ELSEVIER

Journal of Chromatography A, 733 (1996) 497–509

JOURNAL OF CHROMATOGRAPHY A

Use of capillary gas chromatography for measuring fecal-derived sterols Application to stormwater, the sea-surface microlayer, beach greases, regional studies, and distinguishing algal blooms and human and non-human sources of sewage pollution

Peter D. Nichols*, Rhys Leeming, Mark S. Rayner, Val Latham

Division of Oceanography, CSIRO, Marine Laboratories, GPO Box 1538, Hobart, Tasmania 7001, Australia

Abstract

The sterol composition, including the fecal biomarker coprostanol, from a variety of sample types was determined by capillary gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The coprostanol concentration in field samples readily provided an estimate of human fecal pollution. The technique was successfully used for stormwater, the sea-surface microlayer, beach sands and greases, and in regional studies of coastal sediments. Sterol profiles can be used to distinguish human and non-human sources of sewage pollution and algal blooms. Development of appropriate component ratios, both within the sterol fraction and between compounds classes, may provide a useful mechanism to further exploit sterol data. For sewage-containing samples, it may be possible to extend the data comparison, and calculation of key ratios, to include bacteriological parameters. Collectively, the use of sterol compositional data can complement other physical, chemical and biological measurements obtained in environmental studies.

Keywords: Water analysis; Sewage pollution; Environmental analysis; Sediments; Beaches; Sterols; Coprostanol

1. Introduction

Sewage tracers can be used to examine the distribution and transport of sewage in the environment [1]. One such tracer is the sterol coprostanol (5β-cholestan-3β-ol) which is produced in the digestive tract of higher animals and has been shown to be an excellent signature for sewage pollution. A chemical signature such as coprostanol also overcomes many of the shortcomings (e.g. die-off, lack of correlation with fecal pathogens) of classical microbiological indicators of sewage pollution (e.g. [2,3]).

Recent studies have highlighted the usefulness of coprostanol for examining sewage pollution in the marine environment. For example, the dispersal and accumulation of untreated sewage in the canals and lagoons of Venice was investigated [4], as has the transport of sludge-derived organic pollutants to deep-sea dump sites off the coast of

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00807-1

New Jersey [5] and the distribution of sewage contamination in Narragansett Bay [6]. In most studies coprostanol was analyzed by gas chromatography (GC) and/or gas chromatography–mass spectrometry (GC–MS) after extraction and fractionation of samples. In this overview we demonstrate the use of fecal sterols to measure sewage pollution for a variety of sample types (e.g. surface microlayer, beach sand and greases, sediments, particulate matter, stormwater). Samples were from a range of environments, collected mainly in waters off Sydney, Australia. Further background on the Sydney environment with respect to sewage disposal can be found in [7].

2. Experimental

2.1. Sample collection and reagents

Collection of human and animal fecal matter was performed by officers from AWT-Science and Environment, Water Board, Sydney. Samples were freeze-dried. Sample locations for creek and stormwater from the Sydney region are given in Fig. 1. Samples were received in 1-l containers and had been preconcentrated (from 100 l) by continuous membrane filtration. Sites were: Whale Beach stormwater drain, NH103; Malabar stormwater drain, SH71; Devlins Creek, LC235; Penrith STP, T46. Sea-surface microlayer samples were collected at Manly Beach, the North Head plume and at a control site (Long Reef) in November and December 1990 from the Manly region adjacent to the North Head cliff face outfall in Sydney coastal waters as previously described [8] (Fig. 1). Beach sand and grease samples were collected in September 1992 from Sydney beaches by NSW EPA officers and shipped on ice to Hobart. Ship-board sampling was conducted aboard RV Franklin during November 1989 (cruise FR13/89), May 1991 (FR4/91), January 1992 (cruise FR1/92) and December 1993 (cruise FR9/93). Station locations along the Malabar transect are shown in Fig. 1 (see also [8]). The Malabar deep ocean outfall is located approximately 3 km offshore

