

# **Snapshot investigation of likely contaminant sources in the Tilligerry Estuary catchment (Zones 5A and 5B)**

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## Introduction

A report recently submitted to Port Stephens Council by Lucas et al (2007) included water quality results for groundwater and surface drainage waters in a non-sewered sub-catchment within Tilligerry estuary. The monitoring results from June 2006 to December 2006 indicated surface drainage as the main hydrological pathway for contaminant export to the estuary. Indicators of human contamination were found to be negligible from the non-sewered catchment over the period of monitoring.

Nevertheless, the continuing assessment of wastewater systems in the Tilligerry catchment was recommended as the hydrological connection between septic tanks and surface drains had been confirmed at the allotment-scale in the past (Geary, 2005). Furthermore, results from the Lucas et al (2007) study indicated that faecal contamination in the estuary from herbivores or others (native animals and/or domestic pets) was the most predominant source of faecal contamination in surface waters draining to the estuary.

The current study was undertaken in April 2007 to characterise water quality in all major drains entering zones 5A and 5B and at sites within the estuary. The aims of the study were:

- a) To provide a “snapshot” of water quality in major surface waters draining to the estuary and within the estuary after a particularly wet period. The samples were analysed for nutrients (orthophosphate and nitrate), total coliforms, faecal coliforms, *E.Coli*, faecal streptococci and faecal sterols and;
- b) To interpret the most likely sources of faecal contamination from the data obtained as elevated faecal coliform concentrations had been recorded after significant rainfall in the past.

## Methods

### Field methods

On Thursday 26<sup>th</sup> April 2007, fifteen samples were taken from surface drainage waters entering the estuary margin (sites 1 – 7 and 14 – 21) and six samples were taken from within the estuary (sites 8 – 13). Figure 1 shows the location of these sites. Rainfall was monitored using a 0.2 mm tipping bucket rain gauge that recorded data at 6-minute timesteps.

All 21 samples were taken over the 3-hour period approaching low tide on the 26/04/07. Samples taken at each site were tested for pH, temperature and electrical conductivity (EC) *in-situ*. At each site, a 250 mL sterilised container was filled for microbial analyses and a 5 L polyethylene container was filled for faecal sterol analysis (FSA).

### Laboratory methods

In the laboratory, orthophosphate ( $\text{PO}_4^{3-}$ ) and nitrate ( $\text{NO}_3^-$ ) were determined using standard spectrometry methods (Clesceri et al, 2001) using an aliquot from the 5 L container filled for faecal sterol analysis.

Concentrations of total coliforms, faecal coliforms, *E.Coli* and faecal streptococci (all in cfu/100 mL) were obtained by filtering sampled waters through 0.45  $\mu\text{m}$  membrane filters which were then incubated at standard temperatures and durations for each test. Several dilutions were used to determine the final count of viable colonies in each test.

