The use of chemical indicators to source human, animal and bird pollution of Fraser Island lakes and streams.

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Abstract:
The presentation covers the use of a chemical indicator technique to investigate whether tourists are polluting the fresh water ecosystems on the relatively pristine Fraser Island. The objectives to the development and use of the technique included:

- Seeking to quantify and distinguish faecal pollution inputs from human and other, natural animal sources.
- Identify lakes and creeks on Fraser Island that are impacted by human visitation.

The focus of this paper is on the methodology and use of the chemical indicator technique. Samples of surface waters and lake floor sediments were collected from Fraser Island and analysed by GC-MS. The analytical technique is based on using faecal sterols as indicators of faecal pollution. The results showed that the sterols were very low to low in the lake sediments and undetectable in the surface water samples. Ratios of sterols indicated that water fowl are the likely predominate faecal source. Human input appeared to be negligible compared to natural sources.

Introduction:
The research investigated whether tourist visitation of the relatively pristine Fraser Island lakes is polluting the fresh water ecosystems. The other aim of the study was to develop and test the faecal sterols chemical indicators methodology for use at USC. This study was dependant on access to a newly purchased gas chromatograph-mass spectrometer (GC-MS) in the FoSHE research instruments laboratory. If the study was successful, it would develop analytical capability in organic chemical contamination that would be useful to the environmental science and public health programs within USC and the region. While microbial techniques can identify the presence or absence of faecal microbes, then generally don't provide much detail on relative types and amounts of sources.

Objectives and workshop structure:
The aim is to introduce a relatively little used chemical indicator technique which can be applied to local/regional water quality studies. It is particularly useful in determining the likely sources of faecal contamination of surface and subsurface waters and sediments, and potentially capable of distinguishing relative inputs of faecal material. Effectively it duplicates and applies the faecal sterol chemical indicators technique that has been used in CSIRO (Leeming et al., 1996, Leeming et al., 1998) and on lake sediments (Logan et al., 2001), river water (Suprihatin et al., 2003) and groundwater (Roser et al., 2002) within Australia. This technique was also used in a much larger scale by the US Geological Survey to study organic contamination of streams across 30 states in the continental USA (Kolphin et al., 2002). While the technique was initially developed over a decade ago now, it is still only used relatively sparsely as it requires careful attention to trace analyses and the interpretation of results is often difficult. Detection limits for the faecal sterols are required be down in the parts-per-trillion level and interpretation can be confusing due to variability in pollutant and natural sterol levels.

Method:
Samples of surface waters and lake floor sediments were collected from Fraser Island during two field sampling trips to Fraser Island in the summer of 2005. Samples were returned to USC and processed prior to analysis by GC-MS. The analytical technique is based on using faecal sterols as chemical indicators of faecal pollution. Sterols in the diet are biohydrogenated by anaerobic bacterial to a mixture of stanols. The unique ratios of sterols and stanols in faeces, arising through diet and metabolism in each animal species, can be used to track the sources of faecal contamination in water bodies and sediments. The faecal sterols are very surface active chemicals and bond onto sediment particles so are usually found in the sediments or suspended sediments. Their lifetime dissolved in water is short (~ 1 week) compared to months in anaerobic sediments.

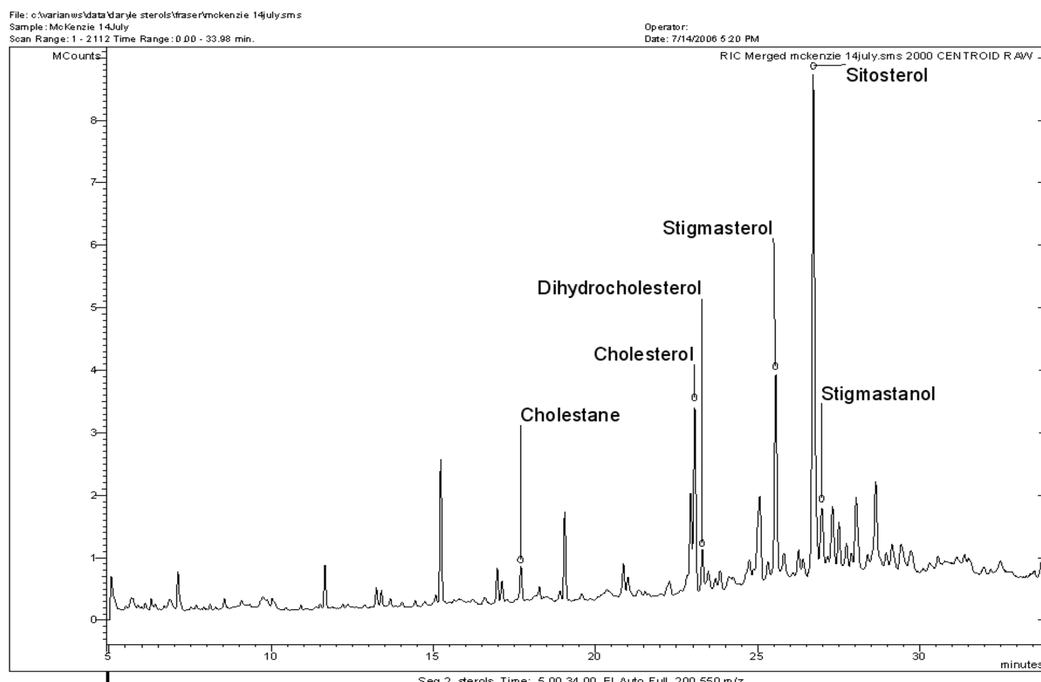
Sediment samples were collected from selected lakes and streams in glass bottles, and transported to the University on ice. They were stored at 4ºC and processed and analysed within 24 hours of collection. Processing included draining of excess water by vacuum filtration. Then each sediment sample was weighed and 50mL of hexane and 50mL of methanol added, together with one gram of sodium carbonate and cholestane internal standard. The samples were then tumbled for 24 hours. The mixtures were vacuum filtered on pre-washed filters, and the filtrate transferred to separating funnels. The lower, aqueous methanol layer was drained away. The hexane layer was washed with water and dried over sodium sulphate. Then the samples were evaporated under nitrogen gas and placed into a freeze drier for one hour at less then 1 milli-bar. The dried samples were then re-dissolved with 1 mL of hexane. To improve the volatility and stability of sterols in the extract and to improve detection limits on the GC-MS, they were converted to their O-trimethylsilyl derivatives prior to analysis through the addition of 20µL trimethylsilyl imidazole. The samples were allowed to stand for three hours before being analysed by GC-MS.