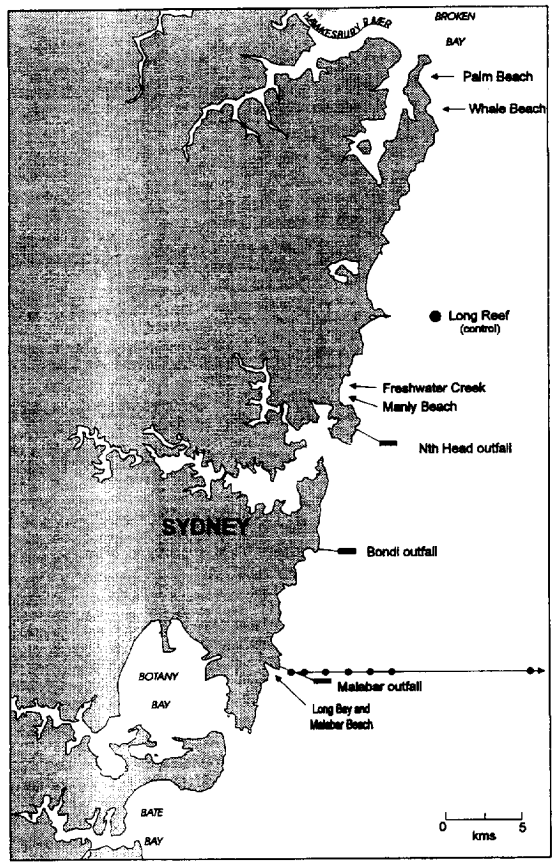

Fig. 1. Sampling sites in the Sydney, Australia region.

and just south of the 3.6 km station (Fig. 1). Sediments were collected using a Smith–McIntyre grab. Surface sediment (0–2 cm) was removed from the grab using a stainless-steel or a sterile plastic spoon. Algal bloom samples were collected in the Derwent Estuary and Orielton Lagoon, Hobart, Tasmania and water was filtered using glass-fibre filters (Whatman GF/F, nominal pore size 0.5 μm). All samples for chemical analyses were transported on ice to the CSIRO Marine Laboratories in Hobart and were stored immediately at −20°C for subsequent analyses.

Nanograde solvents and reagents (Mallinkrodt) were used in the lipid procedures. All glassware was prerinsed with nanograde solvent prior to use. Concurrent analyses of laboratory blanks were undertaken during sample analysis.

2.2. Lipid extraction and fractionation

Samples were extracted quantitatively by the modified one-phase $CHCl_3$–MeOH Bligh and Dyer method [9,10]. After phase separation, the lipids were recovered in the lower $CHCl_3$ layer (solvents were removed in vacuo) and were made up to a known volume and stored under nitrogen at −20°C. Total sterols were obtained following alkaline saponification of a measured aliquot of the total lipids [11]. Products were extracted into hexane–$CHCl_3$ (4:1, v/v) and stored at −20°C. Sterols were converted to their corresponding trimethylsilyl (TMSi) ethers by treatment with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (50 μl, 60°C, 60 min).

2.3. Gas chromatography and gas chromatography–mass spectrometry

Gas chromatographic (GC) analyses were performed with a Hewlett-Packard 5890 GC equipped with a 50 m × 0.32 mm I.D. cross-linked methyl silicone (0.17 μm film thickness) fused-silica HP1 capillary column (Hewlett-Packard), a flame ionization detector (FID) and a split/splitless injector. After addition of methyltricosanoate internal standard, samples were injected (2 μl out of typically 50–1000 μl of sample) in the splitless mode at 50°C; after 1 min the oven temperature was raised to 150°C at 30°C/min, then to 250°C at 2°C/min, and finally to 300°C at 5°C/min. Hydrogen was used as the carrier gas (inlet pressure 70 kPA, 1 ml/min). Selected samples were also analyzed with an HP5 column using similar conditions to those used for the HP1 column. For short (<20 min) GC assays performed using the HP1 column, an initial oven temperature of 200°C was employed and the oven was temperature programmed to 280°C at 10°C/min, then to 310°C at 2°C/min which was then maintained for 5 min. Sterol concentrations were quantified from peak areas (GC–FID assays) determined using chromatography software (DAPA Scientific software) operated using an IBM compatible computer. Sterol identifications were based on comparison of relative retention time data [11] with data obtained for authentic and laboratory standards. Coprostanone eluted after both coprostanol and epicoprostanol under the GC conditions used.

