Distinguishing Wastewater Contamination From On-site Systems In Mixed Land Use Watersheds

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Abstract. Part of a large estuary along the eastern Australian coastline (150 kms north of Sydney, NSW) used for shellfish production has been closed to harvesting for over 18 months. Monitoring has shown viral contamination of oyster tissue on a number of occasions and surveys of drainage channels and the estuary indicate regular exceedances of bacterial standards set for shellfish growing waters, particularly following heavy rainfall. The contamination has been attributed to urban runoff, agricultural wastes and possibly failing on-site wastewater systems.

Investigations have been undertaken to distinguish and estimate contributions from various sources of fecal contamination in this part of the estuary, and whether individual on-site wastewater systems can be linked to the recorded surface and groundwater contamination. Fecal biomarkers such as sterol compounds (e.g. coprostanol), along with microbiological indicators, have been used to determine the sources of the contamination. Hydrological investigations involving both surface and groundwater monitoring have also been completed to determine likely hydraulic pathways between the wastewater systems and the estuary. Apart from an isolated illegal on-site wastewater discharge, the principal sources of contamination appear to relate to other landuse activities within the watershed, principally from herbivores.

Keywords. Contamination, coprostanol, fecal bacteria, fecal sterols, shellfish, wastewater.

Introduction

Over the past decade, increasing development in many catchments near sensitive estuarine environments has resulted in elevated microbial and nutrient loads in natural waterways and nearshore waters (Weiskel et al, 1996). As a consequence of increasing numbers of pathogenic microorganisms in estuarine waters, there are often exceedances of standards set for shellfish growing waters (fecal coliforms) and oyster tissue (E.coli). Of concern in relation to public health is the possible presence of human viruses in shellfish harvested for consumption. The likely sources of microbial contamination in a catchment are often highly contentious and many sources have the potential to contribute to poor quality runoff. While possible sources of contamination include urban stormwater runoff, livestock agriculture from the land, and wastewater pump-outs from marine activities, failing septic tank/absorption trench systems in unsewered areas are often reported as potentially able to contribute fecal material to estuaries in surface and groundwater discharges. There are however difficulties in discerning direct linkages between failing systems and widespread contamination due to effluent dilution and the difficulties which exist in differentiating effluent pathways in the field.
Chemical indicators of contamination including the by-products of human metabolism are difficult to identify at the watershed scale and the standard or common microbiological indicators (commonly used to identify fecal contamination in water) cannot be used on their own to distinguish between human contamination 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 is generally sparse and ambiguous at a watershed or catchment scale (Gardner et al., 2006).

Fecal biomarkers, such as sterol compounds, have been one technique which has been used to distinguish and estimate contributions from various sources of fecal contamination in waters and sediments. All fecal material contains sterols, and their breakdown products, stanols. The distribution of sterols found in feces, 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 feces 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 fecal 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 feces, 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 fecal pollution is of human or animal origin. It has been used to trace fecal pollution in Australia (Suprihatin et al., 2003) and overseas in marine, estuarine and freshwater environments (Gilpin et al., 2002; Reeves and Patton, 2005; Shah et al., in press).

Leeming et al (1998) proposed the use of a three-step ratio analysis to interpret the results of fecal sterol analyses. Typically coprostanol and 24-ethylcoprostanol need to be present in the water sample for further consideration of the source of fecal contamination. If the ratio of coprostanol to cholestanol is greater than 0.4, then fecal contamination from humans and/or herbivores is likely to be present. If this is the case and the ratio of epicoprostanol to coprostanol is greater than 0.3, then the source may be aged human fecal matter such as sewage sludge. The proportion of human versus herbivore fecal contamination can then be calculated as shown in Equation 1.

\[
\frac{\text{coprostanol}}{\text{coprostanol} + \text{24-ethylcoprostanol}} \times 100 = A
\]

