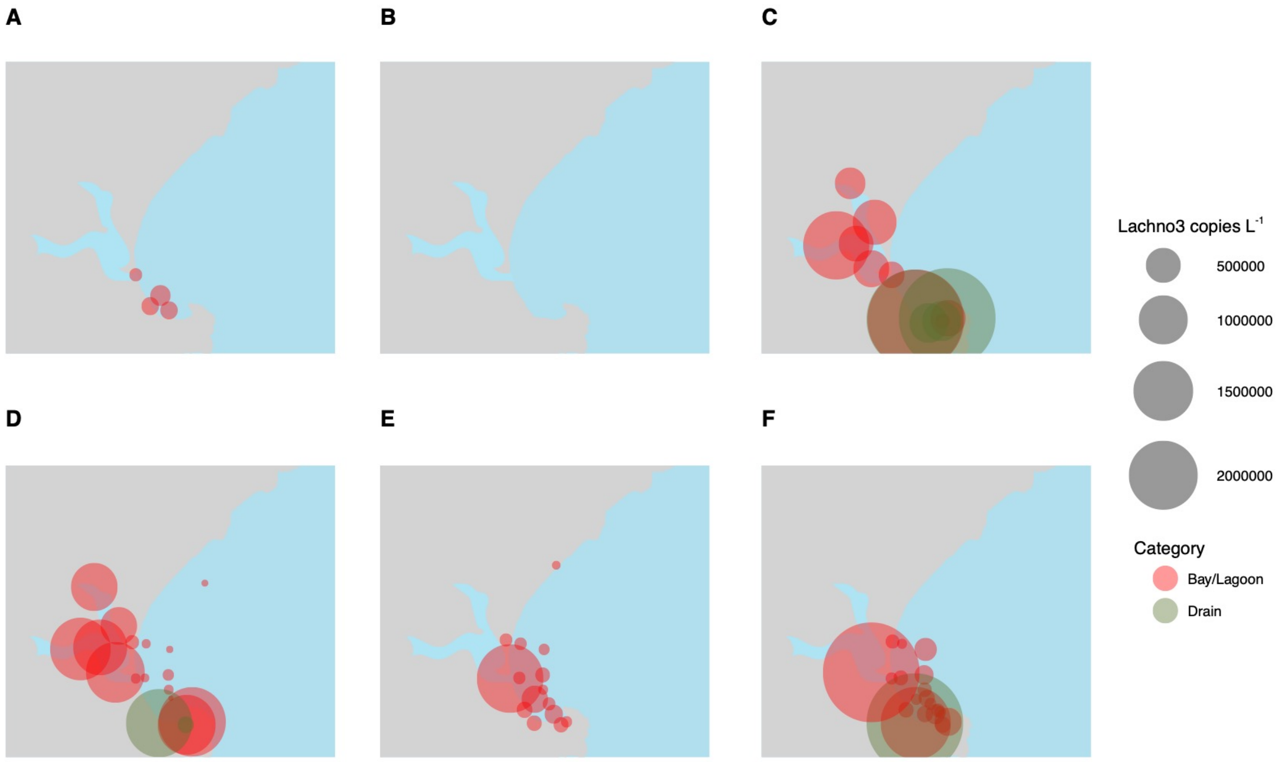

Figure 11: Distribution of Lachno3 marker for the human faecal bacteria *Lachnospiraceae* (sewage marker) across sampling locations within Terrigal Beach on **(A)** 20/5/19 – which had been preceded by 6 mm of rain in the previous 2 weeks; **(B)** 31/5/19 – which had been preceded by only 2 mm of rain in the previous 2 weeks; **(C)** 4/6/19 - which had been preceded by only 20.4 mm of rain in the previous 3 days; **(D)** Morning of 6/6/19 - which had been preceded by only 40.8 mm of rain in the previous 48 hours; **(E)** Afternoon of 6/6/19 – after the mouth of Terrigal Lagoon had been opened; **(F)** 11/6/19 – after 5 days without rain. Bubble size scales to data magnitude bins (refer to side scale). Red bubbles correspond to seawater and lagoon samples, while green bubbles correspond to samples collected in Drains.

4.5 Other Indicators of Anthropogenic Impact

4.5.1 Arcobacter

Arcobacter is a genus of bacteria that includes species associated with human sewage and urban storm-water and sewage pipe infrastructure[17], with their occurrence in coastal waters suggestive of inputs from human wastestreams[18]. *Arcobacter* are therefore indicative of water that has moved through pipes, including both sewage and uncontaminated stormwater. Following relatively dry weather conditions on 20/5/19 and 31/5/19, *Arcobacter* were detected in 95% of samples, but concentrations were not significantly higher ($p > 0.05$) within either Terrigal Beach or Lagoon samples than the 'pristine' control site at Forrester's Beach. Across the data-set, *Arcobacter* concentrations displayed a statistically significant correlation ($r = 0.57$; $p < 0.001$) with *Enterococci* levels.

Following 20.4 mm of rain on 4/6/19, *Arcobacter* levels increased significantly ($p < 0.001$) across all samples, with highest levels observed within drain and lagoon samples (Figure 12). Within nearshore seawater samples, *Arcobacter* levels increased by 16- to over 1000-fold during this rainfall event. During this time, highest levels of Arcobacter were observed in Drain 4, where concentrations were over 14 times higher than the average observed across all other samples.