The FID response was found to be linear in the practical concentration range (0.5–150 ng of individual components injected) used. Samples were routinely analyzed by GC within 1–2 weeks of extraction. Prior to instrumental analysis samples were stored in solvent and were not allowed to go to dryness. During a typical column lifetime (6–12 months), repeat analysis indicated that minimal loss of coprostanol occurred as long as routine injector maintenance was performed. Although synthetic coprostanol was not available, analysis of replicate samples of sewage effluent indicated good agreement for within-day, day-to-day, and longer-term variation samples. The relative standard deviation for replicate samples was generally 25% or better. Duplicate GC assays of the same sample showed 1–10% standard deviation over the concentration range used.

Identification of sterols was verified by GC–MS analysis of samples performed on either an HP 5890 GC and 5970 mass selective detector or a FISONS MD800 GC–MS. Operating conditions are described in detail elsewhere [12,13]. The non-polar column was similar to that described above. The mass spectra of coprostanol and coprostanone were distinguishable.

3. Results and discussion

3.1. General considerations

Use of the fecal sterol method for assessing sewage pollution can be compared to that of the traditional enumeration of fecal coliform and other bacteria that is commonly used to determine sewage contamination. In a 1992 survey of the Sydney inner-shelf region, good correlations were observed for coprostanol and *Clostridium perfringens* spores (linear regression) for water ($r^2 = 0.97$) and sediments ($r^2 = 0.96$) [7]. The correlations were not as good for other indicators (fecal coliforms, $r^2 = 0.8$ for water, 0.91 for sediments; streptococci, $r^2 = 0.79$ for

sediments with no relationship observed for coprostanol and fecal streptococci in water). Although the use of fecal coliforms has been questioned due to their variable die-off in natural waters, the water depth and associated lower light penetration for bottom water samples from Sydney's inner-shelf environment may have reduced die-off. This feature might explain the better correlation observed between coprostanol and fecal coliform abundance when results for the Sydney inner-shelf region are compared to other studies. It was proposed that the findings from the Sydney study validated the use of the coprostanol methodology for estimating sewage pollution for this environment [7]. The results also highlight the potential usefulness of the methodology for application to environmental studies in other regions.

3.2. Distinguishing human and non-human sources of sewage pollution

Detailed analyses have been performed of the sterols of fecal matter from humans and other animals that contribute to urban or rural catchments (e.g. sheep, dogs, cats, pigs, birds) [14]. Results for several animals will be described briefly here. The concentration (μg/g, dry weight) of individual sterols varied markedly between animals and in several cases within groups of animals.

Total sterol content in human feces was 3000–8800 μg/g. The concentration of sterols in human feces was around a factor of five greater than in most other samples. The coprostanol concentration in human feces was also greater (by one to three orders of magnitude) than in other animals. Major sterols in human feces were: coprostanol, ethylcoprostanol, cholesterol, 5β-epistigmasterol, 24-ethylcholesterol and 24-ethylcholestanol (Fig. 2 and Table 1). This differs from effluent, where coprostanol > cholesterol > 24-ethylcoprostanol > 24-ethylcholesterol; the differences may be due to the effect of the microbial community of the treatment plant on the sterols of sewage-derived organic matter. C_{27} sterols were present in human feces at higher levels than C_{29} sterols, whereas the opposite was