Water samples were collected in 10-liter high-density polyethylene (HDPE) containers. The containers were rinsed 3 times with water from ~15cm below the surface of each site before the samples were collected. The samples were then transported to USC and placed into cold storage at 4˚C, and processed and analysed within 36 hours. Each sample was filtered through 142mm/0.45micron glass fibre filter. The sterol-based lipids were extracted from the particulate matter trapped on the glass filters using a similar extraction process as was used above with the sediments.

Sample extracts were analysed on a Varian 3900 gas chromatograph (GC) equipped with a ZB-5ms-30m x 0.25mm capillary column. Technical details: Initial injector temperature was set at 320˚C and the split ratio was initially 50:1 and closed for 30 seconds on injection. The constant column gas flow was 1.0mL/min. The oven temperature was programmed at 200˚C on injection and increased at 20˚C/min to 240˚C, then increased at 3.0˚C/min to 320˚C and held for 5min. The total method run time was 33.67 min. The GC was coupled to a Varian Saturn 2100T mass spectrometer with a transfer line temperature of 310˚C, and compound ionization by 70eV electron impact. The positive fragment ions were analysed over a mass range 200 to 550m/z. The sterols /stanols of interest were identified by retention time against known standards and by their mass spectra. Sterol/stanol standards of cholestane, coprostanol, cholesterol, stigmasterol, sitosterol and sitostanol, were all purchased from Sigma Aldrich, Castle Hill NSW. The 5α-cholestanol and epicoprostanol were purchased from Steraloids inc, Newport Road Island USA.

Validation of the methodology was through the use of spiked samples. Known amounts of some of the standards were added to sediment and water samples and processed and extracted using the same techniques as above. The results from the spiked samples were compared to the unspiked.

An example GC-MS spectra for a sample of Lake McKenzie sediment is included below:



Results and discussion:
We were able to detect the sterol and stanol compounds at sufficient sensitivity and resolution to quantify the amounts and to distinguish the sources of these compounds in samples of lake sediments, but not in water samples. Results for the sampling on Fraser Island on December 5, 2005 are shown together with data, also collected by us, for the North Maroochy river in the Sunshine Coast.

	Lake McKenzie	Lake Birrabeen	Lake Allam	Wabby Lake	Ocean	Septic System	Nth Maroochy
Coprostanol	0.4	0	2.4	1	1	6000	231
Cholesterol	229	317	233	118	204	100000	151
5a-Cholestanol	51	0	25	0	0	5300	74
24-Et. Coprostanol	0	0	2	0	0	29000	96
Sitosterol	793	413	3319	2105	1881	22000	74
Sitostanol	0	418	383	239	406	2000	18
Ratio C27/C29	0.35	0.38	0.07	0.05	0.09	3.1	2.4

Ratio 5b/5a	0.007	0	0.01	0.003	0.003	12.2	3.5
Ratio C27/C29	Septic 3.1	Seagull 3.7	Duck 0.6	Swan 0.25	Possum 0.4	Roo 0.5	Dog 4.9
Ratio 5b/5a	12.2	6.9	0.8	0	13	3.6	0.6

Concentration units: ng/g

The data in the lower part of the table are from the previous studies in Australia (Leeming et al.,1998). This data shows that human faeces (septic systems) are high in coprostanol. Coprostanol is also the major sterol in pig faeces. Cattle, kangaroo, and possums also contain coprostanol in their faeces, but at far lower concentrations than humans. Leeming et al. (1998) note also that while not present in human faeces, epicoprostanol is generated in anaerobic septic and sewerage systems, and can thus be a marker of aged human sewage contamination. Cattle, kangaroo and possum herbivores had much lower C27:C29 ratios compared to humans. Bird species exhibit a broad dietary intake, but Leeming et al., 1998 and Walker et al., 1982 noted low 5α stanols, including coprostanol in a range of bird faeces. The 5β stanols were below detection in dog faeces. Invertebrates reportedly do not exhibit coprostanol in their faeces (Leeming et al., 1998), similarly for fish and reptiles.

Data from Fraser Island showed that the sterols and stanols were very low to low in the lake sediments and undetectable in the surface water samples. Ratios of C27 to C29 sterols, C27/C29, and that of 5β to 5α sterols, 5β/5α, indicated that water fowl are the likely predominate faecal source. Human input appeared to be negligible compared to natural sources on Fraser Island. In contrast, samples and data from the North Maroochy river on the Sunshine Coast gave higher levels of stanols and indicated that human septic emissions were probably contributing to the elevated levels and ratios.

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