EVALUATION OF NUTRIENT/MICROBIAL CONTRIBUTIONS FROM AN UNSEWERED AREA TO THE TILLIGERRY CREEK ESTUARY

FINAL REPORT

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1. Introduction

Increasing development in catchments near sensitive estuarine environments may result in elevated microbial and nutrient loads in natural waterways and nearshore waters. One source of these contaminants may be runoff from unsewered areas. For example, a wastewater system discharging effluent subsurface will create a plume that may enter the groundwater with the contaminants then transported off-site; or after heavy rainfall, effluent may be elevated above ground level and then run off-site if soil storages become saturated. For microorganisms and nutrients to contribute to a management problem in receiving waters, there needs to be a contaminant source where they can be mobilised and transported to an off-site location such as a creek or estuary.

The presence of contaminants in part of the Tilligerry estuary (Zones 5A and 5B) within Port Stephens has resulted in the closure of several oyster farms and the cessation of oyster harvesting due to the presence of human viruses in oyster tissue. On the basis of previously undertaken sanitary surveys and inspections, on-site wastewater systems have been considered to be a source of these contaminants to the Tilligerry Creek estuary. In response, Council has inspected on-site wastewater systems in the area and developed a program to replace and upgrade systems with higher order treatment systems to produce a better quality of effluent for discharge to the environment. Port Stephens Council has more recently had prepared a Catchment Management Plan (Earth Tech, 2006) which, if implemented, should also result in improvements in the quality of stormwater runoff from other land uses, such as urban and agricultural runoff, which also enters the estuary.

There have already been a number of water quality investigations undertaken in the area which can be used to characterise the often poor quality of waters in the Tilligerry estuary (Hunter, 1999; Geary, 2003). Results from previous monitoring programs between 2000 and 2005 have also been summarised by Earth Tech (2006). In addition, there are current water quality monitoring programs underway, namely by Port Stephens Council (PSC) and by the NSW Shellfish Authority (Quality Assurance Program (SQAP)). In all of these studies, it has however been difficult to gain a clear understanding of the sources of the contaminants to the Tilligerry estuary and the contribution that on-site wastewater systems are making to its water quality. These programs have been fragmented by nature and there have been differences in prevailing hydrological conditions at the time of sampling.

In order to successfully determine the sources of contamination in an estuary such as Tilligerry, any study must demonstrate a correlation with bacteriological indicators and other wastewater quality parameters. Also, results must be able to be placed in the context of catchment hydrological conditions and climate (rainfall, drainage flow) and human (wastewater) processes.

The University of Newcastle through the Tom Farrell Institute for the Environment received funding from Port Stephens Council in 2006 to undertake a monitoring study in part of the Tilligerry Creek catchment to identify whether hydrological pathways existed between an unsewered urban development and the estuary, and whether the observed contamination in the estuary could be related to this particular land use activity. The aims of this study were:

- to undertake monitoring and describe water quality variations during a range of climatic conditions within the Michael Drive subdivision;
- to investigate the hydrological linkages between the subdivision and the estuary and examine potential contaminant sources in the context of these existing hydrological linkages.

In this study, potential contaminant export to the Tilligerry estuary was interpreted with respect to the prevailing hydrological conditions, such as rainfall, groundwater levels, surface drain levels and wastewater discharges between June and December 2006. The conditions during this time were then used to identify catchment responses that were most likely to contribute to contaminant transport to the estuarine environment. In addition to chemical wastewater indicators, faecal biomarkers such as sterol compounds (e.g. coprostanol), along with microbiological indicators, were used to determine the likely sources of the contaminants present in surface runoff and groundwater.

2. Background

Over the past decade, increasing urban development in catchments near sensitive estuarine environments has resulted in elevated microbial and nutrient loads in natural waterways (Beal et al, 2003; Valiela et al, 1997; Weiskel and Howes, 1991; Weiskel et al, 1996). The research which has been undertaken has described the important relationship between activities on land, the quality of runoff from different land uses and the contamination of receiving waters.

The likely sources of the contamination in a catchment are often highly varied and many sources are usually identified as having the potential to contribute to poor runoff water quality. Possible sources of contamination potentially include failing septic tank/absorption trench systems in unsewered areas, urban stormwater runoff from directly routed drainage networks, runoff from fertilised agricultural lands containing livestock, waterway activities such as marinas (wastewater pump-outs) and sewerage treatment plant discharges. It is of interest to land managers to know the source of contaminants and their proportionate contribution to the pollutant load to an estuary. Figure 1 depicts the complex interactions which exist between catchment, climate and human processes in a mixed land use catchment contributing runoff to a sensitive environment, similar to the Tilligerry estuary.



Figure 1: Catchment/climate/human processes and flows that drive contaminant transport

The Catchment Assessment Study undertaken by Hunter (1999) for the Karuah/Port Stephens area suggested that failing wastewater systems were likely to be a contributor to contaminant export in surface runoff in Port Stephens. The study identified a number of areas as high risk. The Tilligerry/Salt Ash area was one of these due to the poor performance of wastewater systems, the high groundwater tables and the fact that many

of the failing systems were adjacent to waterways. In response to this, a number of the identified system failures were rectified. In 1999 Smith (1999) undertook a groundwater study within part of the Tilligerry area which reported that, even though there were a number of system failures, there was no nutrient or microbial contamination present in groundwaters collected at a depth of approximately six metres.

Following this, Geary (2003) undertook further monitoring of on-site systems and drains in the area and suggested that contaminants from wastewater systems were able to be transported to drains and the estuary along the water table surface. The study which was undertaken as part of the NSW SepticSafe program concluded that "enteric viruses can be transported to the estuary, particularly during periods of heavy rainfall, and given their published survival times, could be present in estuarine waters used for aquaculture". It also described the potential for the area to contribute to the declining estuarine water guality, and identified a number of hydrological connections for contaminant transport between allotments and the estuary. Using a tracking technique involving antibiotic resistance analysis, Geary and Davies (2003) also concluded that "while no single source emerged as the most significant contributor of faecal contamination to either the oyster leases or to Tilligerry Creek, cattle, human and chicken faeces were all found to be contributing to faecal contamination of the drains and estuary". More recent monitoring however, which was undertaken as part of another research project at the University of Newcastle in 2005, and using another faecal biomarker technique, appeared to clearly indicate that human sourced contaminants were present in some surface drains in the area (Geary et al, 2006).

In terms of the estuary itself, monitoring for faecal bacteria has been undertaken since 1997 as part of the Shellfish Quality Assurance Program. These data have been summarised and analysed by Geary (2003), Hoang Pham (2006) and the SQAP. Generally, the median faecal coliform concentrations in the Tilligerry Estuary have continued to increase indicating that the quality of estuarine waters is declining. There have been regular exceedances of the SQAP standards set for shellfish growing waters (faecal coliforms) and oyster tissue (E.coli) particularly following heavy rainfall. Within Zones 5A and 5B, no sample sites met the Australian Shellfish Quality Assurance Program (ASQAP) statistical criteria for an approved harvest area of < 10 cfu/g (NSW Food Authority, 2005). Given the monitoring history and the results from previous investigations, it was not altogether surprising that human viruses were found in oysters grown in the estuary providing further evidence that various landuse activities in the catchment were having a marked impact on the estuarine water quality.