On the morning of 6/6/19, after 40.8 mm of rain, *Arcobacter* levels remained elevated within drain and adjacent seawater samples, but also became dramatically higher within Terrigal Lagoon, where concentrations increased significantly ($p < 0.001$) by up to 78-fold from the preceding time-point. At this time, highest *Arcobacter* levels were observed in the L2 lagoon sample. After the entrance to Terrigal Lagoon was opened on the afternoon of 6/6/19, *Arcobacter* levels within Terrigal seawater samples associated with transects adjacent to the mouth of Terrigal Lagoon (i.e. transects 4, 5 and 6) increased significantly ($p < 0.001$) relative to conditions prior to the lagoon entrance being opened (Figures 12, 13). At this time, the nearshore sample in transect 6 (the transect immediately adjacent to the mouth of Terrigal Lagoon) increased by 210-fold, and even the off-shore samples of Transect 5 increased by up to 13.6-fold. Five days after any rainfall (11/6/19), Arcobacter levels remained significantly ($p < 0.001$) elevated in all samples relative to conditions prior to the rainfall event, with highest levels observed with Drain 4 and Terrigal Lagoon samples.

Figure 12

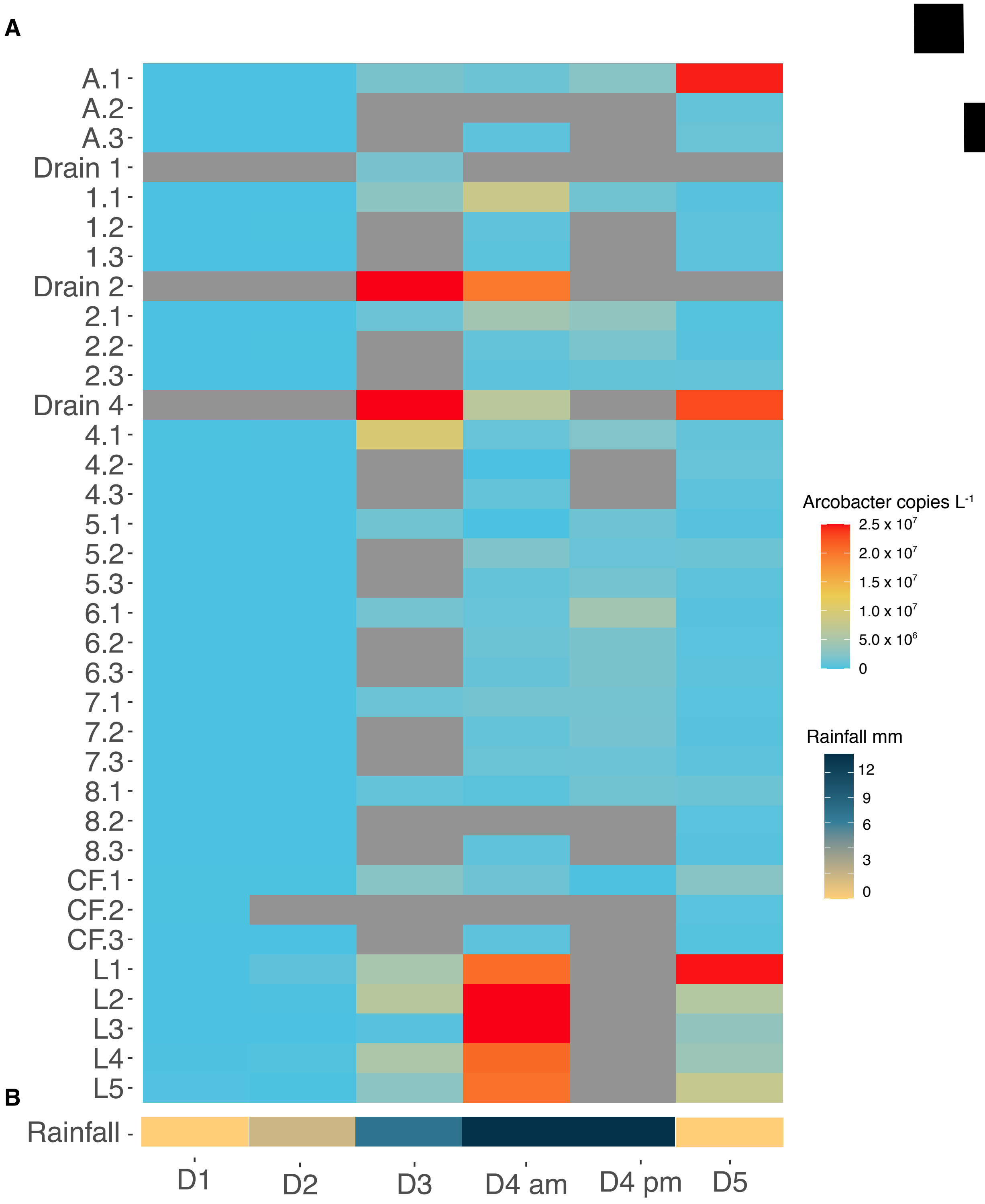

Figure 12: **(A)** Heatmap displaying distribution of *Arcobacter* bacteria across sampling ! locations (Y axis) and days (X axis). Colour scale corresponds to copy numbers L^{-1} defined using qPCR. Grey cells represent samples not collected either due to lack of water flow in ! drains or rough ocean conditions and low safety levels during the rainfall event. **(B)** Heatmap ! displaying intensity of rainfall on each sampling day. !