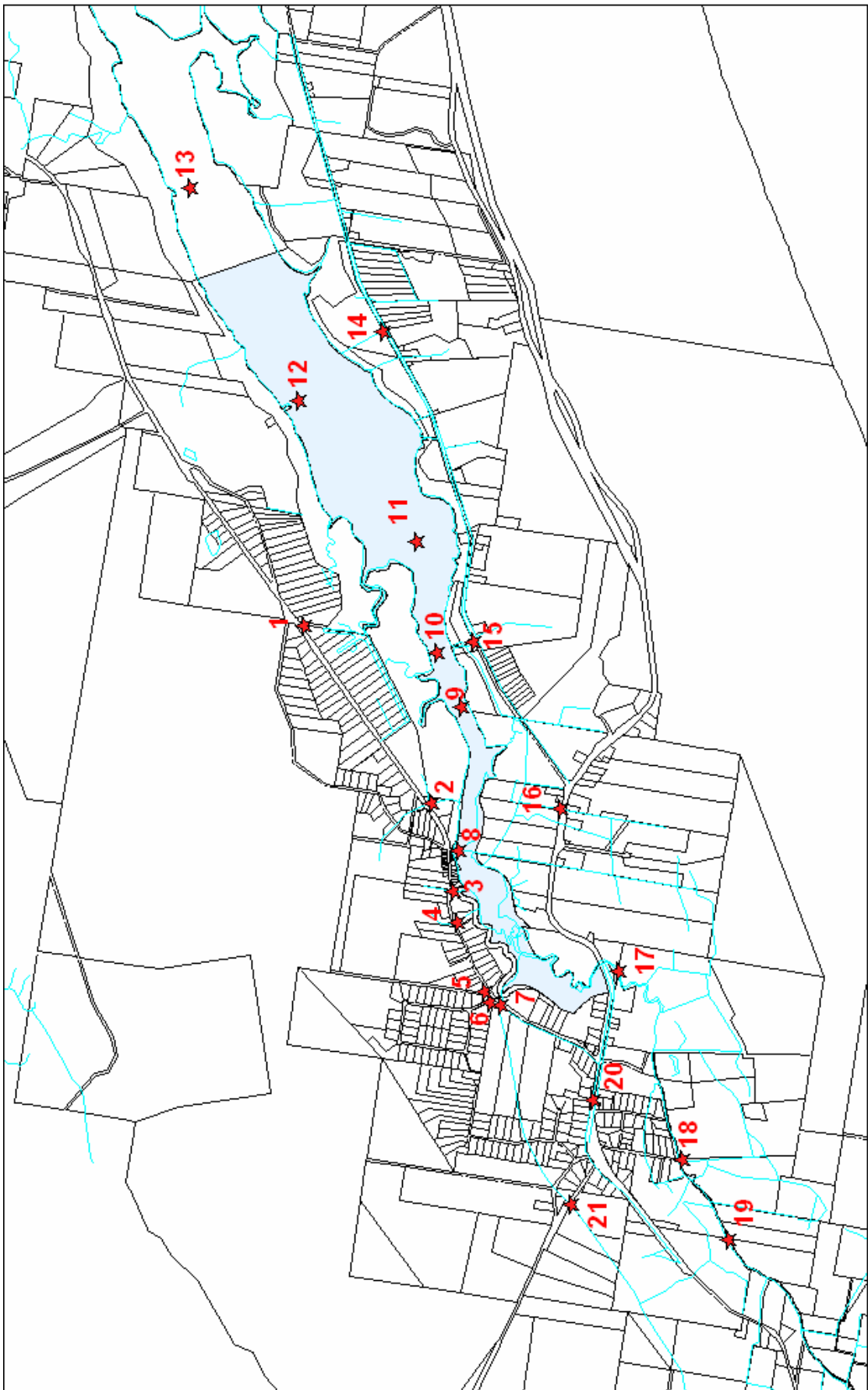


Figure 1: Location of samples sites in the Snapshot Study

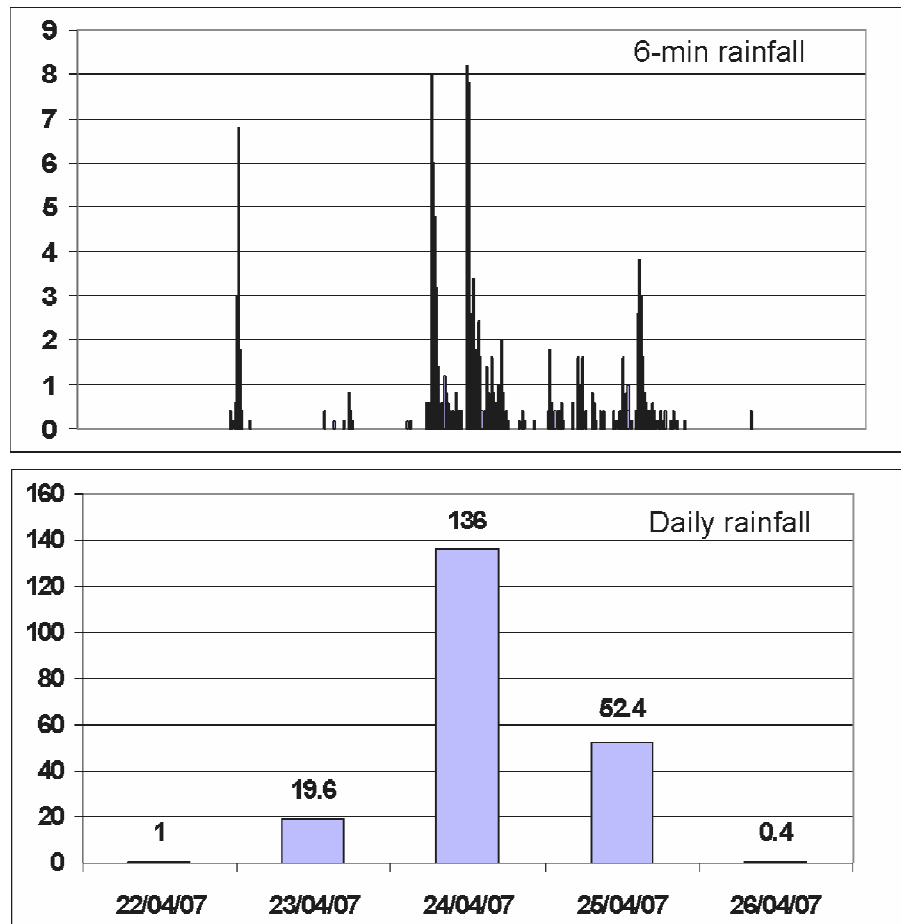
Concentrations of faecal sterols (in ng/L) were obtained by filtering sampled waters through 0.45 µm glass fibre filters. Solvent extraction of sterols from the filter was performed before determination by gas chromatography – mass spectrometry (GC-MS) using the method described by Shah et al (2006).

Statistical analyses were also undertaken to determine significant relationships between measured parameters at and between locations. The purpose of this was twofold. Firstly, the greater the variance between measured parameters at and between sites, the greater the likelihood of faecal contamination originating from a different source. In contrast, the greater the similarity between measured parameters at and between sites, the greater the likelihood of faecal contamination originating from similar sources. Secondly, if many and/or strong relationships are observed in the whole dataset, then the likelihood of faecal contamination originating from a similar source would also be strong.