In catchment scale monitoring studies, there are always difficulties in discerning direct linkages between failing on-site systems and widespread contamination due to effluent dilution and the complexity of detecting effluent pathways in the field. While chemical indicators of contamination, including the by-products of human metabolism, are difficult to identify at the catchment scale, the standard or common microbiological indicators cannot be used on their own to distinguish between human contamination from on-site wastewater systems and that derived from domestic pets, farm animals, and native birds. As a consequence, the evidence for off-site environmental impacts from the failure of numbers of on-site wastewater systems in particular is generally sparse and ambiguous at the catchment scale (Gardner et al, 2006). The case with the Tilligerry estuary illustrates this, as it has been difficult to identify sources of individual contamination in various studies undertaken in the past.

In order to determine to determine the source of the microbiological contamination in catchment waters, a number of source-tracking methods have been utilised by researchers (Shah et al; 2004). One common tracking method is the use of faecal

biomarkers. 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. 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.

Distinguishable sterol profiles. i.e. 'sterol fingerprints" (Leeming et al, 1998) for humans, herbivores and birds have been found to be sufficiently distinctive to be of diagnostic value in determining whether faecal pollution is of human or animal origin. It has been used to trace faecal pollution in Australia (Suprihatin et al, 2003) and New Zealand (Gilpin et al, 2002) in marine, estuarine and freshwater environments (Reeves and Patton, 2005; Shah et al, in press). Leeming et al (1996) provide a spectrum of herbivore, carnivore and omnivore (including humans) faecal sterol profiles whereby they used a ratio approach in interpreting human-sourced contaminants. Selected sterol profiles from Leeming et al (1996) are shown in Appendix A. Figure 2 describes how faecal sterol data is generally interpreted.



Figure 2: Flowchart for faecal sterol analysis (derived from Bull et al, 2000)

This project involved the instrumentation of the small Michael Drive catchment adjacent to the Tilligerry estuary and the monitoring of surface and groundwaters for a variety of indicators. The aim of the project was to determine if contaminants from individual on-site wastewater systems in this part of the catchment were contributing to contamination within the estuary. Of particular importance was the use of sterol compounds as faecal biomarkers in this unsewered area, where failing systems have in the past been considered to contribute to contamination in the Tilligerry estuary.

3. Michael Drive Catchment

The Michael Drive catchment is north of Salt Ash and adjacent to part of the Tilligerry estuary. It is not within the Tilligerry Creek catchment upstream of the floodgates but does contribute runoff to the estuary. While it does contain a number of on-site wastewater systems, there are significantly more in unsewered areas within the Tilligerry Creek catchment. Within the Michael Drive catchment, there are 40 one hectare allotments (Figure 3) which currently use rainwater tanks for all indoor uses and aroundwater extraction for outdoor uses. All indoor water demand is sourced from rainwater tank systems, usually with tank sizes > 50 kL. The large allotments (1 ha) have minimal impervious area (0.3%, including the roof) and most homes utilise groundwater bores for outdoor use. Many homes also have in-ground swimming pools. No treated wastewater is currently reused for household demand however several homes have aerated systems that allow surface irrigation or sub-surface irrigation. The septic tank density for the study area is approximately one wastewater system per hectare. Wastewater flows are treated by most households using a septic tank with effluent disposed to subsurface absorption trench systems, typically between 12 and 15 metres in length. The area is subject to high groundwater levels which potentially act as a subsurface flow path for septic tank discharge. The study area comprises medium to coarse sandy soils away from the estuary margin and numerous estuarine muds and fine sand horizons comprise soil profiles near the estuary margin.

4. Methods

4.1 Hydrology

The potential pathways for contaminant export to the estuary are considered to be through groundwater and/or surface runoff from the catchment. Groundwater quality is most likely to be impacted upon by contaminants that rapidly dissolve into percolating water and reach the water table. Surface water quality is most likely to be impacted upon as surface contaminants are directly routed to surface drains and/or prolonged rainfall fills the soil water storage to capacity and transports contaminants adsorbed in the soil to the surface. Groundwater may also intersect with the surface water drains before entering the estuary.

A monitoring program was developed to investigate water quality which involved both hydrological pathways in the Michael Drive subdivision. The monitoring period started on the 9/6/06 and finished on the 31/12/06. Figure 3 shows the layout and monitoring points in the study area. Also shown is the direction of groundwater movement, culverts and connecting drains and existing drainage lines. Rainfall was continuously monitored (6-minute timesteps) using a 0.2 mm tipping bucket rain gauge which was located near site M (see Figure 3) and water use was monitored at four homes within the subdivision. Water quality and water level monitoring were undertaken for all groundwater and surface water sites. The monitoring sites are described below.

4.1.1 Groundwaters

Five groundwater bores were drilled by qualified contractors and a multi-depth sampling configuration was used which allowed for three samples to be collected from different depths (typically between 1 and 2 m below the surface). The locations of the sites at M, B, F, H and T are shown in Figure 3, while the configuration of the boreholes is shown in Figure 4. A pressure transducer was placed within one of the deeper piezometers at the greatest depth to monitor changes in groundwater level at each site over the study period. The groundwater monitoring sites were not located next to or near wastewater disposal systems, but were located at sites selected to represent groundwater leaving the catchment in the direction of the estuary. Two sites were located hydraulically above the

subdivision and one was located within the subdivision. Two sites were located hydraulically downgradient and along the direction of groundwater flow near the margin of the estuary (Figure 3). Figure 5 indicates the elevation (m AHD) of the selected groundwater locations and values in brackets indicate the distance from the estuary of the groundwater monitoring sites. Direction of groundwater flow shown in Figure 3 was determined by triangulation between borehole levels. Groundwater levels were conditioned in reference to metres above Australian Height Datum (m AHD).



Figure 3: The Michael Drive catchment near Tilligerry estuary – showing the location of monitoring and sample points, existing culverts and connecting drains, and existing drainage flow paths

4.1.2 Surface waters

Surface water samples were collected from Drains 1A, 2 and 2A as shown on Figure 3. The sample site at Drain 2 was fitted with a pressure transducer as this was considered to be the main drainage pathway routing surface flow from within the study area to the estuary. Drain 2 was surveyed to provide a slope based on points at either end of a straight reach, and coupled with drain water level data, a surface profile was calculated that shows the extent of tidal excursion in the Michael Drive sub-catchment. Drain 1A routes runoff from a large catchment adjacent to the subdivision. While its catchment includes some unsewered development in the Salt Ash area (with some larger lot sizes) and upslope forested areas towards Williamtown, it does not contain runoff from the

Michael Drive subdivision. Drain 2 however does route surface runoff to the estuary from part of the unsewered subdivision in Michael Drive, but it also includes runoff from areas above the subdivision, including grazing land and Hunter Water Corporation owned land used to buffer the Tomago Sandbeds area.



Figure 5: Relative elevation (m AHD) and distance from estuary (in brackets) of selected groundwater locations

В

Μ

estuary

Т

4.1.3 Water demand

Н

F

0

Water use was monitored at the homes at sites M, B, F, and T using ""smartmeters"" (Hauber-Davidson and Idris, 2006). The meters provided water demand from each household rainwater tanks at 5 litre increments and at 6-minute timesteps, resulting in diurnal water use patterns for the four monitored homes. For the homes monitored, two had two occupants; one had four, while the other had five occupants. Since only indoor demand was sourced from the rainwater tank, it was assumed that after uses in the home, "smartmeter" results reflected actual discharge to the septic systems. Each home produced an individual diurnal water use profile. However for this study all data was summed for each 6-minute timestep to obtain:

- an actual diurnal pattern of water use from the rainwater tank from the four individual sites, and;
- rainwater profiles summed from the four allotments as a surrogate for discharge to the septic tank.