Figure 13

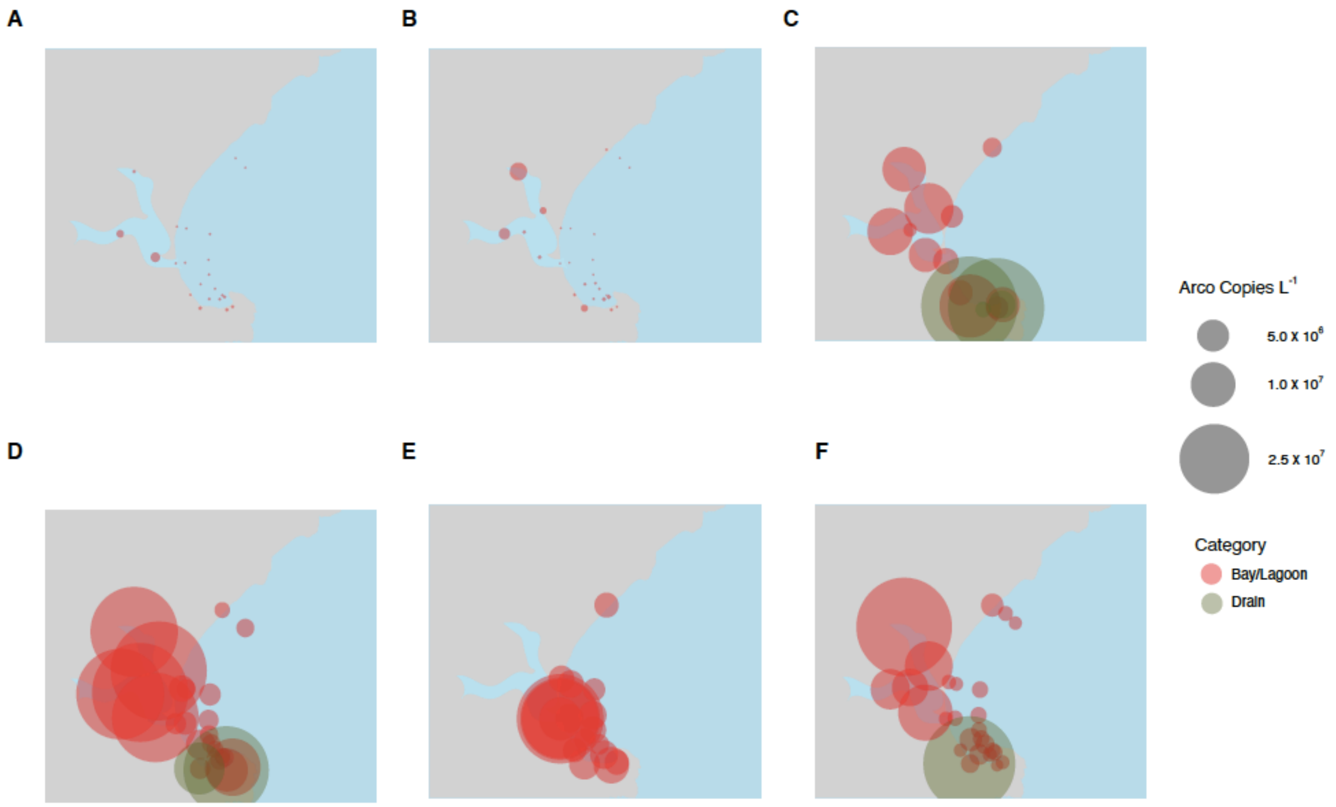

Figure 13: Distribution of Arcobacter bacteria determined using qPCR across sampling locations within Terrigal Beach on **(A)** 20/5/19 – which had been preceded by 6 mm of rain in the previous 2 weeks; **(B)** 31/5/19 – which had been preceded by only 2 mm of rain in the previous 2 weeks; **(C)** 4/6/19 - which had been preceded by only 20.4 mm of rain in the previous 3 days; **(D)** Morning of 6/6/19 - which had been preceded by only 40.8 mm of rain in the previous 48 hours; **(E)** Afternoon of 6/6/19 – after the mouth of Terrigal Lagoon had been opened; **(F)** 11/6/19 – after 5 days without rain. Bubble size scales to data magnitude bins (refer to side scale). Red bubbles correspond to seawater and lagoon samples, while green bubbles correspond to samples collected in Drains.