## Results

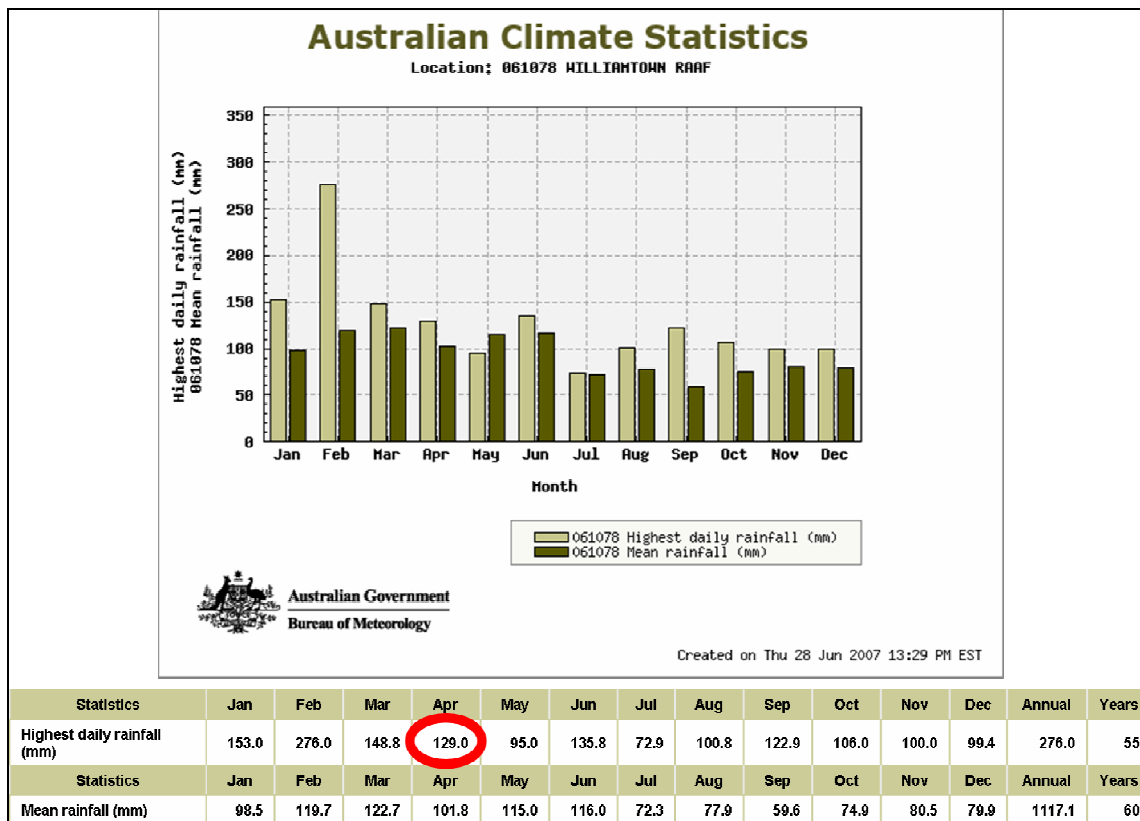
### Climate and hydrology

Figure 2 shows that approximately 200 mm of rain fell in the four days prior to sampling. The 6-minute rainfall data shows that two large rainfall events occurred on the 24/04/07, with several events of lesser intensity occurring on the day before sampling.



**Figure 2: 6-minute rainfall and daily rainfall for the 22/04/07 – 26/04/07**

Rainfall measured at Tilligerry on the 24/04/07 (136 mm) was similar to the highest ever daily rainfall recorded for April (Williamstown data, 1972 – 2007) (see Figure 3). Furthermore, rainfall that occurred during the four days prior to sampling reflected total monthly rainfall exceeding the 90<sup>th</sup> percentile for the month of April.



**Figure 3: Williamtown rainfall records (1972 – 2007)**

### Chemical and nutrient data

Table 1 shows the chemical and nutrient data obtained from the snapshot study on the 26/04/07. The Shellfish Quality Assurance Program (SQAP) sites (sites 8 – 13) and the main Tilligerry creek channel sample locations (sites 17, 18 and 19) are indicated by shaded areas. Other locations represent other major surface drains entering Tilligerry estuary. Note that averages and standard deviations are not used in interpretation as this was a “snapshot” study consisting of one sample from many sites within zones 5A and 5B.

Sites 1 to 7 represent surface drainage waters discharging from the western side of the estuary. Sites 1 and 2 showed a relatively elevated EC compared to other surface drains which was most likely due to tidal excursion from the previous high tide. EC at sites 14 and 15 also indicated tidal excursion in drains on the eastern side of the estuary. The EC of all other drainage waters entering the estuary were indicative of stormwater runoff in rural areas (Wong, 2006). Of particular interest is the increasing EC gradient from site 8 to 13, which effectively indicates the influx of fresh stormwater runoff to the estuary.

Nitrate (NO<sub>3</sub><sup>-</sup>) concentrations were less than Australian and New Zealand Guidelines for Freshwater and Marine Water Quality (< 10 mg/L, ANZECC/ARMCANZ, 2000) threshold values for all sites (sites 1 – 7). Orthophosphate (PO<sub>4</sub><sup>3-</sup>) concentrations at sites 3, 17, 18 and 19 neared or exceeded PO<sub>4</sub><sup>3-</sup> concentrations in the estuary (sites 8 – 13). It must be noted that PO<sub>4</sub><sup>3-</sup> (bioavailable phosphorus) > 0.2 mg/L is likely to induce algal blooms under certain conditions.

**Table 1: Chemical and nutrient data (26/04/07)**

	Site No.	Time	Temp.	pH	EC $\mu\text{S/cm}$	$\text{NO}_3^-$ mg/L	$\text{PO}_4^{3-}$ mg/L
403 LTP Rd	1	11:35	19.6	6.76	2810	0.7	<0.05
Up from boat ramp	2	11:45	20.4	6.24	14350	0.8	<0.05
Chicken drain	3	11:55	18.7	6.85	790	0.8	3.33
181 LTP Rd	4	12:05	18.8	6.61	645	0.8	0.18
D2A	5	12:10	18.4	6.43	920	0.1	0.17
D2	6	13:00	20.4	6.85	376	0.3	0.25
D1A	7	13:05	19.6	6.80	250	0	<0.05
QAP53	8	11:30	20.3	7.25	1523	0.2	1.33
QAP54	9	11:35	21	7.13	4130	0.2	0.41
QAP54A	10	11:40	21.5	7.02	9370	0	0.20
QAP55	11	11:50	21.9	6.95	20700	0.2	0.18
QAP56	12	11:55	22.5	6.49	35800	0	<0.05
QAP57	13	12:00	22.7	6.52	36400	0	<0.05
Shepherd's drain	14	13:20	20.8	5.62	6580	0	<0.05
68 Marsh Rd	15	13:30	20.2	5.87	1950	0.3	0.40
Nelson Bay Rd (Pet shop)	16	13:40	19.6	6.33	208	0.7	0.98
Main Floodgate	17	13:45	19.5	6.28	332	0.4	1.40
F9/bridge	18	13:50	20	6.21	246	0.6	2.10
Upper Tilligerry	19	14:50	19.2	6.28	149	0.1	1.20
Nelson Bay Rd (drain at Salt Ash)	20	14:05	19	6.22	297	0.3	0.20
D1A/Richardson Rd	21	14:35	19.4	6.54	100	0.4	<0.05