4.2 Water quality

Both groundwaters and surface waters sampled during a range of rainfall event based conditions were analysed based on standard analysis methods for pH, electrical conductivity (EC, μ S/cm), ammonium (NH₄⁺, mg/L), nitrate (NO₃⁻, mg/L) and orthophosphate (PO₄³⁻, mg/L). The enumeration of total coliforms (TC cfu/100 mL), faecal coliforms (FC cfu/100 mL), *E.Coli* (cfu/100 mL) and faecal streptococci (FS cfu/100 mL) in water samples was determined by membrane filtration following standard methods (Clesceri et al, 1998). Water samples for faecal sterol analysis (ng/L) were filtered using pre-baked glass fibre 0.7 µm filters. Filters with particulate matter were freeze dried and then kept at -20°C until analysis. Analysis was by GC-MS using 5α-Cholestane as an internal standard.

4.3 Statistics

Using matrices correlation, ANOVA and discriminant function analysis, statistical analysis was undertaken on the complete surface water dataset to identify:

- any significant relationships between monitored parameters;
- any significant relationships between surface water sites (drains);
- any significant differences between drain sites;
- any significant variance between monitored parameters and between drain sites and;
- any significant differences in contaminant profiles between wet and dry periods.

5. Results

5.1 Hydrological monitoring

Making the hydrological connection and identifying contaminant sources impacting estuarine water quality is a fundamental prerequisite in being able to manage catchment land uses. Apart from rainfall, the extent of contaminant transport from the Michael Drive subdivision will depend on:

- the wastewater system used and resulting water quality and volume produced;
- the means of eventual disposal (surface or sub-surface);
- the volume of stormwater produced at the allotment-scale available to transport contaminants;
- the ability of the environment to assimilate contaminants.

5.1.1 Rainfall

Sampling occurred between June and December 2006 and during this time the subdivision received a total of 741.6 mm of rainfall. Figures 6 and 7 show the daily and monthly rainfall recorded in the Michael Drive subdivision at site M over this monitoring period. Several months had rainfall much greater than the long-term median (50th percentile) recorded at Williamtown. The numbers of raindays recorded for each month are also shown in brackets in Figure 7. In context of past years, the rainfall received from June – December 2006 can be considered well above average and very wet.





Figure 6: Daily Depth and distribution of rainfall (June – December 2006)



Figure 7: Monthly distribution of rainfall from June – December 2006 compared to long-term median monthly rainfall at Williamtown. The numbers of raindays are shown in brackets.

Further analysis of the data (Figure 8) indicates that of the 214 days of the monitoring period, 117 days were dry (< 0.2 mm, 55 % of the time), 55 days had rainfall of > 2 mm and < 5 mm (26 % of the time), 15 days had rainfall > 5 mm and < 10 mm (7 % of the time), 18 days had rainfall > 10 mm and < 25 mm (8 % of the time), while nine days had rainfall > 25 mm (4 % of the time). The figure of 25 mm/day is the rainfall threshold used by the SQAP to initiate water quality sampling in the estuary. The number of raindays confirms that the subdivision experienced very wet periods during this monitoring period and that a wide range of rainfall conditions was captured which produced runoff to the estuary.



Figure 8: Number of dry days and raindays from June – December 2006

5.1.2 Groundwater levels

Table 1 summarises groundwater levels at monitoring sites throughout the subdivision. The monitoring sites have been listed from furthest from the estuary (site H) to the closest to the estuary (site M) as shown in Figure 5. Whilst average, maximum and minimum groundwater levels decrease approaching the estuary, the range of groundwater level remained relatively consistent across all sites. This suggests that the response of groundwater level to rainfall is similar across the subdivision. Groundwater flow direction has been interpreted as east from the subdivision towards the estuary as shown on Figure 3.

	m AHD	m AHD	m AHD	m AHD
	Average	Maximum	Minimum	Range
Site H	2.017	2.165	1.815	0.350
Site F	1.300	1.468	1.158	0.310
Site T	1.122	1.361	1.001	0.360
Site B	0.035	0.256	-0.084	0.340
Site M	-0.049	0.186	-0.144	0.330

Table 1: Summary of groundwater levels in the Michael Drive subdivision

5.1.3 Hydrology

The hydrological connection between rainfall, groundwater levels and runoff in surface drains has been clearly identified in the monitoring undertaken in the Michael Drive subdivision and a number of examples are presented below for Drain 2.

Figure 9 summarises rainfall (mm), drain level (at D2, in m AHD) and groundwater level (at site M, in m AHD) from the 9/6/06 to 9/9/06 and the 10/9/06 to 31/12/06 at 6-minute timesteps. The arrows and dates represent sampling occasions. Unfortunately there were problems with the drain level data logger (pressure transducer) from 14/11/06 onwards and no data was available for the remainder of the study period (to 31/12/06). Also, battery failure was the cause for the lack of groundwater level data from mid-October until the 14/11/06.





The water level in Drain 2 reflected the stage of stormwater runoff after rainfall, and the observed Drain 2 response (change in water level) compared to rainfall at 6-minute timesteps provided an insight into the rate at which the soil water store was filled under differing rainfall conditions before surface runoff would be induced. Once the soil store becomes full and the soil water unable to move downwards due to the high groundwater level, surface runoff will occur even after small rainfall events. Therefore, the lag time between rainfall and drain level reflects the recharge rate of the soil water storage.

Comparisons with changes in groundwater levels can also be made providing the surface water/soil water/groundwater linkages for potential contaminant transport. In Figure 9, the response rate of drain level and groundwater level to rainfall was very rapid. Significant intra-daily variations in both drain level and groundwater level indicate that other factors also contribute to processes governing the movement of contaminants. The response rate of the catchment in producing surface runoff is an important consideration determining contaminant transport to the estuary.

Figure 10 shows a shorter time period (15/7/06 - 31/8/06) which highlights the consistent cyclic nature of changes in groundwater and drain level and suggests tidal influence also plays a role. Tidal influence can be seen to occur even after rainfall. Tidal influence would most likely act as a natural barrier to the rapid discharge of stormwater from the catchment complicating the determination of contaminant sources. The reason for this is that there appears to be a high potential for contaminants to be imported to the surface drains from the estuary from low to high tide, particularly during baseflow conditions; and an increased potential for mixing of pooled contaminants from previous rain events at the estuary margin at high tides.



Figure 10: Monitoring results for rainfall (mm), drain level (m AHD) and groundwater level (m AHD) from the 15/7/06 to the 31/8/06

The influence of tides was found to be important in groundwater and surface water movement in the Michael Drive catchment. Figure 11 shows the extent of tidal excursion based on the slope of the drain and maximum and minimum drain levels. At high tides the maximum drain level recorded over the monitoring period was approximately 0.50 m AHD and at low tides the minimum drain level was approximately 0.20 m AHD. The calculated surface profile shows that tidal influence can extend up to 370 m from the

estuary shoreline affecting both groundwater and drain levels. As a result, tidal excursion could hold up or impede stormwater drainage at high tides during rain events that result in surface drainage. Alternatively, on other occasions, the tidal influence could potentially result in the transport of wastewater away from individual allotments within this zone.



Figure 11: Surface profiles for Drain 2

In summary, Figure 12 conceptually describes the major catchment processes considered to drive contaminant transport in the unsewered catchment under baseflow (A) and high rainfall conditions (B).