4.5.2 Class 1 Integron-integrase gene

The Class 1 Integron-integrase gene (*IntI1*) has been identified as an excellent microbial measure of anthropogenic contamination in aquatic habitats[19]. During relatively dry weather conditions on 20/5/19 and 31/5/19, the *IntI1* gene was detected in 98% of samples, but at very low levels. Notably, *IntI1* gene levels were not significantly ($P > 0.05$) higher within either Terrigal Beach or Lagoon samples than the 'pristine' control site at Forrester's Beach, indicating a low level of anthropogenic impact within Terrigal Beach water samples under dry weather conditions, which is in accordance with both the Enterococci levels and sewage marker (i.e. HF183 and Lachno3) results described above. Indeed, *IntI1* gene levels displayed a statistically significant correlation with Enterococci counts ($r = 0.63$; $p < 0.001$).

During the rainfall event on 4/6/19, average levels of the *IntI1* gene increased significantly ($p < 0.001$) by 159 times. During this time, highest levels of the *IntI1* gene were recorded in the Drain and Terrigal Lagoon samples, with highest concentrations observed in Drain 4 and the immediately adjacent seawater sample. During this rainfall event, levels of the *IntI1* gene also increased significantly ($p < 0.05$) within Terrigal Lagoon samples, with the most substantial increase (223-fold) occurring in the L5 sample (Figures 14, 15).

On the morning of 6/6/19, after 40.8 mm of rainfall over 48 hours, highest levels of the *IntI1* gene occurred in Terrigal Lagoon and in the seawater sample immediately adjacent to Drain 1. Following opening of the entrance of Terrigal Lagoon on the afternoon of 6/6/19, very high levels of the *IntI1* gene were observed in transects adjacent to the mouth of Terrigal Lagoon (i.e. transects 6 and 7), where levels of this marker of anthropogenic input increased significantly ($p < 0.001$) relative to conditions prior to the lagoon entrance being opened. Notably, these increases were experienced in Transect 5, 6 and 7 samples located in 5 and 10m depth water, situated approximately 100 m and 300 m from the shore-line, further indicating that the flushing Terrigal Lagoon into the ocean has a spatially extensive impact on the surrounding coastal environment. Five days after any rainfall (11/6/19), the proportion of samples that the *IntI1* gene was detectable in decreased dramatically to 33%, but high levels remained within Drain 4 and its adjacent seawater sample and several Terrigal Lagoon samples, in particular L5.