As with results in Lucas et al (2007), nutrient analyses did not indicate that nitrogen (as nitrate) and phosphorus (as orthophosphate) were significant contaminants from land use activities in the catchment. With this large amount of rainfall, stormwater runoff waters significantly diluted those in the estuary.

### Microbial data

Table 2 shows microbial data for the Tilligerry snapshot study. All sites except site 14 exceeded the threshold concentration for thermotolerant bacteria (*E.Coli* > 150 cfu/100 mL) in estuarine waters according to Australian and New Zealand Guidelines for Freshwater and Marine Water Quality (ANZECC/ARMCANZ, 2000). The majority of the highest total coliform, faecal coliform, *E.Coli* and faecal streptococci concentrations occurred within the estuary (sites 8 – 13) and the sites at and upstream of the floodgate in the main channel (sites 17, 18 and 19). The concentrations of faecal coliforms recorded in the estuary were similar to those recorded after significant rainfall (> 25 mm/day) in the past (NSW Food Authority, 2005; Geary 2003). The faecal coliform test for site 20 had overlapping colonies and poor colour stability which resulted in ambiguous colony counts. However greater confidence was placed on the *E.Coli* test (with separated colonies and strong colour stability) and subsequent value (as a subset of faecal coliforms).

**Table 2: Microbial data (26/04/07)**

	Site No.	Time	Total C cfu/100 mL	Faecal C cfu/100 mL	E.Coli cfu/100 mL	F. Strept. cfu/100 mL
<b>403 LTP Rd</b>	<b>1</b>	<b>11:35</b>	<b>80000</b>	<b>4000</b>	<b>1900</b>	<b>650</b>
<b>Up from boat ramp</b>	<b>2</b>	<b>11:45</b>	<b>48000</b>	<b>3000</b>	<b>900</b>	<b>510</b>
<b>Chicken drain</b>	<b>3</b>	<b>11:55</b>	<b>96000</b>	<b>10300</b>	<b>3550</b>	<b>1870</b>
<b>181 LTP Rd</b>	<b>4</b>	<b>12:05</b>	<b>46000</b>	<b>1800</b>	<b>1250</b>	<b>460</b>
<b>D2A</b>	<b>5</b>	<b>12:10</b>	<b>33000</b>	<b>6300</b>	<b>2550</b>	<b>1940</b>
<b>D2</b>	<b>6</b>	<b>13:00</b>	<b>56000</b>	<b>5200</b>	<b>2100</b>	<b>260</b>
<b>D1A</b>	<b>7</b>	<b>13:05</b>	<b>34000</b>	<b>1900</b>	<b>500</b>	<b>100</b>
QAP53	8	11:30	128000	22000	5900	2750
QAP54	9	11:35	115000	16000	5850	2510
QAP54A	10	11:40	136000	21000	6950	2780
QAP55	11	11:50	84000	11000	6250	3260
QAP56	12	11:55	35000	4850	2500	880
QAP57	13	12:00	26000	4050	2250	560
<b>Shepherd's drain</b>	<b>14</b>	<b>13:20</b>	<b>11000</b>	<b>100</b>	<b>100</b>	<b>10</b>
<b>68 Marsh Rd</b>	<b>15</b>	<b>13:30</b>	<b>118000</b>	<b>2800</b>	<b>800</b>	<b>380</b>
<b>Nelson Bay Rd (Pet shop)</b>	<b>16</b>	<b>13:40</b>	<b>154000</b>	<b>7500</b>	<b>2800</b>	<b>1130</b>
Main Floodgate	17	13:45	148000	8350	6250	3240
F9/bridge	18	13:50	79000	2800	2300	2000
Upper Tilligerry	19	14:50	54000	4300	1300	420
<b>Nelson Bay Rd (drain at Salt Ash)</b>	<b>20</b>	<b>14:05</b>	<b>144000</b>	<b>4300?</b>	<b>7700</b>	<b>960</b>
<b>D1A/Richardson Rd</b>	<b>21</b>	<b>14:35</b>	<b>27800</b>	<b>1200</b>	<b>500</b>	<b>50</b>

### **Faecal sterol analysis (FSA)**

Faecal biomarkers, such as sterol compounds, have been a technique which has been used to distinguish and estimate contributions from various sources of faecal contamination in waters and sediments (Leeming et al, 1998; Reeves and Patton, 2005). All faecal material contains sterols, and their breakdown products, stanols. The distribution of sterols found in faeces, and hence their source-specificity, is caused by a combination of diet, an animal's ability to synthesise its own sterols, and the conversion of sterols by intestinal microbiota in the digestive tract. Coprostanol constitutes about 60% of the total sterols in human faeces and is produced by biohydrogenation of cholesterol by anaerobic bacteria in the intestines of humans and higher mammals. It is unaffected by physical factors such as temperature and salinity (Sargeant, 1999). 24-ethylcoprostanol has been found to be the principal faecal biomarker in the excreta of herbivores, whereas other animals which are ubiquitous in urban areas, such as dogs and birds, either do not have coprostanol in their faeces, or it is present in trace and/or smaller amounts, thus providing a diagnostic dichotomy of presence/absence.