Figure 12: Catchment processes driving contaminant transport in the study area baseflow (A) and high rainfall conditions (B)

Figure 12A shows a shallow unsaturated soil profile potentially capable of providing improved wastewater treatment after discharge of effluent from the septic tank/adsorption system. In contrast, the same soil profile is near saturation during high rainfall periods (Figure 12B) often resulting in considerable surface flow, particularly in constructed drains routing surface runoff from outside the subdivision around the study area.

Within the study area, surface flow is predominantly directed through Drain 2, thus any flows along this drain after rain are most likely to show a stronger "signal" for humansourced contaminants than the peripheral drains (Drains 1A and 2A). Figure 12 also highlights the potential change in gradient as the fresh/groundwater interface will increase and decrease as governed by the tide (lunar cycles).

5.1.4 Water demand

The use of the "smartmeters" to monitor water use from rainwater tanks at 5 L increments (summed at 6-minute timesteps) has allowed the determination of a range of existing diurnal water use patterns from the four households. The volume and timing of wastewater discharged from the septic tank to the environment is an important diurnal pattern in connecting hydrological pathways to groundwater, surface drains and the estuary. Table 2 summarises daily water demand from the rainwater tanks with respect to the occupancy of the household in January 2007. Average daily water demand is very low with a range from 65 to 155 L/p/day with an average of 92 L/p/day. These figures are significantly lower than the design figure of 145 L/p/day used for non-reticulated supply developments in AS/NZS 1547 (2000).

SITE	Daily Water Demand L/day	Daily Water Demand L/p/day	Occupancy
М	215.80	107.90	2
В	309.45	154.73	2
Т	412.83	82.57	5
F	260.12	65.03	4

Table 2: Daily indoor water demand from the rainwater tank for January 2007

Figure 13 shows the diurnal water use pattern from the rainwater tank for each of the four monitored sites. An example of the daily diurnal pattern for each site is shown in Figure 14. The variation between intra-daily patterns of rainwater use is clearly visible. Since rainwater provides all indoor demands, it has been assumed that all indoor wastewater goes to the septic tank. As a result, the water use monitored by "smartmeters" from the rainwater tank has been used as a surrogate for daily wastewater discharged to each wastewater system. With respect to determining actual human-sourced wastewater flows, rainwater from the four monitored homes has been summed for each 6 minute period as shown in Figure 15.

Since 51 homes exist in the subdivision and only four homes were monitored, the discharge from wastewater systems has been assumed to represent approximately 8 % of the total wastewater flows from the subdivision. The average occupancy for monitored homes was 3.3 persons per household. Wastewater contributions from the four homes have been extrapolated to the 51 homes by multiplying wastewater discharge by 12.5 to provide an estimate of total wastewater discharge from wastewater systems in the subdivision. Based on actual monitoring data, the average wastewater produced was calculated at 92 L/p/day. For 51 homes at an occupancy rate of 3.3, the number of people contributing to wastewater systems equalled 168 within the subdivision.







June 07



Figure 15: Intra-daily wastewater discharge from wastewater systems as summed from household water demand monitoring of four homes in the Tilligerry area for January 2007

Therefore, the wastewater generated at 92 L/p/day for 168 people provides approximately 15,456 L of wastewater discharged to the groundwater each day. Considering the subdivision area of 400,000 m², the depth of wastewater discharged is equivalent to approximately 0.04 mm/day. Over a 12 month period this equates to only 14 mm/yr which when placed in context of an annual average rainfall of approximately 1070 mm/yr suggests that the total wastewater discharges from the subdivision potentially represents approximately 1 % of runoff flows in an average rainfall year.

At the allotment-scale however, contaminant loading is reflected by the point source nature of wastewater discharge from on-site wastewater systems. In this study, the soil adsorption trench for each property was estimated from the homeowner's knowledge on their wastewater systems. As such, calculations of wastewater loading were based on a typical soil adsorption trench 15 m in length and 600 mm to 1 m in width, although it is known that a number of systems have smaller trench lengths. For a trench length of 15 m there is a surface area of between approximately $9 - 15 \text{ m}^2$ using the above dimensions.

Based on a 3.3-person household generating 92 L/p/day, the loading rate to the on-site wastewater systems can be up to 34 mm/day (304 L / 9 m²/day). The upper end of this range is substantially higher than that recommended by AS/NZS1547 (2000) for a sandy soil. If in fact trench lengths are shorter, then loading rates will be higher. As groundwater has been found to be a major hydrological pathway, it is very likely that allotment-scale human-sourced contaminants from wastewater systems could enter the groundwater with little opportunity for treatment within the soil. On this basis, the important consideration becomes whether the contaminants are transported toward the estuary and able to be detected at the catchment scale.

5.2 Water quality

Water sampling occurred on 14 occasions between the 9/6/06 and 31/12/06. Both groundwaters and surface waters were sampled during a range of rainfall event based conditions. An example of conditions when sampling was undertaken is shown in Figure

16 for the 18/07/06 at Site M. This figure clearly shows the variation in groundwater levels both due to the tide and in response to rainfall. The response of the drain level to the rainfall and groundwater inflows and the timing of the water sampling can also be seen in the figure.



Figure 16: Rainfall (mm), groundwater level (m AHD) and drain level (m AHD) at Site M on the 18/7/06

The following tables in this report list averages and standard deviations of the water quality results from each site. The results for individual sampling occasions for all water samples analysed are contained in Appendix B in this report.

5.2.1 Groundwater quality

Multi-depth sampling occurred at each of the groundwater sites. Results from chemical, microbial and sterol analysis for each groundwater site are summarised in Tables 3, 4 and 5 respectively. The data have been arranged in order of the proximity of each site to the estuary, for example, Site M was nearest the estuary, while Site H was furthest from the estuary. The number used at each location represents whether the samples were collected from the shallowest to deepest multi-sample point, for example F1 was closest to the surface while F3 was furthest from the surface.

Sites B1 and T1 did not ever have an adequate volume of water to sample and have been excluded from Table 1. Site M1 had water present on two occasions only. Site T was installed later following the first sampling occasion, so not as many samples were collected at this site.

The groundwater samples collected were typically of low pH (around 5 - 5.5 units) and of low electrical conductivity (188 – 1278 μ S/cm). In comparison to other sites, EC at sites M and B was approximately 3 - 4 times higher than at sites T, F and H, possibly reflecting their locations closer to the estuary margin. A decrease in EC with depth was observed at all sites. During drilling at several of the sites, a coffee rock layer containing fine indurated sands was encountered and this has a marked impact on the turbidity and colour of the water sampled. Sites with coffee rock horizons coinciding with groundwater sample depths included M, B and T. Sites H and F were predominantly comprised of sand horizons of varying grain size. At all sites with respect to ammonium (NH₄⁺), nitrate

 (NO_3) , ammonium + nitrate $(NH_4^+ + NO_3)$ and orthophosphate (PO_4^3) , concentrations were low and generally decreased with depth. Nitrogen species concentrations were marginally higher with increasing proximity to the estuary. There were no groundwater sites either above or below the subdivision where water quality concentrations for the nutrients were elevated and above acceptable levels relative to standards used to assess water for ecosystem health.