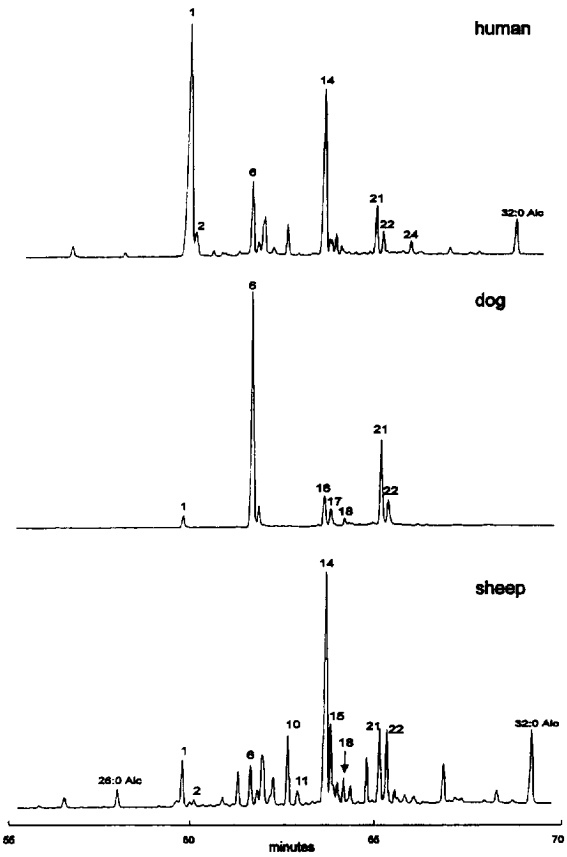

Fig. 2. Partial gas chromatograms of sterols from human, dog and sheep feces. Peak numbers refer to Table 1. HP1 capillary column. Alc denotes long-chain alcohol.

observed for herbivores and other omnivores (e.g. birds) (Fig. 2).

The sterol profile of pig feces was similar to that of human feces, although the absolute concentration of sterols was lower. A difference was that the levels of C_{29} sterols, including 24-ethylcholestanol, were greater than those of C_{27} sterols. For birds, the major sterols generally were: 24-ethylcholesterol, 24-ethylcholestanol, 24-ethylcoprostanol, 24-methylcholesterol and cholesterol. Coprostanol was absent or present at low levels.

For herbivores such as cows and sheep (Fig. 2), major fecal sterols were: 24-ethylcholesterol, 24-ethylcoprostanol, 24-ethylcholestanol, 24-methylcholesterol, cholesterol and coprostanol. C_{29} sterols were generally in greater concen-

Table 1
Sterol names and peak identification numbers

Peak number	Sterol	Common name
1	5β-cholestan-3β-ol	coprostanol
2	5β-cholestan-3α-ol	epicoprostanol
3	5β-cholestanone	coprostanone
4	cholesta-5,22E-dien-3β-ol	dehydrocholesterol
5	cholest-22E-en-3β-ol	dehydrocholestanol
6	cholest-5-en-3β-ol	cholesterol
7	5α-cholestan-3β-ol	cholestanol
8	24-methylcholesta-5,22E-dien-3β-ol	brassicasterol
9	24-methyl-5α-cholest-22E-en-3β-ol	brassicastanol
10	24-ethyl-5β-cholest-22E-en-3β-ol	5β-stigmastanol
11	24-ethyl-5β-cholest-22E-en-3α-ol	5β-epistigmastanol
12	4α-methyl-5α-cholest-7-en-3β-ol	lophenol
13	24-methylcholesta-5,24(28)E-dien-3β-ol	24-methylenecholesterol
14	24-ethyl-5β-cholestan-3β-ol	24-ethylcoprostanol
15	24-ethyl-5β-cholestan-3α-ol	24-ethylepicoprostanol
16	24-methylcholest-5-en-3β-ol	24-methylcholesterol
17	24-methyl-5α-cholestan-3β-ol	24-methylcholestanol
18	24-ethylcholesta-5,22E-dien-3β-ol	stigmasterol
19	24-ethyl-5α-cholest-22E-en-3α-ol	stigmastanol
20	4,24-dimethyl-5α-cholest-22E-en-3β-ol	
21	24-ethylcholest-5-en-3β-ol	24-ethylcholesterol
22	24-ethyl-5α-cholestan-3β-ol	24-ethylcholestanol
23	24-ethylcholesta-5,24(28)E-dien-3β-ol	isofucosterol
24	24-ethylcholest-7-en-3β-ol	
25	4,24-dimethyl-5α-cholestan-3β-ol	
26	4,23,24-trimethylcholesta-5,22-dien-3β-ol	dehydrodinosterol
27	4,23,24-trimethyl-5α-cholest-22-en-3β-ol	dinosterol
28	4,23,24-trimethyl-5α-cholest-24(28)-en-3β-ol	
29	4,23,24-trimethyl-5α-cholest-7-en-3β-ol	
30	4,23,24-trimethyl-5α-cholestan-3β-ol	dinostanol

Peak numbers refer to Figs. 2–6. C_{24} stereochemistry not determined.

tration than C_{27} sterols for most herbivores. Sheep could be distinguished from other herbivores based on the presence of higher relative levels of 24-ethylepicoprostanol. Cats and dogs (carnivores) differed markedly in their sterol composition. Cat feces contained coprostanol, cholesterol, 24-ethylcoprostanol, 24-ethylcholesterol and 24-ethylcholestanol, whereas in dog feces, cholesterol, 24-ethylcholesterol and 24-methylcholesterol were abundant; coprostanol was only a trace component. C_{27} sterols were present at higher levels than C_{29} sterols in both carnivores in comparison to herbivores and omnivores.