Table 3 shows results from faecal sterol analysis and circled values highlight the likelihood of human contamination. Sites 9, 10, 11, 17, 18 and 19 generally showed the highest sterol concentrations of all sites. The likelihood of human and herbivore + other contamination was interpreted using Figure 4.

Table 3: Faecal sterol data (26/04/07)

	Site No.	Coprostanol	Epicoprostanol	Cholesterol	Cholestanol	24-Ethylcoprostanol	Camposterol	Stigmasterol	beta-Sitosterol	Cop/Cholestanol	Cop/(Cop+24-ethylcop) X 100	Cop/(Cop+24-ethylcop)	Epicop/Cop.	% Human	% Herbivore + Other
403 LTP Rd	1	12	0	638	60	44	154	484	508	0.20	21.5	0.22	0.00	0	100
Up from boat ramp	2	24	0	679	119	97	85	187	613	0.20	20.0	0.20	0.00	0	100
Chicken drain	3	68	12	1066	183	176	267	663	818	0.37	27.8	0.28	0.17	0	100
161 LTP Rd	4	15	0	339	44	48	88	433	351	0.34	24.3	0.24	0.00	0	100
DZA	5	21	0	864	42	70	89	280	295	0.51	23.5	0.23	0.00	0	100
D2	6	14	0	475	51	69	113	503	680	0.27	16.7	0.17	0.00	0	100
D1A	7	10	0	518	38	62	203	677	776	0.27	13.5	0.13	0.00	0	100
QAP53	8	570	107	2982	628	1046	607	1555	2278	0.91	35.3	0.35	0.19	0	100
QAP54	9	369	71	1532	310	573	326	867	1225	1.19	39.2	0.39	0.19	3	97
QAP54A	10	319	61	1532	253	515	293	781	1021	1.26	38.3	0.38	0.19	1	99
QAP55	11	189	32	865	145	305	114	291	542	1.30	38.2	0.38	0.17	1	99
QAP56	12	64	5	556	70	115	78	75	320	0.91	35.9	0.36	0.08	0	100
QAP57	13	47	1	433	51	92	60	129	306	0.92	33.9	0.34	0.03	0	100
Shepherd's drain	14	3	0	363	55	48	83	297	426	0.05	5.8	0.06	0.00	0	100
88 Marsh Rd	15	83	7	715	83	215	141	564	667	1.01	30.3	0.30	0.08	0	100
Nelson Bay Rd (Pet shop)	16	43	18	3797	104	166	626	1779	1175	0.41	20.6	0.21	0.37	0	100
Main Floodgate	17	310	50	1436	183	468	576	779	1085	1.69	39.8	0.40	0.16	5	95
F9/bridge	18	267	46	1564	194	412	501	790	1341	1.38	39.4	0.39	0.17	4	96
Upper Tilligerry	19	237	43	1428	153	354	848	713	919	1.54	40.1	0.40	0.18	6	94
Nelson Bay Rd (drain at Salt Ash)	20	16	0	623	51	75	153	609	789	0.31	17.2	0.17	0.00	0	100
D1A/Richardson Rd	21	5	0	756	18	41	266	1207	1011	0.33	11.4	0.11	0.00	0	100

Using Figure 4 as a guide, human and/or herbivore contamination may be present in waters when the coprostanol / cholesterol ratio is > 0.4. Further estimation of human and/or herbivore contamination was calculated using the coprostanol / coprostanol + 24-ethylcoprostanol (x 100) value. Ratio values > 73 are interpreted as 100 % human and ratio values < 38 are deemed 100 % herbivore (Bull et al, 2002; Shah et al, in press). Percentage contributions for ratio values between 38 and 73 can be calculated using the  $(73 - \text{ratio value}) \times 2.86$  (see Figure 4).