	eito	n (chemicel)	Temp (oC)	pH	EC (u\$/cm)	NHL+ (mg/L)	NO3- (mg/L)	NO3- + NH4+ (mg/L)	Turbidity (NTL)	PO43- (mg/L)
	M 1	2	15.3	4.80	1278	1.38	0.80	2.18	3	0.06
	M2	5	1 6.0	5.03	1055	0.72	0.54	1.26	4	0.11
	MЗ	5	16.0	5.07	823	0.54	0.44	0.98	4	0.09
	B 2	5	15.2	5.71	1431	1.40	0.70	2.10	22	0.13
	В	5	15.4	5.71	837	0.51	0.64	1.15	14	0.07
	T2	4	16.0	5.43	473	0.38	0.53	0.91	119	0.18
Average	Ę	4	16.2	5.37	295	0.30	0.60	0.90	100	0.10
	F1	5	15.1	5.83	360	0.24	0.38	0.62	127	0.13
	F2	5	15.6	5.68	203	0.19	0.50	0.69	57	0.11
	З	5	16.0	5.43	188	0.18	0.38	0.56	21	0.09
	H1	5	15.4	5.65	263	0.14	0.64	0.78	89	0.15
	H2	5	15.7	5.61	262	0.13	0.68	0.81	34	0.09
	Ε	6	16.9	5.76	270	0.22	0.74	0.96	36	0.10
	M 1	2	-	0.14	39	0.53	0.14	0.67	1	0.01
	M2	5	1.4	0.32	298	0.31	0.17	0.40	1	0.07
	ß	5	1.2	0.22	251	0.07	0.17	0.19	1	0.06
	82	5	1.1	0.30	190	0.59	0.23	0.66	11	0.14
	ß	5	0.8	0.24	110	0.12	0.17	0.19	8	0.03
	T2	4	0.7	0.12	90	0.28	0.17	0.29	52	0.22
SD	ĘЗ	4	0.3	0.10	42	0.02	0.24	0.23	58	0.07
	F1	5	1.2	0.29	81	0.18	0.15	0.30	92	0.11
	F2	5	0.7	0.25	17	0.06	0.19	0.23	56	0.05
	Ę	5	0.6	0.22	9	0.04	0.11	0.10	26	0.05
	H1	5	1.7	0.30	21	0.03	0.11	0.13	61	0.15
	H2	5	1.7	0.21	35	0.07	0.30	0.31	25	0.10
	HЗ	5	1.1	0.13	12	0.13	0.26	0.31	28	0.04

Table 3: Chemical analysis results (Groundwaters)

Total Coliforms existed in appreciable numbers in all the groundwaters sampled (Table 4). Of importance was the fact that faecal organisms (*E.Coli*, Faecal Coliforms and Faecal Streptococci) were not found in any of the groundwaters sampled, except in several samples collected at Site M. At this site the average *E.coli* concentration was low < 5 cfu/100 mL, FC concentration < 25 cfu/100 mL and FS < 35 cfu/100mL. The lack of these indicator organisms at sites (other than at Site M) suggests that landuse activities were not contributing these organisms to the groundwater, or that if they were present, they were not able to survive in large numbers. Overall, groundwater is not considered to be a major pathway for nutrient or microorganism export to the estuary.

Table 5 shows the faecal sterol analysis results. The commonly accepted indicator sterol related to human-sourced wastewater is coprostanol. Ratios of coprostanol to other faecal sterols have been used to attribute percentage contributions from detected sources (Bull et al, 2002; Leeming et al, 1998). As discussed previously, sterol ratios have been used to interpret the relative likelihood of contaminant contributions from humans and herbivores (see Figure 2).

	site	n (micro)	Total C (cfu/100mL)	E.Cali (cfu/100mL)	F.Coliforms (cfuMQUmL)	F.Strep (cfu/100mL)
	M1	1	6800	5	10	-
	M2	4	333	3	25	35
	MЗ	4	330	1	1	5
	B2	4	1371	0	0	0
	B3	4	695	O	0	0
	T2	3	3020	0	0	0
Average	ТЗ	3	1437	0	0	0
	F1	4	1098	0	0	0
	F2	4	220	0	0	0
	F3	4	54	0	0	0
	H1	4	733	0	0	0
	H2	4	2703	0	0	0
	H3	4	2925	0	0	0
	M1	1	-	-	-	-
	M2	4	514	5	50	27
	M3	4	581	1	1	4
	B2	4	2555	0	0	0
	B3	4	1151	0	0	0
	T2	3	4501	0	0	-
SD	ТЗ	3	2394	0	1	-
	F1	4	2135	0	0	0
	F2	4	388	0	0	0
	F3	4	104	0	0	0
	H1	4	774	0	0	0
	H2	4	4880	0	0	0
	H3	4	4784	0	0	0

Table 4: Microbial analysis results (Groundwaters)

Table 5: Faecal sterol analysis results (Groundwaters)

	cito	п	Coprostanci (ng/L)	Epicoprostanci (ng/L)	Cholesterol (ng/L)	Choisetanol (ng/L)	24-Ethylcoprostanol (ng/L)	Cempestarol (ng/L)	Stigmasterol (ng/L)	bete-Sitosterol (ng/L)
	M1	0	-	-	-	-	-	-	-	-
	M2	3	0	0	457	10	78	49	304	2970
	MЗ	3	0	0	461	3	84	48	285	2830
	B2	3	0	0	365	6	79	16	50	277
	B 3	3	O	0	208	0	19	3	21	222
	T2	2	0	O	860	101	829	241	492	1835
Average	Т3	2	0	0	37 1	33	311	69	102	541
	F1	З	0	0	5180	105	133	35	101	240
	F2	3	0	0	266	12	102	26	75	263
	F3	3	O	0	203	14	120	32	73	255
	H1	3	0	0	623	43	486	75	246	496
	H2	3	4	78	225	10	88	25	60	193
	HЗ	3	0	0	211	8	63	16	40	153
	M1	0	-	-	-	-	-	-	-	-
	M2	3	O	O	254	12	121	54	429	4913
	MЭ	3	0	0	260	5	35	58	423	4584
	B2	3	0	0	249	10	35	14	30	164
	B3	3	0	0	154	0	17	5	24	163
	T2	2	0	0	964	111	885	245	525	1891
SD	T3	2	0	0	354	13	72	43	56	205
	F1	3	0	O	8419	115	145	33	112	1 42
	F2	3	0	0	1 94	11	99	23	70	197
	F3	3	0	0	239	25	104	32	66	195
	H1	3	0	0	724	55	530	75	261	398
	H2	3	7	135	171	10	59	18	45	101
	HЗ	3	0	0	128	7	36	15	18	53

The faecal sterol analysis results for groundwaters also resulted in low or negligible concentrations. Due to the low concentrations overall and the large number of samples recording zero for coprostanol, the use of ratio analysis to interpret contaminant source is limited. In this study the only groundwater site where ratio analysis could be undertaken was for Site H on 15/08/06 where a coprostanol concentration of 12 ng/L was measured. According to the ratio method previously outlined, and because the epicoprostanol concentrations were dominant over coprostanol, the source of contamination appeared to be most likely from herbivores. The concentration measured however is very low relative to those levels likely to be found in faecally contaminated waters (Shah et al., in press).

In summary, relatively low nutrient concentrations, negligible microbial counts and very low or zero coprostanol concentrations characterised all groundwaters sampled throughout the Michael Drive catchment during the study period. Groundwater analysis results suggest that if there are contaminants present from the landuse activity, then transport to the estuary is most likely due to another hydrological pathway. As a result the sampling frequency at groundwater sites was decreased during the study in favour of increasing surface water sampling in adjacent drains. The proximity of Site M to Drains D1A, D2 and D2A meant that at high groundwater levels, flow in the drain was most likely to include the groundwater/surface water interface.