Figure 14

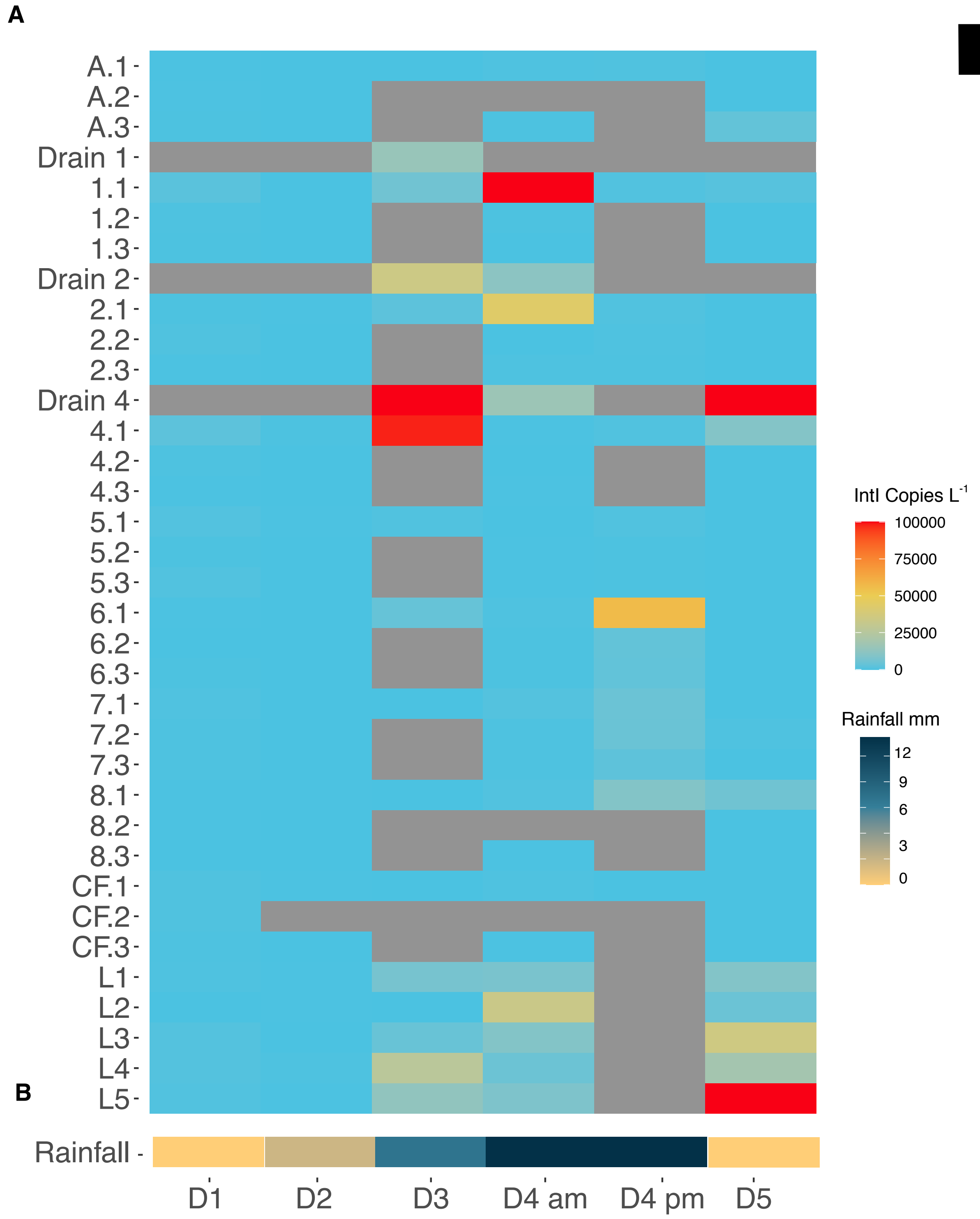

Figure 14: (A) Heatmap displaying distribution of the *IntI1* gene (marker for anthropogenic impact) across sampling locations (Y axis) and days (X axis). Colour scale corresponds to copy numbers L^{-1} defined using qPCR. Grey cells represent samples not collected either due to lack of water flow in drains or rough ocean conditions and low safety levels during the rainfall event. **(B)** Heatmap displaying intensity of rainfall on each sampling day.

Figure 15

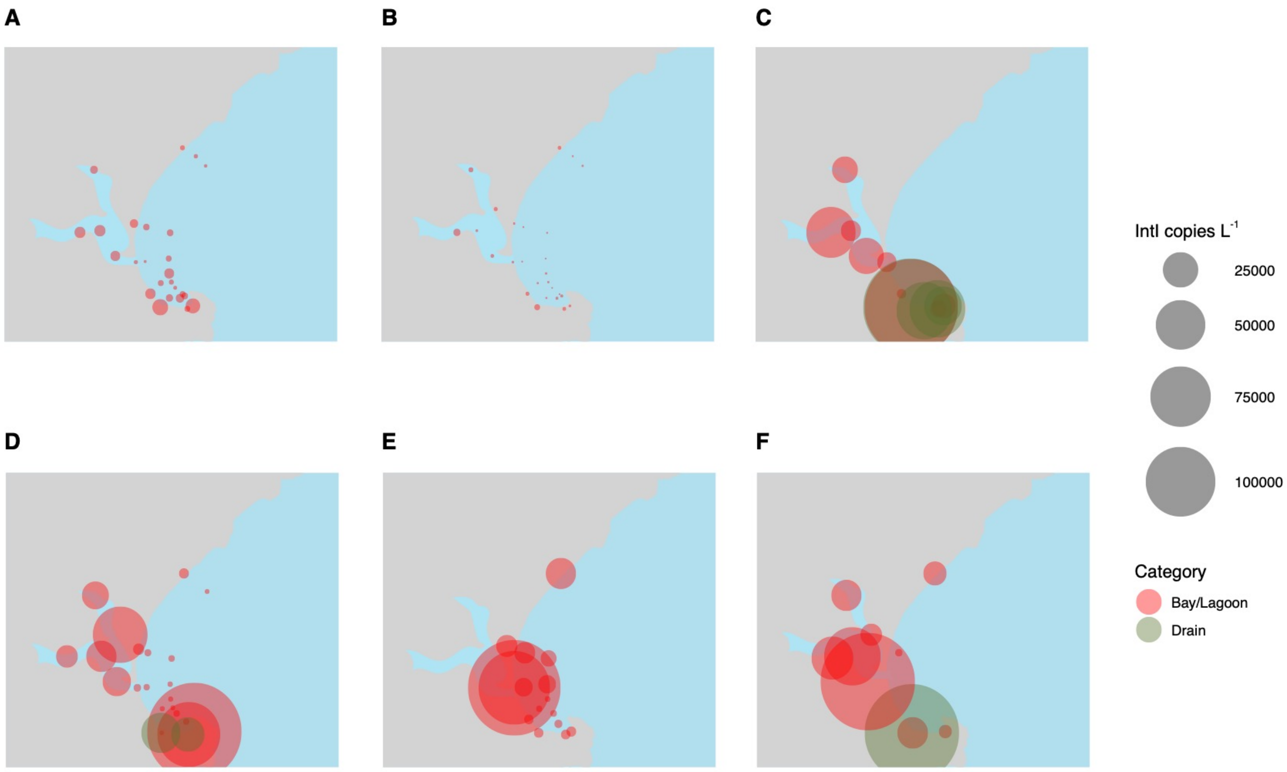

Figure 15: Distribution of the *IntI1* gene (marker for anthropogenic impact) across sampling locations within Terrigal Beach on **(A)** 20/5/19 – which had been preceded by 6 mm of rain in the previous 2 weeks; **(B)** 31/5/19 – which had been preceded by only 2 mm of rain in the previous 2 weeks; **(C)** 4/6/19 - which had been preceded by only 20.4 mm of rain in the previous 3 days; **(D)** Morning of 6/6/19 - which had been preceded by only 40.8 mm of rain in the previous 48 hours; **(E)** Afternoon of 6/6/19 – after the mouth of Terrigal Lagoon had been opened; **(F)** 11/6/19 – after 5 days without rain. Bubble size scales to data magnitude bins (refer to side scale). Red bubbles correspond to seawater and lagoon samples, while green bubbles correspond to samples collected in Drains.