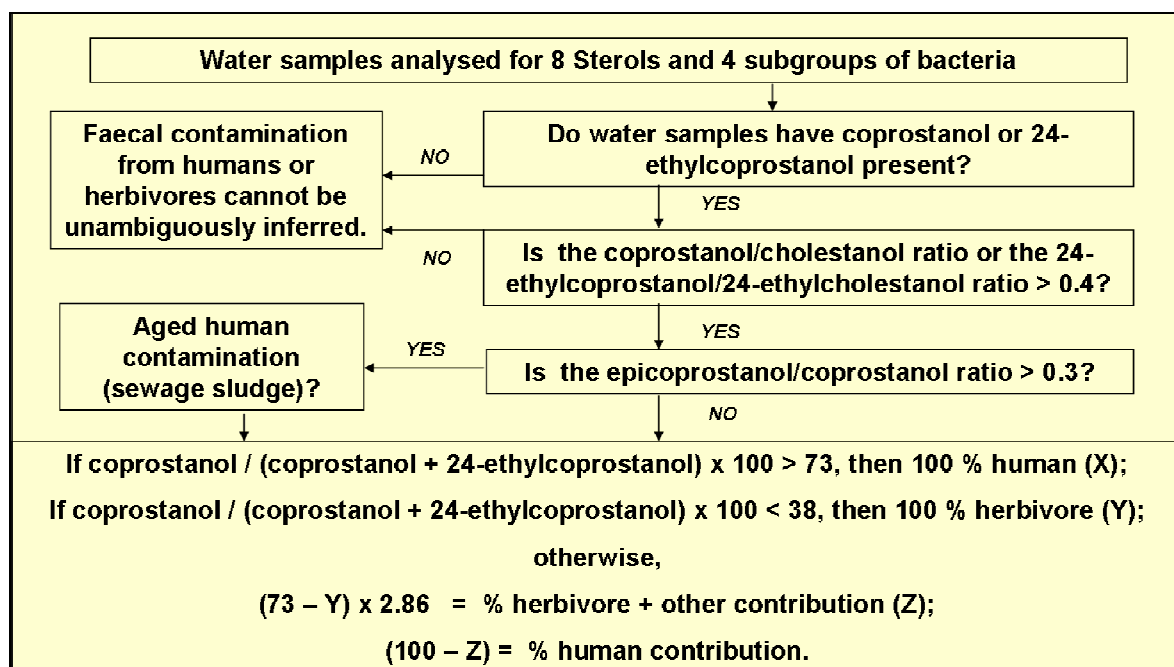


Figure 4: Flowchart for interpreting faecal sterol analysis



## Discussion

The aims of the snapshot study were (a) to gain insight into the water quality of major surface drains, Tilligerry Creek and of the estuary after a particularly wet event; and (b) to identify the most likely sources of faecal contamination.

### Climate and hydrology

The rainfall record showed approximately 200 mm had fallen in the four days prior to the sampling day. In addition to large areas of ponded surface water throughout the catchment, considerable flows were also observed in all surface drains towards the estuary on the sampling day.

### Chemical and nutrient data

Nutrient results at all sites draining to the estuary and within the estuary did not reflect any specific source of faecal contamination. Nitrate ( $\text{NO}_3^-$ ) concentrations did not exceed 0.9 mg/L and thus fell well below the threshold guideline of 10 mg/L for recreational waters (ANZECC/ARMCANZ, 2004). Bioavailable phosphorous (as  $\text{PO}_4^{3-}$ ) concentrations however were shown to exceed 0.2 mg/L, the concentration threshold for potential algal blooms in natural waterways.

Of particular interest was the increasing EC gradient from site 8 to 13. These results illustrate the salinity gradient along the terminal reach of the estuary and suggest that oyster leases in this reach (zones 5A and 5B) are effectively subject to little more than water quality indicative of rural stormwater runoff. Furthermore, bacterial and viral die-off are most effective in waters with higher electrical conductivities (i.e. those typical of seawater) (Hoang Pham, N.K., 2006), and the poor mixing of catchment runoff and estuarine waters in the Tilligerry catchment have considerable implications with respect to die-off rates in this reach of the estuary. Bacterial transport and die-off rates within the Tilligerry catchment can only be further clarified by 3-D modelling of hydrological pathways and mixing regimes to and within the estuary.

### Microbial data

Sites in the estuary (sites 8, 9, 10, 11, 12, 13) displayed the highest faecal coliform numbers of all locations. The relative high faecal coliform numbers recorded at sites entering the estuary indicate that faecal contamination from most surface drains is likely to contribute to elevated faecal coliform counts in the estuary.