5.2.2 Surface drains

Results from chemical, microbial and sterol analysis for each of the surface water sites are summarised in Tables 6, 7 and 8 respectively. The monitoring results for pH indicate that drain waters are typically around 6.5 units. For EC concentrations are typically higher than for groundwaters. Nutrient levels in drain waters were found to be low and only marginally higher than those found in groundwaters. Reference has also been made to the summarised monitoring data collected from drains in the vicinity of Michael Drive and presented in the Earth Tech (2006) Catchment Management Plan. The concentration data for nutrients (Total Nitrogen and Total Phosphorus) for the period May 2000 to July 2005 are not particularly elevated. They do not, in association with the data contained in this report, necessarily indicate that runoff from the unsewered development is necessarily any higher than that from other landuse activities in the area.

Data in Table 6 suggests that the chemical signature of D1A and D2 is similar, with D2A having a considerably higher EC and slightly higher $NH_4^+ + NO_3^-$ than at the other two sites. This was most likely a function of differing flow regimes in each drain and the influence of the tide at the time of sampling. Flow through these drains was observed to be highest through D1A, followed by D2A then D2. Since D2 drained the Michael Drive catchment only, it would be expected that the greatest potential for recording a human-sourced contaminant profile would occur within this drain; however, this cannot be interpreted from the chemical results obtained.

Table 7 presents microbial data obtained from sampling and analysis of D1A, D2 and D2A. The microbial counts for *E.Coli*, faecal coliforms and faecal streptococci are however as expected considerably higher for the surface waters than those in groundwaters. All surface drains recorded average values for *E.Coli* and faecal coliforms that neared or exceeded national water quality guidelines for recreational waters (> 150 cfu/mL) (ANZECC/ARMCANZ, 2000). In addition, high average concentrations for faecal streptococci were observed in all drains.

	Site	n (chemical)	Temp (oC)	рĦ	EC (uS/cm)	NH4+ (mg/L)	NO3- (mg/L)	NO3- + NH4+ (mg/L)	Turbidity (NTU)	PO43- (mg/L)
AVGE	D1A	18	17.2	6.6	758	0.62	0.40	1.02	7	0.06
	D2	22	16.5	6.4	451	0.58	0.51	1.09	8	0.08
	D2A	16	17.1	6.5	2071	0.86	0.49	1.35	7	0.06
	D1A	18	2.7	0.8	677	0.40	0.21	0.44	5	0.03
SD	D2	22	1.8	0.6	337	0.56	0.22	D.66	10	0.06
	D2A	18	2.1	0.6	5849	1.34	0.29	1.44	3	0.02

Table 6: Chemical analysis results (Surface waters)

Table 7:	Microbial	analysis	results	(Surface waters)	
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	Site	n (micro)	Total C (cfu/100mL)	E.Coll (cfu/100mL)	F.Collform s (cfu/100mL)	F.Strep (cfu/100mL)
	D1A	16	1681	334	659	192
AVGE	D2	22	3436	192	639	236
	D2A	16	2162	198	527	921
	D1A	18	2119	434	1569	420
SD	D2	22	2212	268	1030	410
	D2A	16	2965	171	1252	2461

Faecal coliform counts for these drains are also similar to the data presented by Earth Tech (2006), but not as high relative to those typically experienced in runoff from agricultural land in the Tilligerry catchment following heavy rainfall (Geary, 2003). While microbial results indicate that faecal contamination is present in drainage waters entering the estuary, results from other sites within the catchment on land surrounding the estuary are often significantly higher. The estuary itself, while saline, can also contain higher concentrations of faecal bacteria following heavy rainfall. The actual source of the microbial contamination in surface waters in the unsewered area still cannot be determined based on these results.

Table 8 presents the average faecal sterol concentrations for surface drains D1A, D2 and D2A for the 14 samples collected during the monitoring period. The sterol concentrations for surface waters in the drains (D1A, D2 and D2A) were all very low in comparison with the high concentrations found in a drain in the subdivision in 2005 (Geary et al, 2006) which are shown for comparison in Table 9. Of the three drains, 1A had the highest average coprostanol concentration (23 ng/L; SD 47 ng/L) relative to those determined for the D2 (3 ng/L; SD 5 ng/L) or D2A (4 ng/L; 6 ng/L) sites. Overall the levels of the measured coprostanol concentrations in the Michael Drive catchment were very low and were consistent with measured coprostanol concentrations in the Michael Drive catchment were very low and were consistent with measured coprostanol concentrations in the Michael Drive catchment were very low and were consistent with measured coprostanol concentrations in the Michael Drive catchment were very low and were consistent with measured coprostanol concentrations in the Michael Drive catchment were very low and were consistent with measured coprostanol concentrations in forested human-free environments (Shah et al, in press).

	Ste	n	Coprostanol (ng/L)	Epicoprostanol (ng/L)	Cholesterol (ng/L)	Cholestanol (ng/L)	24-Ethyi coprostanol (ng/L)	Campesterol (ng/L)	Stigmasterol (ng/L)	beta- Sitosterol (ng/L)
	D1A	14	22	49	652	38	67	51	165	390
AVGE	D2	14	4	4	963	32	94	99	131	2504
	D2A	14	4	o	538	19	24	41	75	431
	D1A	14	45	119	305	46	58	32	117	182
SD	D2	14	5	12	731	29	196	120	65	4950
	D2A	14	6	9	582	24	141	95	62	3620

Table 8: Faecal sterol analysis results (Surface waters)

Sampia Date	Coprostanol (ng/L)	Epicoprostanol (ng/L)	Chalesterol (ng/L)	Cholestanol (ng/L)	24- Ethylcoprostanol (ng/L)	%Human/ Herbivore Contribution	
25/02/05	1144286	0	1061600	53291	15511	100/0	
31/03/05	2443	72	5335	5173	631	100/0	

Table 9: Faecal stero	concentrations obtained	in surface drai	n in 2005
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The previously described ratio method has been applied to the surface water data set to determine whether the low sterol contaminant sources were likely to be human or from herbivores. Sterol ratios for surface water data (D1A, D2 and D2A) are shown in Table 10. The percentage (%) contribution from human and/or herbivore + other is also given. "Other" sources of faecal contamination include birds, domestic pets and native animals). Within the data set there were only several results where the ratio of coprostanol/cholestanol was greater than 0.4 (when faecal contamination from humans and/or herbivores is likely to be present). Unlike the groundwater sterol ratios, interpretable ratios were common amongst sampled surface waters at all sites. On the 8/8/06 and the 5/9/06 samples were taken at the same site and at hourly intervals for several hours.

The sampling occasion on the 8/8/06 was undertaken during mid-to-high tide, while on the 5/9/06 and the other occasions were sampled approaching low tide. Sampling while approaching low tide was believed the most likely time to capture the "signature" of stormwater runoff only, while near high tide it was believed that tidal excursion may potentially import contaminants from the estuary. For the two samples collected on the 08/08/06, the source of the sterol compounds found in the surface drains in the unsewered area was found to come from sources other than human. This suggests that herbivores + others may have been responsible for the faecal contamination measured.