If \( A > 73\% \), the fecal pollution may be contributed by 100% human sources and if \( A < 38\% \), the fecal pollution may be contributed by 100% herbivore sources. Depending on the ratio calculated, it is then possible to determine the relative percentage contributions by human and/or herbivores, for example, the contribution of human sources can be calculated as (Leeming et al., 1997, 1998),

\[
(A - 38) \times 2.86 = \% \text{ human pollution}
\]

**Previous Investigations**

The problem of possible contamination from failing on-site wastewater systems in the area adjacent to Tilligerry Creek, NSW, Australia was identified in a study conducted in 1997/98 by Hunter (1999) and was even then considered to pose a high risk. This was due to the fact that the seasonal water table was usually close to the ground surface and the soils consisted of deep coarse white crystalline sands. Extrapolated and generalised water table contours show groundwater levels between 0 and 3 m below the land surface with interpreted flow direction towards Tilligerry Creek. The medium coarse sands had high transmissivities and hydraulic conductivities (10 – 20 m/day). In addition, many areas used for on-site effluent disposal were in close proximity to waterways and drainage lines and buffer distances were inadequate. The report by Hunter (1999) also concluded that the sources for the high numbers of fecal coliform bacteria regularly present in surface and estuarine waters were considered to be septic tank contamination of surface waters, rural runoff from grazing activities along the channel and runoff from bush and forested areas.

Recent research on individual systems using added lithium and bromide tracers and nitrogen/ammonium monitoring by Geary (2004) has demonstrated that hydraulic connections exist between on-site wastewater systems, ground and surface waters and the estuary. Most recently a study by Hoang Pham (2006) reported that the estuarine monitoring data for fecal coliforms and E. coli conducted as part of the Shellfish Quality Assurance Program regularly exceeded the water quality guidelines stipulated for
aquaculture. The recent testing of oysters in this part of the estuary by the Foodsafe NSW has confirmed the presence of several human viruses in oyster tissue and as a result, this part of the estuary has been closed for shellfish harvesting. While runoff from other landuse activities enters the estuary, failing on-site wastewater systems have been considered to be the source of the viral material given that sanitary surveys and the research by Geary (2004) have both indicated that they represent the likely source of human derived contaminants.

In 2005 a number of surface water samples were collected during wet weather from one of the surface drains in the unsewered area adjacent to the estuary. Two of the samples which were tested for microorganisms and fecal sterols (Geary et al., 2006) recorded very high results (for coprostanol and cholesterol) as shown in Tables 1 and 2. Using the ratio approach previously outlined, an estimate was obtained of the relative percentage contributions from human or animal (herbivore) sources. On these two occasions, this method confirmed that human wastewater contributions were present in drainage waters in the unsewered area near the estuary.

Table 1. Sterol Concentrations Obtained at Surface Drain.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>CO (ng/L)</th>
<th>EP (ng/L)</th>
<th>CH (ng/L)</th>
<th>CS (ng/L)</th>
<th>24-EC (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/02/05</td>
<td>1144286</td>
<td>0</td>
<td>1061600</td>
<td>53291</td>
<td>15511</td>
</tr>
<tr>
<td>31/03/05</td>
<td>2443</td>
<td>72</td>
<td>5335</td>
<td>5173</td>
<td>631</td>
</tr>
</tbody>
</table>

CO – Coprostanol; EP – Epicoprostanol; CH – Cholesterol; CS – Cholestanol; 24–EC-Ethylcoprostanol

Table 2. Coliform Bacteria Counts and Calculated Sterol Ratios at Surface Drain.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>FC (cfu/100L)</th>
<th>TC (cfu/100mL)</th>
<th>CO/CS</th>
<th>EP/CO</th>
<th>CO/C+24-EC</th>
<th>%Human/Herbivore Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/02/05</td>
<td>950</td>
<td>19000</td>
<td>21.47</td>
<td>0.00</td>
<td>0.99</td>
<td>100/0</td>
</tr>
<tr>
<td>31/03/05</td>
<td>110</td>
<td>4200</td>
<td>0.47</td>
<td>0.03</td>
<td>0.79</td>
<td>100/0</td>
</tr>
</tbody>
</table>

TC – Total Coliforms; FC – Fecal Coliforms; FS – Fecal Streptococci; CO – Coprostanol; EP – Epicoprostanol; CH – Cholesterol; CS – Cholestanol; EC–24-Ethylcoprostanol

In 2006 a research project was commenced which involved the instrumentation of a small unsewered 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 were contributing to watershed scale contamination within the estuary. Of particular importance was the use of sterol compounds as fecal biomarkers in this unsewered area where failing systems were considered to contribute to contamination within the estuary. A number of the results from this investigation are reported in this paper.