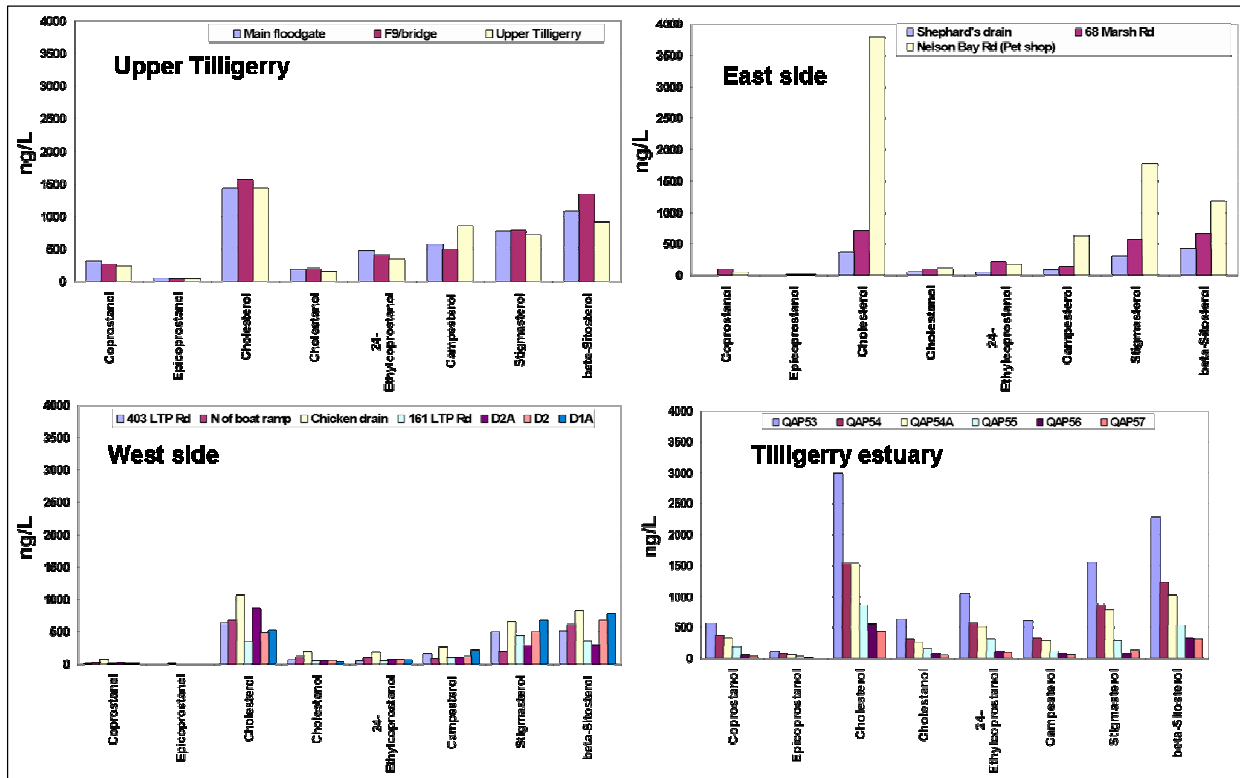
### Faecal sterol data

Figure 5 summarises faecal sterol concentrations for Tilligerry creek at and upstream of the main floodgates (sites 17 – 19), sites on the western side of the estuary (sites 1 – 7), sites on the eastern side of the estuary (sites 14 - 16) and sites within the estuary (sites 8 – 13). It can be seen that sites upstream from the main floodgates (“Upper Tilligerry”) generally have relatively higher sterol concentrations when compared to other sample sites draining to the estuary. Since the creek channel is likely to provide the greatest flows to the estuary, then the highest faecal sterol loads to the estuary are also likely to emanate from the main Tilligerry creek channel.

Compared to surface drainage waters, faecal sterol concentrations in the estuary (sites 8 – 13) were amongst the highest recorded in the study and there are two possible explanations:

1. Faecal sterols accumulate in the freshwater “tongue” that enters the estuary after heavy rainfall and/or;
2. Faecal sterols accumulated in sediment around the estuary margin are re-suspended by turbulence from stormwater runoff after heavy rainfall events.

Further research is required to improve our understanding of these processes and the potential impact to water quality in Tilligerry estuary.



**Figure 5: Sterol concentrations from major surface drains and the estuary**

Interpretation of faecal sterol ratios was based on Figure 4. Interpretation of sterol analysis suggested negligible contributions from human sources at most sites, with the exception of sites 9, 10 and 11 in the estuary and sites 17, 18 and 19 in the main channel of Tilligerry Creek. Coprostanol concentrations at these sites may indicate a likelihood of human contamination however the relative abundance of 24-ethylcoprostanol (> order of magnitude) strongly suggested herbivores + other were most likely to be the dominant contributors to faecal loads to Tilligerry creek. However, as a result of high coprostanol concentrations found in Tilligerry creek, the unsewered area upstream from the floodgates and adjacent agricultural lands require further investigation. These projects may include:

- the sampling/analysis of estuary sediments to see if coprostanol is present and/or accumulates on the estuary margin;
- the further monitoring of waters from shallow groundwater bores, surface drains, Tilligerry Creek and the estuary to further clarify the likely source/s of faecal contamination, and;
- the measurement of drainage flows ( $m^3/s$ ) (over a full tidal range) to gauge the actual volume of water (hence contaminant loads) passing through the main floodgate.

Statistical analysis was undertaken to determine the likelihood of contaminant sources to validate this interpretation. A correlation matrix was created that highlighted significant relationships ( $P < 0.05$ ) within the whole dataset (Table 4). The heavily bordered area shown in Table 4 highlights the main biomarker indicators of human and/or herbivore + other faecal contamination.

The significant relationships between faecal coliforms, *E.Coli*, faecal streptococci and faecal sterols are highlighted by the heavily bordered area in Table 4. Due to the number of significant correlations observed, results suggest that faecal contamination is from a

similar source. For example, when the relative proportion of contaminants at-a-site and between sites shows similar variance over time and space, then it is likely that all sites received contaminant “inputs” from similar sources at that time. In contrast, many poor correlations would have indicated the increased likelihood of faecal contaminants coming from a number of different sources.

Leeming et al (1998) have shown that faecal sterols can be used to distinguish sources of faecal pollution in inland coastal waters, where relatively higher coprostanol concentrations may indicate the likelihood of human contamination. Shah et al (in press) have shown that waters containing mixed faecal sterol signatures (e.g. from human, cow, dog, native animals, etc) are likely to provide ambiguous interpretations of contaminant sources. In this study, 24-ethylcoprostanol concentrations are generally an order of magnitude greater than coprostanol concentrations and this indicates that the most likely source of faecal contamination was from herbivore + other sources.

While the presence of epicoprostanol may indicate the potential presence of aged human contamination (sewage sludge, perhaps from resuspended sediments), the significant relationships (at  $P < 0.05$ ) between coprostanol, 24-ethylcoprostanol, faecal coliforms and *E.Coli* suggest faecal contamination is predominantly from a similar source; and that source is most likely to be herbivore + other.