For the sample collected in Drain 1A on 05/09/06, the ratio analysis method suggests that human sourced waste was present in the surface water drain, along with waste from herbivore + other sources. On this occasion, sterol ratios indicate human contributions to contaminant export (5/9/06, 11:55) as the coprostanol / cholestanol ratio exceeded 0.4 and the coprostanol / coprostanol + 24-ethylcoprostanol (x 100) value was > 38 but < 73. Human contributions were interpreted as comprising 23 % of the contaminants present. Subsequently, the remaining contributing contaminants were deemed from herbivores + other sources (77 %). However, on this day several samples were also taken at D1A, D2 and D2A at 10:55 and 12:55 (one hour either side of the 11:55 sample), and at these sample times ratios suggest no human contributions. The fact that hourly sampling at the same sites produced different interpretations when compared with the outcome for the significantly higher concentrations collected in the Michael Drive drain in 2005 (Table 9).

While it is quite clear that faecal microbial contamination was present in the drain waters on this occasion and on others (Tables 8 and 9), it is still not clear as to whether wastewater from on-site systems was a contributing factor during this monitoring period in 2006. No samples collected from Drains 2 and 2A, which drain the unsewered development, contained high faecal sterol concentrations (particularly coprostanol) or exhibited ratios which would suggest that failing wastewater systems were the source of the contaminants present, while the only result where the ratio approach indicated some human contribution was from Drain 1A, where only part of the catchment contains unsewered development.

Date	18/7/06	8/8/06	8/8/06	8/8/06	8/8/06	15/8/06	5/9/06	5/9/06	5/9/06	12/9/06	19/9/06	10/10/06	14/11/06
Time	6:50	17:55	18:55	19:55	20:65	10:20	10:55	11:55	12:55	10:02	10:20	9:38	9:20
Site	D1A	D1A	D1A	D1A	D1A	D1A	D1A	D1A	D1A	D1A	D1A	D1A	D1A
Coprostane// Cholestanol (>0.4?)	0.00	0.00	0.00	0.59	1.24	0.00	0.68	1.12	0.46	0.00	0.00	0.00	0.00
Epicoprostanol/ Coprestanol (>0.3?)	0.00	0.00	0.00	0.00	18.53	0.00	8.61	0.18	0.00	0.00	0.00	0.00	0.00
Coprostanol/ Cop+24- Ethylycoprostanol x100 [<30% or >75%?]	0.00	0.00	0.00	25.85	32.02	0.00	26.48	45.94	24.51	0.00	0.00	0.00	0.00
% Human	¢	0	0	0	0	o	٥	23	0	a	0	٥	0
% Herbivare + Other	100	100	100	100	100	100	100	$\overline{\mathcal{D}}$	100	100	100	100	100
Date	18/7/06	8/8/06	8/8/06	8/8/06	8/8/06	15/8/06	5/9/06	5/9/06	5/9/06	12/9/06	19/9/06	10/10/06	14/11/06
Time	7:00	18:01	19:01	20:01	21:01	10:14	11:02	12:02	13:02	10:08	10:26	9:25	9:40
Site	D2	02	02	02	02	02	02	02	02	02	D2	02	02
Coprostanel/ Cholestanel (>0.4?)	0.00	0.27	0.00	0.00	0.00	0.00	0.25	0.20	0.09	0.00	0.00	0.00	0.00
Epic oprostanol/ Coprostanol (>0.3?)	2.30	12.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coprostanol/ Cop+24- Ethylycoprostanol x100 (<30% or >15%?)	19.59	16.89	0.00	0.00	0.00	0.00	5.53	16.89	8.31	0.00	0.00	0.00	0.00
% Human	O	٥	0	0	0	0	0	0	0	0	0	0	0
% Herbivare + Other	100	100	100	100	100	100	100	100	100	100	100	100	100
Date	18/7/06	8/8/06	8/8/06	8/8/06	8/8/06	15/8/06	5/9/06	5/9/06	5/9/06	12/9/06	19/9/06	10/10/06	14/11/06
Time	7:14	18:07	19:07	20:07	21:07	10:08	11:07	12:07	13:07	10:14	10:32	9:32	9:50
Site	D2A	D2A	D2A	D2A	D2A	D2A	D2A	D2A	D2A	D2A	D2A	D2A	D2A
Coprostanol/ Cholestanol (>0.4?)	0.00	0.81	0.32	0.48	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0,12	0.00
Epicoprostanol/ Coprostanol (>0.3?)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coprostanol/ Cop+24- Ethylycoprostanol x100 (<30% or>75%?)	٥	19	22	31	31	٥	Q	0	0	Q	o	11	a
% Human	٥	D	0	0	0	0	٥	D	٥	a	0	a	0
% Herbivore + Other	100	100	100	100	100	100	100	100	100	100	100	100	100

Table 10: Sterol ratios from surface water data and % source contributions

5.3 Statistics

Statistical analysis was undertaken on all surface water data to determine significant relationships between drain sites and monitored parameters. Correlation matrices were used to identify any significant relationships between all monitored drain data (Table 11).

Table 11: Significant correlations between all drain data

Surface Water only						
Correlations significant at p < 0.05	n = 39					
Related Variables	r ²					
Groundwater level and Drain level	0.96					
Hours since last rain (Dry (hrs)) and NO_3^-	0.51					
Hours since last rain (Dry (hrs)) and PO_4^{3-}	0.41					
Electrical conductivity and $(NH_4^+ + NO_3^-)$	0.88					
Total Coliforms and Faecal Coliforms	0.73					
Faecal Coliforms and E.Coli	0.45					
E.Coli and Coprostanol	0.70					
Coprostanol and 24-ethylcoprostanol	0.69					
E.Coli and 24-ethylcoprostanol	0.48					
Faecal Coliforms and 24-ethylcoprostanol	0.53					
Cholesterol and 24-Ethylycoprostanol	0.47					
Cholesterol and Campesterol	0.70					
Cholesterol and Stigmasterol	0.32					
Cholesterol and beta-Sitosterol	0.86					

The most significant relationship is that of groundwater level and drain level ($r^2 = 0.96$) reflecting their rapid response to rainfall. Correlations with nitrate (NO₃⁻) and orthophosphate (PO₄³⁻) with the number of hours since rainfall (Dry (hrs)), most likely reflects accumulation over the dry time and flushing of nutrients during wetter times.

However, the relationship between electrical conductivity and $NH_4^+ + NO_3^-$ ($r^2 = 0.88$) strongly suggests that $NH_4^+ + NO_3^-$ is imported into the surface drains from the estuary; or sourced from rising groundwater within the tidal excursion zone as shown by $NH_4^+ + NO_3^-$ concentrations in Table 3 (Sites M and B).

Significant relationships were also observed for Total Coliforms and Faecal Coliforms ($r^2 = 0.73$) and Faecal Coliforms and *E.Coli* ($r^2 = 0.45$). This means that over the monitoring period, the analyses of either Total Coliforms, Faecal Coliforms or *E.Coli* would have provided similar indicator profiles for contaminant export. *E.Coli* also showed a significant correlation to coprostanol ($r^2 = 0.70$) which is in contrast to several other studies where there was a poor relationship between faecal indicator bacteria and faecal sterol concentrations.

If water quality in several drains was similar and a group of monitored parameters showed a similar variability over time, then it is most likely that the drain waters comprised contaminants from similar sources. In contrast, if water quality in several drains was similar and the same group of monitored parameters varied differently over time, then it is most likely that the drain waters comprised contaminants from different sources. When all monitoring data was considered, it appeared that the quality of water in Drain D2 was more like that in Drain 2A (P < 0.05), rather than that in Drain 1A. The variance describes the group of monitored parameters at-a-site and their similarity to variance within the same group of monitored parameters at a different site, in this case between D1A, D2 and D2A. No similarity in population profile existed between D1A and either D2 or D2A. Figure 17 highlights the significant similarity between D2 and D2A and the dissimilarity of D1A to both D2 and D2A.