Materials and Methods

There are 330 residences in the unsewered community in and around Tilligerry Creek at a density of approximately 70/km². The small unsewered subdivision centered around Michael Drive (51 residences each on 1 hectare allotments) was examined in this study (Figure 1). The residents use rainwater tanks for all indoor uses and groundwater extraction for outdoor uses. Within the subdivision, the majority of wastewater flows are treated by septic tank systems and effluent disposed of via subsurface absorption trench systems. A few homes have aerated secondary systems that allow surface irrigation of treated effluent. The monitoring period started on the 9/6/06 and finished on the 31/12/06. The figure below shows the surface and groundwater monitoring points in the study area, the direction of groundwater movement (measured at 0.45 m/day), 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.
Figure 1. Unsewered Subdivision near Tilligerry Estuary, Port Stephens, NSW.

Five groundwater piezometers 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). 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 sited along the direction of groundwater flow to represent groundwater leaving the subdivision 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 1). Figure 2 indicates the elevation (in meters relative to the Australian Height Datum) of the selected groundwater locations and values in brackets indicate the distance from the estuary to each of the groundwater monitoring sites. Groundwater monitoring was undertaken at Sites M, B, F, H and T and water sampling occurred on 14 occasions between the 9/6/06 and 31/12/06.

Two surface drains which collect all runoff waters leaving the subdivision were also sampled (Drains 2 and 2A) during this period, as well as another drain entering the same part of the estuary (Drain 1A). This latter drain collected runoff from an unsewered urban development and from a forested area. 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, μS/cm), ammonium (NH₄⁺, mg/L), nitrate (NO₃⁻, mg/L) and orthophosphate (PO₄³⁻, mg/L). The enumeration of total coliforms (TC cfu/100 mL), fecal coliforms (FC cfu/100 mL), E.Coli (cfu/100 mL) and fecal streptococci (FS cfu/100 mL) in water samples was determined by membrane filtration following standard methods (Clesceri et al, 1998). Water samples for fecal sterol analysis (ng/L) were filtered using prebaked 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. The following sections report on some of the overall results obtained from the study.

Figure 2. Relative elevation (m AHD) and distance from estuary (in brackets) of groundwater locations.
Results

Hydrology

The hydrological connection between rainfall, groundwater levels and runoff in surface drains was clearly identified in the monitoring undertaken. An example of the connection is the rapid recharge of shallow groundwater following rainfall as is shown in Figure 3. The data in the figure highlights the interrelated nature of changes in groundwater and drain level (Drain 2) and suggests that a cyclic tidal influence also may play a role in the transport of land-derived contaminants to the estuary. Depending on the tide, contaminants from the land surface may quickly infiltrate into the shallow groundwater and be rapidly transported to surface drains and the estuary itself.

Groundwater Quality

The groundwater samples collected were typically of low pH (around 5 - 5.5 units) and of low electrical conductivity (188 – 1278 µS/cm). There were no sites either above or below the subdivision where water quality concentrations for the nutrients (orthophosphate, nitrate or ammonium) were elevated or above acceptable levels relative to standards used to assess water for ecosystem health. While TC existed in appreciable numbers in the groundwaters, E. Coli, FC and FS were not found in any of the samples, except in several collected at site M (Figure 1). 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 this site in groundwaters suggests that this means of transport of any contaminants from the subdivision is not likely to be a major pathway for microorganism export to the estuary.