5.0 Interpretation of Results & Conclusions

This project applied a microbial source tracking approach to understand the causes and spatiotemporal extent of poor recreational water quality at Terrigal Beach (NSW) during wet-weather conditions. A previous microbial source tracking study at this location provided evidence that the waters of Terrigal Beach become significantly contaminated with sewage, as indicated by molecular markers for human faecal bacteria, following rainfall[6]. Here we expanded on this previous work by applying a more comprehensive sampling design, incorporating high temporal resolution sampling, coupled with measurements of the microbiological characteristics of Terrigal Beach across a series of onshore-offshore sampling transects, to examine the extent of water contamination during rainfall events. Below we discuss the findings of this study within the context of our 3 primary research objectives.

(1) Are high Enterococci levels at Terrigal Beach during rainfall primarily caused by human (i.e. sewage) or animal (dog or bird) sources of faecal contamination?

During this study, Enterococci levels at Terrigal Beach and within Terrigal Lagoon displayed a significant increase from very low levels during a period of relatively dry weather (8mm of rain over the preceding 4 weeks) to very high levels following 20.4 mm of rain. Following this rainfall event, Enterococci levels within all sampled stormwater drains reached the maximum limit of detection, and became highly elevated within several nearshore seawater samples and within Terrigal Lagoon, where levels in many cases exceeded the NHMRC maximum threshold for significant risk of illness. Using a microbial source-tracking approach, our goal was to determine whether these high Enterococci levels were driven by sewage inputs or other potential sources (e.g. animal) of faecal contamination.

Notably, all of the microbial source-tracking markers employed in this study displayed statistically significant correlations to Enterococci levels. However, the GFD marker for bird Enterococci generally displayed spatial and temporal patterns that were de-coupled from total Enterococci levels, with levels of this marker not increasing notably within either stormwater drain or Terrigal Beach seawater samples during the rainfall event. In contrast, within Terrigal Lagoon, rainfall led to both increases in the proportion of samples that the GFD marker was detected in and a significant increase in concentrations of this marker. Furthermore, under dry weather conditions (5 days after the rainfall event), concentrations of the GFD marker in one Terrigal Lagoon sample (L1) were 70 times higher than the average GFD levels observed across the study and, perhaps notably, coincided with elevated total Enterococci counts observed in this sample. These patterns in Terrigal Lagoon are consistent with the very high numbers of birds (pelicans, cormorants, ducks, and gulls) observed within this site during the sampling campaign and suggest that Terrigal Lagoon is a hotspot for bird faecal material in this region. However, given that even after the mouth of Terrigal Lagoon was opened, levels of the GFD marker were not significantly elevated within Terrigal Beach water samples, we conclude that rainfall does not lead to an increase in the impact of bird faecal contamination of Terrigal Beach waters above natural base-line levels.

In a previous microbial source tracking study conducted at Terrigal Beach, the DG3 marker for dog-faeces associated *Bacteroides* was undetectable[6], but in the present study it was detected, albeit in only 18% of samples. Under dry weather conditions, very low levels of this marker were detected on Terrigal Beach, indicating a negligible influence of dog faecal material on water quality at this site. However, during the rainfall event, significantly higher concentrations of this marker occurred in Drains 1 and 4 and their adjacent seawater samples, as well as within Terrigal Lagoon samples, with highest concentrations observed in Drain 1. Notably, spikes in the abundance of the DG3 marker often coincided with increased levels of the human faecal markers and were largely restricted to stormwater drain samples. This pattern is consistent with other recent microbial source tracking studies conducted at coastal sites within NSW[20,21], which we propose is indicative of either: (a) dog faeces from the catchment being washed into the Terrigal Beach stormwater network, or (b) a secondary sewage signal resulting from dog faeces being flushed down domestic toilets. In either case, we argue that it is unlikely that dog faecal material from the local environment negatively impacts water quality at Terrigal Beach and Haven. Five days after the rainfall event, the DG3 marker was below detection limit in all samples, with the exception of Drain 4 and one lagoon sample (L5), where very high levels occurred – but once again these coincided with significantly elevated levels of the human faecal markers and the human waste-water infrastructure markers (*Arcobacter* and *IntI1*), further indicating a link to sewage contamination.

Across the entire data-set, both of the human faecal markers (HF183 and Lachno3) displayed significant correlations to Enterococci levels and during the rainfall event there was clear correspondence between the samples exhibiting high Enterococci levels and elevated concentrations of the human faecal markers. The dynamics of the HF183 and Lachno3 markers observed here indicate that during the rainfall event, sewage contamination was released into stormwater drains and subsequently into adjacent seawater, with Drain 4 displaying both the highest levels of these sewage markers and the most notable impact on surrounding seawater samples. Notably, the significant sewage signal within Drain 4 and adjacent seawater samples was not restricted to the rainfall event, with very high concentrations of both human faecal markers observed in this drain 5 days after the rainfall event, potentially indicating on-going post-wet weather sewage contamination into this drain – potentially as a result of sewage draining out of soils and/or groundwater and into the stormwater system.