Figure 17: Variance between drain sites showing the significant similarity between D2 and D2A (P < 0.05) and dissimilarity to D1A (P >> 0.05)

In terms of the faecal sterols, the parameters showing the most significant variance (P < 0.05) between sites included cholesterol, campesterol and stigmasterol (Figure 18). Seagulls, magpies, ducks, rosellas, dolphins, penguins, dogs and cats all contain greater or considerably greater cholesterol (by dry weight) than humans (1 - 5 times greater); particularly birds (Leeming et al, 1996) (see Appendix A). Campesterol from other

sources is also considerably greater than humans and stigmasterol is most common in plants (Leeming et al, 1996) (see Appendix A). Therefore, there is no indication from the sterol analyses that any sampled surface waters contained any human-sourced contamination.

Figure 18 shows that D2, the drain most likely to contain human-sourced contamination, has 24-ethylcoprostanol, cholesterol, campesterol and stigmasterol signatures that are typical of herbivore + other sources. Therefore, the sterol signature that dominated surface water sampling over the study period is most likely from herbivore + other sources, particularly as coprostanol signals were low. However, further analysis has been undertaken to investigate the most likely hydrological conditions for contaminant export.



Figure 18: Parameters exhibiting significant variance between drain sites (D1A, D2, D2A)

In this study, data was categorised by "wet" and "dry" periods to determine the difference in contaminant concentrations over the hydrological conditions sampled throughout the monitoring period. Drain levels > 0.21 m AHD (n = 18) were classed as wet and drain levels \leq 0.21 m AHD were classed as dry (n = 21). "Wet" conditions were deemed to reflect the highest probability of human-sourced contaminants being entrained in surface flow after the available soil storage had been filled. "Dry" conditions represented periods where drain water were sampled at minimum levels.

The significant variances (P < 0.05) between wet and dry periods occurred with Faecal Coliforms and 24-Ethylcoprostanol, however no significant difference was statistically found between wet or dry periods. Figure 19 shows Faecal Coliform results for wet (drain level > 0.21 m AHD) and dry (drain level \leq 0.21 m AHD) periods. Figure 20 shows 24-Ethylcoprostanol results for wet (drain level > 0.21 m AHD) and dry (drain level \leq 0.21 m AHD) and dry (d

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Figure 19: Faecal Coliform results for wet (drain level > 0.21 m AHD) and dry (drain level ≤ 0.21 m AHD) periods

Figure 20: 24-Ethylcoprostanol results for wet (drain level > 0.21 m AHD) and dry (drain level \leq 0.21 m AHD) periods

Significant correlation existed between Faecal Coliforms and *E.Coli* ($r^2 = 0.45$), *E.Coli* and 24-Ethylcoprostanol ($r^2 = 0.48$), Faecal Coliforms and 24-Ethylcoprostanol ($r^2 = 0.53$) and Coprostanol and 24-Ethlycoprostanol ($r^2 = 0.69$). Since it has been previously established that monitored coprostanol concentrations relative to monitored cholesterol, campesterol and stigmasterol concentrations are typical of contaminant export in forested catchments (Shah et, in press), the Coprostanol and 24-Ethlycoprostanol relationship is also most likely to reflect the same source. In addition, average 24-Ethylcoprostanol concentrations were approximately an order of magnitude lower than those typically found in human faeces.

6. Conclusions

This investigation has analysed a number of the hydrological linkages between surface and groundwaters in the Michael Drive catchment and part of the Tilligerry estuary. It has provided further insights into the dynamic nature of processes driving contaminants from the catchment to the estuary. For the monitoring period in 2006, the data presented suggests that groundwaters from the unsewered development in the Michael Drive catchment have negligible human-sourced chemical and microbiological contaminants present. It appears that, at this location, surface flow is the dominant pathway for contaminant transport from the land to part of the estuary. The chemical data for the surface drains also suggests that any human sourced contaminant transport to the estuary is minor.

An attempt has been made in this work to distinguish wastewater contributions from the unsewered development in Michael Drive using a developing source-tracking technique involving the use of faecal sterol profiles. Results which were obtained from a drain in this unsewered subdivision in 2005 using this technique clearly indicated that human contaminants were present in surface drainage waters. On these occasions, very high concentrations of a number of sterol compounds were measured in a limited number of samples. Since then, further contamination of the estuary and aquaculture beds has occurred.

The faecal sterol data for surface drains from monitoring in 2006 indicates that the unsewered area is not significantly contributing human sourced contaminants in drainage waters and that herbivores + other were determined to be the sources of faecal sterols on 14/39 occasions. If human-sourced contaminants were present, then a significant coprostanol signature would be expected to be found (as they were in 2005) in at least some of the monitored waters, particularly considering the hydrological connections in the subdivision and the wet weather during the monitoring period. The relative abundance of natural sterols sharing similar and significant variance coupled with relatively low coprostanol indicates that the contaminant export which was found during the study period was most likely from herbivore + other sources.

The detailed investigations of ground and surface waters which have been reported here have not been able to ascertain that on-site wastewater systems are currently contributing to the contamination in the estuary. At this density of unsewered development (40 systems/ha), no contamination of groundwater has been found and the majority of the surface water contamination is considered to emanate from herbivore + other sources. It has not been possible to attribute any significant contamination in the estuary to failing on-site wastewater systems throughout this monitoring period given that the faecal sterol concentrations were so low.

It is clear however that, based on data collected in 2005, there have been occasions when domestic pumpout of wastewater systems to surface drains may have occurred. This may have occurred wilfully (an illegal practise) or most likely by "de-watering", an innocent practise of removing excess water from backyards containing septic tanks. This would explain the high coprostanol concentrations recorded at this time. While this supposition is based on anecdotal evidence, the occurrences of high coprostanol signals appear to have ceased since knowledge of this study and contamination in the estuary have been made public.

Most of the faecal material being exported to the estuary appears to be related to agricultural and herbivore + other sources from the larger Tilligerry catchment. The monitoring results presented in this report do not suggest that this unsewered development (at this time) is responsible for the reported human viral contamination of oysters which are cultivated in the estuary. However, this may not have always been the case, given the fact that some human pathogens, such as enteric viruses, have the ability to survive in the environment for long periods of time (days to months).

Human derived pathogens in this particular part of the estuary could be from wastewater systems where there is an inadequate residence time or treatment of effluent in the

subsurface. If there are inadequate vertical separation and horizontal buffer distances, then contaminants in wastewater can more quickly enter surface drains and be rapidly transported to the estuary in runoff. If there is an illegal discharge of wastewater directly to a surface drain, then the transport of contaminants is also more likely to quickly reach the estuary.

The factors which affect the survival of microorganisms in the environment are very complex and various modelling approaches (see Appendix C) may be undertaken to determine appropriate setback and buffer distances for unsewered development. In terms of existing and any future wastewater systems in the vicinity of Tilligerry Creek, it is important that they meet current best management practice requirements with regard to their siting, sizing and buffer distance above the groundwater table, and the horizontal distance of systems from surface drains. Better management of agricultural land uses, as well as improved management of unsewered urban development, is clearly required if the oyster industry is to survive at this location into the future.

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