**Table 4: Correlation matrix for all data obtained during the snapshot study**

	pH	EC uS/cm	NO3- mg/L	PO43- mg/L	Total C cfu/100 mL	Faecal C cfu/100 mL	E.Coli cfu/100 mL	F. Strep. cfu/100 mL	Coprostanol	Epicoprostanol	Cholesterol	Cholestanol	24-Ethylcoprostanol	Campesterol	Stigmasterol	Beta-Sitosterol
Temp	0.16	0.85	-0.46	0.31	-0.12	0.24	0.16	0.14	0.20	0.17	-0.09	0.13	0.17	-0.27	-0.31	-0.12
pH	1.00	0.03	-0.07	-0.18	0.19	0.69	0.44	0.45	0.43	0.46	0.21	0.50	0.44	0.06	0.24	0.36
EC uS/cm	0.03	1.00	-0.34	0.46	-0.33	-0.01	0.02	0.00	-0.10	-0.14	-0.28	-0.12	-0.13	-0.42	-0.55	-0.42
NO3- mg/L	-0.07	-0.34	1.00	-0.09	0.24	-0.17	-0.13	-0.04	-0.19	-0.17	0.18	-0.04	-0.15	0.09	0.21	0.10
PO43- mg/L	-0.18	0.46	-0.09	1.00	-0.65	-0.48	-0.55	-0.57	-0.48	-0.49	-0.44	-0.40	-0.48	-0.43	-0.35	-0.39
Total C cfu/100 mL	0.19	-0.33	0.24	-0.65	1.00	0.60	0.73	0.60	0.53	0.54	0.64	0.51	0.55	0.47	0.58	0.58
Faecal C cfu/100 mL	0.69	-0.01	-0.17	-0.48	0.60	1.00	0.75	0.78	0.80	0.82	0.56	0.83	0.82	0.33	0.41	0.62
E.Coli cfu/100 mL	0.44	0.02	-0.13	-0.55	0.73	0.75	1.00	0.81	0.59	0.60	0.35	0.53	0.58	0.20	0.22	0.41
F. Strep. cfu/100 mL	0.45	0.00	-0.04	-0.57	0.60	0.78	0.81	1.00	0.74	0.73	0.45	0.65	0.71	0.32	0.23	0.46
Coprostanol	0.43	-0.10	-0.19	-0.48	0.53	0.80	0.59	0.74	1.00	0.99	0.57	0.92	0.99	0.62	0.45	0.79
Epicoprostanol	0.46	-0.14	-0.17	-0.49	0.54	0.82	0.60	0.73	0.99	1.00	0.64	0.93	0.99	0.65	0.52	0.83
Cholesterol	0.21	-0.28	0.18	-0.44	0.64	0.56	0.35	0.45	0.57	0.64	1.00	0.63	0.63	0.76	0.85	0.76
Cholestanol	0.50	-0.12	-0.04	-0.40	0.51	0.83	0.53	0.65	0.92	0.93	0.63	1.00	0.96	0.53	0.51	0.84
24-Ethylcoprostanol	0.44	-0.13	-0.15	-0.48	0.55	0.82	0.58	0.71	0.99	0.99	0.63	0.96	1.00	0.61	0.51	0.84
Campesterol	0.06	-0.42	0.09	-0.43	0.47	0.33	0.20	0.32	0.62	0.65	0.76	0.53	0.61	1.00	0.71	0.72
Stigmasterol	0.24	-0.55	0.21	-0.35	0.58	0.41	0.22	0.23	0.45	0.52	0.85	0.51	0.51	0.71	1.00	0.82
beta-Sitosterol	0.36	-0.42	0.10	-0.39	0.58	0.62	0.41	0.46	0.79	0.83	0.76	0.84	0.84	0.72	0.82	1.00

The actual size of contributing drainage catchments may also influence the water quality recorded during the snapshot study. For example, the drainage area of Tilligerry creek upstream from the main floodgate (site 17) is considerably larger than the drainage area of site 5. However, since the volume of runoff from respective drains was not measured it is difficult to comment further on the dynamics of conservative/non-conservative contaminants recorded during the snapshot study. For example, the estuary recorded the highest coprostanol concentrations and without a major coprostanol source being identified, indicates that these concentrations may have been a result of accumulated sterols from all drains. In contrast, the dynamics of bacteria growth and die-off will be different to those of sterols or nutrients. To gain an insight into these processes and the subsequent response of the estuary a detailed 3-D modelling approach would most likely

be required, in addition to further faecal sterol sampling along the main channel of Tilligerry Creek and within the estuary.

## Conclusion

The snapshot study provided insight into the water quality of major surface drains entering the estuary (and the estuary itself) after a particularly wet period in 2007. Nutrient data, microbial data and faecal sterol analysis were used to interpret the most likely sources of faecal contamination to Tilligerry estuary. In general, nutrients showed poor relationships with either microbial or faecal sterol analysis. In contrast, many strong relationships were observed between microbial and faecal sterol analysis whose presence represented the most likely source of faecal contamination to the estuary.

The correlation matrix using data from all sites indicated numerous significant relationships. In particular, the water quality “finger-print” was found to be similar between microbial data and faecal sterol results. Statistical analyses determined significant relationships between measured parameters both at-a-site and between locations. The relative proportions of faecal contaminants were observed to be similar between sites. Since many strong relationships between microbial and faecal sterol data were observed, the likelihood of faecal contamination originating from a similar source was likely. The relative proportion of bacteria (total coliforms, faecal coliforms, *E.Coli* and faecal streptococci) to faecal sterols indicated herbivore + other as being the most likely source of faecal contamination in the estuary.

The similarity in variance between measured parameters at-a-site also reflected a greater likelihood of faecal contamination originating from the same source. Relatively low coprostanol concentrations compared to 24-ethylcoprostanol concentrations suggested the likelihood of faecal contamination from herbivore + other sources.

Furthermore, Tilligerry Creek would be expected to provide one of the largest hydrological inputs to the estuary. However, further investigations, possibly including 3-D modelling of the estuary, will need to be undertaken to confirm estuary flows and mixing regimes. Since the highest faecal sterol signatures were found in the estuary (sites 9, 10 and 11) and to a lesser extent Tilligerry Creek (sites 17, 18, 19,) there is considerable hydrological evidence to suggest that the majority of the contaminant export concentrations (hence loads) originate from sites upstream from the main floodgate in Tilligerry Creek and therefore further water sampling is recommended. However, the high microbial concentrations observed in major surface drains on the western and eastern side of the estuary also warrant further investigation, however it is clear that the majority of faecal contamination in the estuary is from agricultural landuses. A management program to control and mitigate runoff sources from agricultural lands in the catchment is therefore seen as an integral part of any plan to reduce faecal contamination in Tilligerry estuary.

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