In light of the patterns described above, we conclude that diminished water quality at Terrigal Beach following rainfall is primarily driven by sewage contamination. While markers for animal faeces were observed at Terrigal Beach during this study, we suggest that their presence was reflective of either: (a) natural background levels of faecal material that have minimal effect on increased Enterococci levels during rain – in the case of bird faeces, and (b) faecal material flushed into the Terrigal Beach stormwater network, either from the surrounding catchment or through sewage contamination – in the case of dog faeces.

(2) What are the primary points of contamination input at Terrigal Beach?

Following the rainfall event examined here, both Enterococci and the human faecal marker (HF183 and Lachno3) concentrations were most elevated within stormwater drains, their adjacent seawater samples, and within Terrigal Lagoon. Among the stormwater drains, highest levels of sewage contamination were apparent in Drain 4, where concentrations were up to 54 times higher than the other drains. This observation is consistent with a previous microbial source tracking study conducted at Terrigal Beach, which also identified Drain 4 as the major source of sewage contamination at this site[6]. Notably, high levels of the human faecal markers were observed within this drain and the adjacent seawater sample both during the peak of the rainfall event and five days after rainfall, indicating that this stormwater drain can be a significant source of sewage contamination to Terrigal Beach both during and after wet weather. This stormwater drain represents the outlet from a junction of drains that service run-off from Terrigal's urban center and are potentially exposed to wet weather sewage overflow. We recommend that the stormwater infrastructure upstream of Drain 4 be prioritized in any efforts to resolve the causes of sewage contamination at Terrigal Beach. Notably, despite these high levels of faecal indicator bacteria in Drain 4, levels of these markers remained relatively low within the Beachwatch reference site (5.1), indicating that at least during this event, the influence of Drain 4 did not extend to this reference sampling location.

In addition to the stormwater drains, Terrigal Lagoon also represents a source of sewage contamination to northern parts of Terrigal Beach. Following rainfall, very high levels of Enterococci and elevated concentrations of both human faecal markers were present within several Terrigal Lagoon sampling locations, indicating input of sewage into the lagoon. Notably, after the entrance to the lagoon opened to the ocean on the afternoon of June 6, a significant increase in Enterococci levels and concentrations of both human faecal markers was observed in samples within transect 6, located immediately adjacent to the mouth of the lagoon. This result indicates that when the entrance of Terrigal Lagoon opened during the rainfall event, contaminated lagoon waters impacted water quality in adjacent Terrigal Beach waters. However, it should be noted that a relatively small lagoon water discharge occurred prior to sampling, as a consequence of tidal conditions. Evidence from lagoon water level measurements indicate that a more significant flushing of the lagoon may have in fact occurred after sampling was conducted.

(3) What is the spatial extent and temporal persistence of water contamination at Terrigal beach during a wet weather event?

The sampling design employed during this study was developed to examine the spatial extent (i.e. how far out to sea and along the beach) and temporal persistence (i.e. how long after the rain event) of compromised water quality at Terrigal Beach during a rainfall event. Clear patterns in water contamination linked to inputs from stormwater drains and Terrigal Lagoon were apparent during the course of the event, however, rough ocean conditions during the peak of the rainfall event unfortunately precluded vessel based collection of samples from off-shore sampling points on June 4. This limited our capacity to understand the spatial dynamics of contamination at Terrigal Beach during the period when contamination at the shoreline was greatest. In light of this, we propose that a future study, utilizing a similar sampling design applied during a period of significant rain, but calmer seas, would provide a valuable extension to this work. Nevertheless, the present data-set provides important new insights into water quality dynamics within Terrigal Beach over the course of a major rainfall event.

On June 4, following 20.4 mm of rain in the preceding 3 days, Enterococci levels, along with concentrations of the markers for human faecal material (HF183 and Lachno3) and human wastewater infrastructure (Arcobacter and IntI1) were highly elevated within stormwater drains and Terrigal Lagoon. Within nearshore seawater samples collected at several points along Terrigal Beach, highest levels of this suite of contamination markers occurred within samples immediately adjacent to stormwater drains, with highest levels adjacent to Drain 4. Notably, the seawater sample in the transect adjacent to the closed entrance to Terrigal Lagoon (sample 6.1) also displayed elevated levels of each of these markers. However, low to negligible levels of these markers were observed in samples collected from sites both north (Forresters Beach, Wamberal Beach) and south (North Avoca Beach) of Terrigal Beach, indicating that seawater contamination remained relatively restricted to Terrigal bay.

Somewhat surprisingly, given that a further 20.4mm of rain occurred in the preceding 48 hours, Enterococci levels and concentrations of human faecal markers decreased significantly within the drains that could be sampled (Drains 2 and 4) on June 6. Possible explanations for this pattern are: (i) a build-up of sewage contaminated water within stormwater drains was completely flushed into the environment during the first day of the rainfall event; (ii) potential sewage overflow events affecting the stormwater infrastructure were restricted to the first day of heavy rainfall; (iii) the very large surf conditions during the second part of the rainfall event led to dispersal and/or dilution of sewage-contaminated water. Despite this reduction in the concentration of human faecal markers within drains, seawater samples immediately adjacent to drains retained elevated abundances of these markers. Notably, levels of the human faecal markers and the human wastewater infrastructure markers (Arcobacter and IntI1) were moderately elevated within both near-shore and off-shore samples (i.e. samples situated in 5 and 10m depth water, situated approximately 100 m and 300 m from the shore-line) in transect 6 adjacent to the entrance of Terrigal Lagoon, indicating an off-shore influence of the region surrounding Terrigal Lagoon that was not apparent closer to the stormwater drains in Terrigal bay. This discrepancy could be explained either by (i) higher volumes of contaminated water being released from the shoreline near to Terrigal Lagoon or (ii) differences in hydrodynamic mixing conditions between the northern part of Terrigal Beach and the waters of Terrigal bay.