The source specificity of fecal biomarkers is due to a combination of diet, biosynthesis and biotransformations. The combination of these factors determines 'the sterol fingerprint' of fecal matter. Examination of sterol profiles from animal feces indicates that there can be considerable differences between warm-blooded animals. It should be possible to exploit such differences to distinguish sources of fecal pollution in aquatic environments.

3.3. Algal blooms

In summer field studies of estuarine waters, we have determined the spatial or temporal distribution of the fecal sterol coprostanol; algal

blooms also were observed during several studies and the simultaneous measurement of sewage-derived and algal-derived sterols provided an insight into the environment under investigation. The Derwent estuary in Hobart (population approximately 180 000) has 13 sewage treatment plants along its shores. Effluent of varying levels of treatment enters the estuary from these point sources. In recent years, blooms of the toxic dinoflagellate *Gymnodinium catenatum* have occurred during summer [15]. Although nutrients, and other components, derived from sewage may not be the primary factors triggering blooms, such inputs may affect bloom intensity. Examination of sterol profiles for Derwent River particulate matter collected during the summer period confirmed the presence of sewage-derived organics and also revealed the presence of blooms of *G. catenatum* based on the distinctive sterols present in this alga [15] (Fig. 3; Table 2).

In similar studies at Orielton Lagoon near Hobart, assay of water column particulate matter also revealed, along with the presence of coprostanol, the occurrence of algal-derived sterols, C_{30} and C_{32} long-chain diols and unsaturated alcohols and other possibly related unidentified novel components (Fig. 3). In taxonomic investigations, the various components have been restricted to members of the Eustigmatophyta [16]. Their presence in samples from Orielton Lagoon provided an early indication of the identity of the bloom-forming alga in this environment.

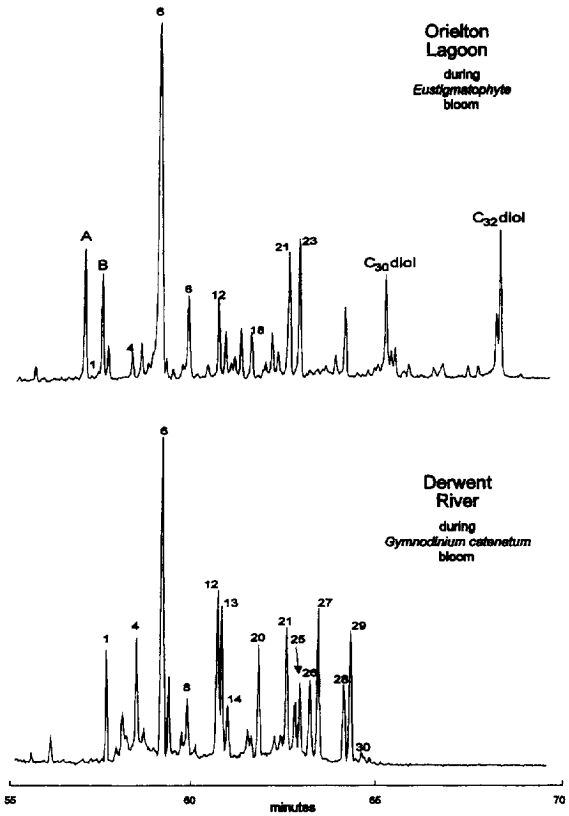

Fig. 3. Partial total ion mass chromatograms of sterols from algal-bloom samples from Derwent River and Orielton Lagoon, Hobart. Peak numbers refer to Table 1. HP1 capillary column. Peaks A and B are unidentified. Sterol 21 also contained fucosterol.