Surface Water Quality

The monitoring undertaken in the surface drains for nutrients does not indicate that contamination from wastewater systems is present as the concentrations were found to be low and only marginally higher than those found in groundwaters. The microbial counts for E. Coli, fecal coliforms and fecal streptococci are however as expected, considerably higher for the surface waters than those in groundwaters (Table 3). All surface drains recorded average values for E. Coli and fecal coliforms that neared or exceeded national water quality guidelines for recreational waters, and high average concentrations for fecal streptococci were observed in all drains. While microbial results indicate that fecal contamination is present in drainage waters entering the estuary, results from other monitoring sites near agricultural land surrounding the estuary are

Figure 3: Monitoring results for rainfall (mm), drain level and groundwater level (all levels in meters relative to Australian Height Datum).
significantly higher (Geary, 2003). The estuary itself, while saline, can after heavy rainfall also contain higher concentrations of fecal bacteria. The actual source of the microbial contamination in surface waters in the unsewered area still however cannot be determined based on these results.

In the monitoring period, sterol profiles were determined on 14 occasions. The sterol concentrations for surface waters in the drains (D1A, D2 and D2A) were all very low in comparison with the concentrations found within a nearby drain in 2005 (Table 1). 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.
Table 3. Average Microbial Quality in Surface Water Drains.

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>No.</th>
<th>TC (cfu/100 mL)</th>
<th>E. coli (cfu/100 mL)</th>
<th>FC (cfu/100 mL)</th>
<th>FS (cfu/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1A</td>
<td>16</td>
<td>1661</td>
<td>334</td>
<td>659</td>
<td>192</td>
</tr>
<tr>
<td>D2</td>
<td>22</td>
<td>3436</td>
<td>192</td>
<td>639</td>
<td>236</td>
</tr>
<tr>
<td>D2A</td>
<td>16</td>
<td>2162</td>
<td>198</td>
<td>527</td>
<td>921</td>
</tr>
</tbody>
</table>

No. – Number of Samples; TC – Total Coliforms; FC – Fecal Coliforms; FS – Fecal Streptococci

As indicated previously the sterol concentrations found in surface waters were very low. Using the described ratio method, an attempt has been made to determine whether the sterol contaminant sources were likely to be human or from herbivores. Within the data set there were only three results where the ratio of coprostanol/cholestanol was greater than 0.4 (when fecal contamination from humans and/or herbivores is likely to be present) and these are shown in Table 4. The calculated ratios for each of the samples are then presented in Table 5.

Table 4. Selected Fecal Sterol Concentrations in Surface Water Drains.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Sample Site</th>
<th>CO (ng/L)</th>
<th>EP (ng/L)</th>
<th>CH (ng/L)</th>
<th>CS (ng/L)</th>
<th>24-EC (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/08/06</td>
<td>D1A</td>
<td>13</td>
<td>0</td>
<td>530</td>
<td>23</td>
<td>38</td>
</tr>
<tr>
<td>08/08/06</td>
<td>D2A</td>
<td>14</td>
<td>0</td>
<td>641</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>05/09/06</td>
<td>D1A</td>
<td>165</td>
<td>27</td>
<td>1199</td>
<td>146</td>
<td>194</td>
</tr>
</tbody>
</table>

CO – Coprostanol; EP – Epicoprostanol; CH – Cholesterol; CS – Cholestanol; EC–24-Ethylcoprostanol

Table 5. Selected Fecal Sterol Ratios for Surface Water Drains.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Sample Site</th>
<th>FC (cfu/100L)</th>
<th>TC (cfu/100mL)</th>
<th>CO/CS</th>
<th>EP/CO</th>
<th>CO/CO+24-EC</th>
<th>%Human/Herbivore Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/08/06</td>
<td>D1A</td>
<td>60</td>
<td>900</td>
<td>0.57</td>
<td>0.00</td>
<td>0.26</td>
<td>0/100</td>
</tr>
<tr>
<td>08/08/06</td>
<td>D2A</td>
<td>250</td>
<td>1700</td>
<td>0.52</td>
<td>0.00</td>
<td>0.32</td>
<td>0/100</td>
</tr>
<tr>
<td>05/09/06</td>
<td>D1A</td>
<td>1380</td>
<td>3200</td>
<td>1.13</td>
<td>0.16</td>
<td>0.46</td>
<td>23/77</td>
</tr>
</tbody>
</table>