On the afternoon of June 6, when the entrance to Terrigal Lagoon was opened, there was a pronounced impact on samples in the immediately adjacent region of Terrigal Beach. Within both nearshore and several offshore (situated approximately 100 m and 300 m from the shore-line) samples of transects 5 and 6, which were located in the region adjacent to the mouth of the lagoon, the Lachno3 and HF183 markers for human faeces as well as the *Arcobacter* and IntI1 markers, increased significantly relative to conditions in the morning prior to the lagoon entrance being opened. These patterns indicate that the opening of the entrance of Terrigal Lagoon, resulting in the flushing of lagoon waters into the ocean, impacts the surrounding coastal environment immediately south of the lagoon entrance. Indeed, the off-shore extent of impact from the mouth of Terrigal Lagoon was more pronounced than that associated with the stormwater drains, even though concentrations of faecal indicators were generally higher within the drains than the lagoon. However, it again should be noted that offshore sampling was not possible when highest concentrations of faecal markers and highest flow rates occurred in the stormwater drains on June 4, meaning that further sampling during a wet weather event coinciding with calm ocean conditions is required before precluding a spatially extensive impact of the stormwater drains on water quality at Terrigal Beach.

Following five days without further rainfall, Enterococci levels within all Terrigal Beach seawater samples decreased to the lowest limit of detection, indicative of very low levels of stormwater contamination. However, while both the markers for human faecal material (Lachno3 and HF183) and urban wastewater infrastructure (*Arcobacter* and *IntI1*) decreased significantly relative to those observed during the rainfall event, levels of all markers remained significantly elevated above those measured prior to the rainfall event, indicating a persistent signature of sewage and stormwater that was not detected by standard Enterococci measurements. This persistent signal is potentially significant given that levels of *Arcobacter* and *IntI1* have both been shown to be associated with conditions presenting a potential human health risk[22,23] that standard Enterococci counts are insensitive to.

In addition to the persistence of low levels of markers for sewage across a number of beach and bay samples, very high levels of the markers for human faecal material

(Lachno3 and HF183) and urban wastewater infrastructure (*Arcobacter* and *IntI1*) and the marker for dog faecal (DG3) material occurred within Drain 4, despite 5 days without rain. We propose that these patterns are potentially indicative of a persistent post-rain sewage discharge within this drain. Finally, the Lagoon 5 sample also displayed highly elevated levels of Lachno3, HF183, DG3 and IntI1 at this time, which similarly is indicative of continued post-rain sewage input.

Overall Conclusions

Following an extended period of low rainfall levels, water quality at Terrigal Beach was generally good, with little impact from sewage or animal faecal contamination. However, following rainfall, water quality decreased significantly, as indicated by very high Enterococci levels and microbiological signatures for human faecal material (sewage) and wastewater infrastructure. While the entrance to Terrigal Lagoon was closed, the principal source of contamination was stormwater drains, with Drain 4, which represents the output from a junction of drains that are exposed to run-off from Terrigal's urban centre and adjacent housing, displaying both the largest sewage signature and the most pronounced impact on adjacent water samples. Unfortunately, rough ocean conditions precluded an examination of the off-shore impact of contamination from the Terrigal Beach stormwater drain network during the peak of the rainfall event, but an along-beach assessment of near-shore samples indicated that contamination was relatively restricted to the Terrigal bay region, with little signature of contamination observed at Forresters Beach, Wamberal Beach or North Avoca Beach. On the other hand, high levels of contamination occurred within Terrigal Lagoon samples throughout the rainfall event, with the opening of the entrance to Terrigal lagoon resulting in a significant impact on water quality at adjacent Terrigal Beach sites. Several samples from transects adjacent to the mouth of Terrigal Lagoon, including some located up to 300m from the shoreline, displayed significant increases in sewage and wastewater infrastructure markers, suggesting that opening Terrigal Lagoon during rain has a substantial impact on the surrounding environment. Although very low Enterococci levels were observed 5 days after the rainfall event, moderate levels of human faecal markers and signatures for urban waste-water infrastructure were indicative of a persistent signature of contamination in many Terrigal bay seawater samples, while highly elevated levels of these markers were observed in Drain 4 and a Terrigal Lagoon sample, indicating that continued sewage input can occur for at least a week after large rainfall events.

Acknowledgements

We thank Caitlan Williams and Melanie James from the Central Coast Council and Shivanesh Rao, Chris Baiada, Michael Orr and Adam Wethered from the Estuaries and Catchment Team within DPIE for assistance with field sampling during this study.

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