3.4. Stormwater

There is considerable debate on whether fecal pollution is derived from sewage or other sources, including stormwater, for a range of locations in Australia. The potential exists to determine the source of such inputs; however, limited data exists on the sterols of stormwater and related samples. Total sterol content of Whale Beach and Malabar Beach stormwater (dry weather conditions) and Devlins Creek samples varied between 0.04–2.3 μg/l (Table 2). Representative gas chromatograms illustrating sterol profiles are shown in Fig. 4. The concentration of total sterols for these three sites showed the trend, Whale Beach stormwater > Devlins Creek > Malabar Beach stormwater. An exception was that the sterol concentration in Whale Beach 3 stormwater (November 5) was lower than for all three Devlins Creek samples. Differences were observed at some sites over the three samplings. The greatest difference, a factor of six, was for Whale Beach, whereas samples from Malabar Beach showed little difference in total sterol concentration between samplings. The Penrith STP samples contained higher absolute concentrations of total sterols (mean 1650 μg/l; Table 2) than measured for stormwater and Devlins Creek water.

All stormwater and Devlins Creek samples

Table 2
Sterol, including the fecal biomarker coprostanol, concentration for human, animal, beach grease, microlayer and algal-bloom samples

Site	Sterol concentration[a]					Total sterols		Enrichment
	1	6	14	21	27	(ng/g)	(ng/l)	factor
Human and animal feces (January and February, 1994) (μg/g)								
Human ($n = 6$)	3430	290	1250	121	–	5610	–	–
Effluent (STP, $n = 7$)	401	270	66	44	–	880	–	–
Sheep ($n = 6$)	270	111	246	196	–	1310	–	–
Dog ($n = 7$)	8	1570	–	274	–	2190	–	–
Cat ($n = 5$)	397	750	97	240	–	1770	–	–
Bird (seagull, $n = 3$)	13	746	132	67	–	1020	–	–
Stormwater and related samples (October and November, 1992) (ng/l)								
Penrith STP effluent ($n = 3$)	737	411	170	63	–	–	1650	–
Malabar stormwater ($n = 3$)	0.0056	0.034	0.0041	0.018	–	–	0.11	–
Whale Beach stormwater								
replicate 1 (WB1)	0.98	0.25	0.39	0.12	0.025	–	2.28	–
replicate 2 (WB2)	0.097	0.48	0.17	0.27	0.017	–	1.65	–
replicate 3 (WB3)	0.0078	0.12	0.021	0.074	–	–	0.39	–
Devlins Creek water ($n = 3$)	0.032	0.29	0.046	0.28	–	–	1.05	–
Beach grease (September, 1991) (ng/g)								
Seven Mile Beach sand ($n = 3$)	–	21.7	5.4	14.0	8.8	113	–	–
Palm Beach ($n = 3$)	–	28.4	8.1	24.3	8.7	152	–	–
Freshwater Beach								
sand ($n = 3$)	24	51.5	29.8	39.2	17.4	363	–	–
grease ($n = 3$) (μg/g)	7.8	38.5	11.9	66.7	3.4	147	–	–
Microlayer (November and December, 1990) (μg/l)								
Manly 1 (6/11/90)								
surface	48.2	30	13.4	7.4	0.62	–	124	40
subsurface	1.2	0.70	0.34	0.18	0.025	–	3.5	–
Manly 2 (6/11/90)								
surface	3.1	2.0	0.87	0.95	0.091	–	10.1	2.4
subsurface	1.3	0.82	0.35	0.21	0.031	–	3.9	–
North Head plume (6/11/90)								
surface	29.3	14.3	7.6	3.3	0.13	–	63.7	0.82
subsurface	36.9	18.7	9.6	4.3	0.23	–	77	–
Control 1 (27/11/90)								
surface	0.11	0.41	0.038	0.45	–	–	1.4	>10
subsurface	–	0.10	0.009	0.22	0.017	–	0.3	–
Control 2 (27/11/90)								
surface	0.06	1.06	0.11	0.19	0.11	–	3.5	>10
subsurface	–	0.082	0.009	0.024	0.18	–	0.3	–
Control 3 (5/12/90)								
surface	0.086	0.51	0.44	0.041	0.035	–	1.2	>10
subsurface	–	0.032	–	0.19	–	–	0.2	–
Algal blooms (μg/l)								
Derwent River *G. catenatem* (March, 1990)	0.11	0.58	0.0003	0.357	0.27	–	3.5	–
Orielton Lagoon Unidentified Eustigmatophyte (November, 1993)	0.19	53.2	–	5.86	–	–	94.6	–