TC – Total Coliforms; FC – Fecal Coliforms; FS – Fecal Streptococci; CO – Coprostanol; EP – Epicoprostanol; CH – Cholesterol; CS – Cholestanol; EC–24-Ethylcoprostanol

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 or household pets may have been responsible for the fecal 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 animal sources. While it is quite clear that fecal microbial contamination was present in the drain water, it is still not clear as to whether wastewater from on-site systems was a contributing factor given that only part of the watershed of Drain 1A contains unsewered development and that upstream forested areas also contribute runoff. No samples collected from Drains 2 and 2A (which drain the unsewered subdivision) contained high fecal sterol concentrations (particularly coprostanol) or exhibited ratios which would suggest that failing wastewater systems were the source of the fecal material present.

Overall the contribution of the unsewered subdivision to the declining quality of estuarine waters at Tilligerry needs to be placed in the context of the total watershed area and other contributing landuse sources. Wastewater use was monitored in four homes within the 51 home subdivision during the monitoring period. Based on measured water demand and an average occupancy of 3.3 persons per household, it is assumed that approximately 15,484 L of wastewater is discharged to the environment through the on-site
wastewater systems each day. Considering the subdivision area of 510,000 m², the depth of wastewater discharged would be equivalent to approximately 0.03 mm/day. Over a 12 month period this equates to 11 mm/yr. Placed in context of an annual average rainfall of approximately 1070 mm/yr for the area, the total wastewater discharges from the subdivision potentially represent approximately 1 % of runoff flows in an average rainfall year. Runoff from other landuses, particularly agriculture, is important to the overall fecal load to estuarine waters and wastewater systems at this low density do not appear to contribute significantly to the declining water quality in the estuary, although the presence of human viruses in oysters grown there is of great concern.

Conclusion

In this investigation an attempt has been made to distinguish wastewater contributions from on-site wastewater systems in a mixed land use watershed using a developing tracking technique involving the use of fecal sterol profiles. Results obtained during 2005 clearly indicated that human waste was present in surface drainage waters in this small watershed as a number of very high concentrations of fecal sterol compounds were recorded in a limited number of samples. The contamination in the aquaculture beds by human viruses was confirmed by viral testing of oyster tissue. More detailed investigation of ground and surface waters in 2006 using the same technique has however not been able to ascertain that on-site wastewater systems are contributing to the contamination in the estuary. At this low density of unsewered development, no contamination of groundwater has been found and the majority of the fecal contamination of surface water is considered to emanate from agricultural runoff and domestic pets and not the on-site wastewater systems. It has been difficult using this methodology to establish the contribution that failing on-site wastewater systems are specifically making to the fecal load of the watershed given that the fecal sterol concentrations are so low. It is clear however that illegal practices such as pumpout appear to have ceased since the residents became aware that this investigation was underway. The monitoring results do not suggest that this unsewered development is responsible for the reported human viral contamination, however this may not have always been the case. Following an illegal discharge, some human pathogens, in particular enteric viruses, have the ability to survive in the environment for long periods of time and could clearly travel to the estuary.

The factors which affect the survival of microorganisms in the environment are very complex and various modelling approaches may be undertaken to determine appropriate setback and buffer distances for on-site wastewater systems. 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 separation distance above the groundwater table, and the horizontal buffer distance from surface drains. Better management of agricultural land uses, as well as improved management of unsewered urban development, is clearly required if the aquaculture industry is to survive at this location into the future.

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References


Hunter, M. 1999. The Karuah River/Port Stephens Catchment Assessment Program, Final report prepared for Port Stephens Council, Raymond Terrace, NSW.