[a] Note for feces, microlayer and bloom samples, sterol concentrations in μg/g or μg/l.
Sterol: 1 = coprostanol; 6 = cholesterol; 14 = 24-ethylcoprostanol (may include 24-methylcholesterol for some samples); 21 = 24-ethylcholesterol; 27 = dinosterol. Peak numbers refer to Figs. 2–7 and Table 1.
Enrichment factor for coprostanol (microlayer samples): surface/subsurface.

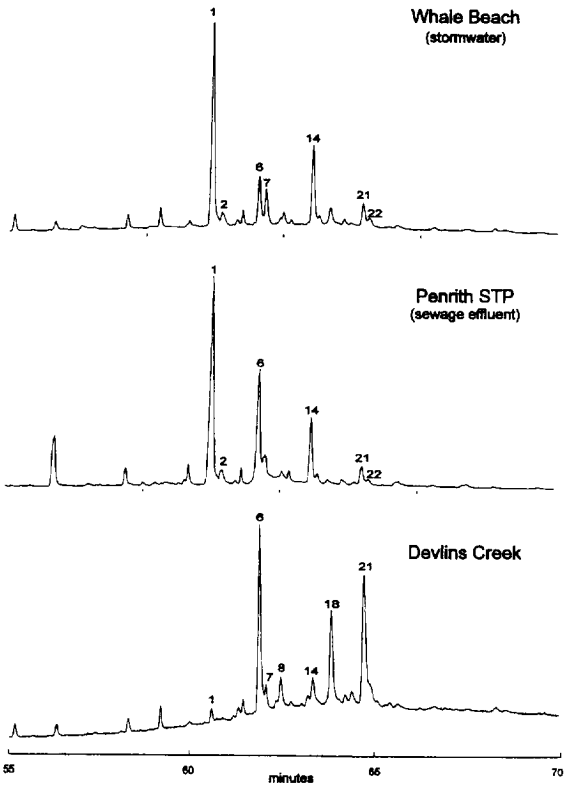

Fig. 4. Partial gas chromatograms of sterols from Whale Beach stormwater, sewerage effluent and Devlins Creek water from the Sydney region. See also Fig. 1. Peak numbers refer to Table 1. HP1 capillary column.

contained coprostanol (0.006–1 μg/l, 2–43% of total sterols, Table 2). Whale Beach 1 (October 10) was the only stormwater or creek water sample that contained high relative and absolute levels of coprostanol (43%, 1 μg/l); the level was of the order measured for plume samples collected adjacent to the Malabar deep water ocean outfall [7]. The concentration of coprostanol was: Whale Beach stormwater > Devlins Creek > Malabar Beach stormwater. The detection of coprostanol in all samples indicated that sewage-derived material is present in stormwater and waters of Devlins Creek, albeit at very low absolute levels relative to sewage effluent.

The concentration of coprostanol in Penrith STP sewage effluent was 740 μg/l (Table 2); this value is between three and five orders of magnitude greater than in stormwater and Devlins Creek samples. Under dry weather conditions, stormwater drains and urban creeks therefore would be only a very minor source of sewage-derived material in the Sydney region. The coprostanol concentrations for Penrith STP effluent were generally 2–3 times greater than for Malabar effluent (200–350 μg/l) ([3] and related studies). The higher coprostanol concentrations for Penrith effluent are consistent with a higher suspended-solids loading at this STP compared to Malabar; an association between coprostanol and suspended-solids load and/or treatment efficiency has been noted [17].

Besides coprostanol, major sterols detected in the stormwater and Devlins Creek samples were cholesterol, cholestanol, brassicasterol, 24-methylcholesterol, 24-ethylcholesterol and 24-ethylcholestanol. The sterol profile for Penrith effluent differed from stormwater and creek samples (Fig. 4, Table 2); coprostanol, cholesterol, 24-ethylcoprostanol and 24-ethylcholesterol were the major components. The generally higher proportion of 24-ethylcholesterol in stormwater and Devlins Creek samples reflects greater contributions from terrestrial vegetation in these samples. Higher plants typically contain higher relative and absolute concentrations of C_{29} sterols than are present in other sources of organic matter [11,18].

3.5. Beach sands and greases

The occurrence of beach greases was of major public concern prior to the commissioning of Sydney's deep ocean outfalls. Although their incidence has reduced markedly since the deep ocean outfalls have been in operation, interest still exists in their occurrence, formation and source. Sterol content of beach sands from the Sydney region (Fig. 5) and from Seven Mile Beach (control site remote from Sydney), varied between 65–440 ng/g (mean 110–360 ng/g, dry weight basis, Table 2). The concentration of sterols was: Freshwater Beach > Palm Beach > Seven Mile Beach. The Freshwater Beach greases contained 2–4 orders of magnitude greater amounts of sterols (147×10^3 ng/g) than the beach sands (Table 2).

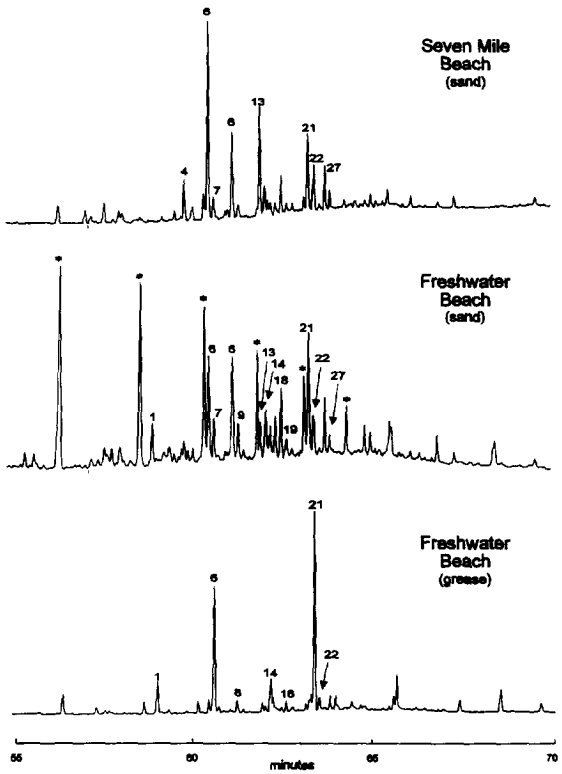

Fig. 5. Partial gas chromatograms of sterols from Seven Mile Beach and Freshwater Beach sands and a sample of Freshwater Beach grease from the Sydney region. See also Fig. 1. Peak numbers refer to Table 1. HP1 capillary column. Peaks marked '*' are *n*-alkanes.

Coprostanol was not detected in the Seven Mile and Palm beach sands (Table 2, Fig. 5). Although samples were collected on only one day, this finding is consistent with the greater distance of these sites from possible point sources. The Freshwater Beach sand and grease samples contained appreciable relative levels of coprostanol (Table 2); the relative level of coprostanol in the Freshwater Beach sand (5.7–8.0%, mean 6.6%) was similar to beach grease at the same site (4.–7.3%, mean 5.3%). The concentration of coprostanol was 24 ng/g in the Freshwater Beach sand and 7.8×10^3 ng/g in the Freshwater Beach grease samples. Theoretically, unpolluted sediments do not contain coprostanol [19]. Our experience also supports this view in practice. Analyses of a range of Australian sediments and sands have not detected coprostanol in samples collected from pristine locations (unpublished data). The results obtained for Freshwater Beach are therefore indicative of significant sewage pollution occurring at this location at the time of sampling. The detection of coprostanol in Freshwater Beach sand indicates that sewage can impact the beach even after commissioning of the deep ocean outfalls.

Besides the striking differences observed between locations in the abundance of coprostanol, variations were also observed in the relative levels of other sterols (Table 2, Fig. 5). Freshwater Beach grease contained higher levels of 24-ethylcholesterol (42–51%) than all sand samples (8.6–20.6%). Similarly, the Freshwater Beach grease samples showed lower relative levels of stigmasterol than the sands. The results for these two sterols are consistent with a greater contribution of vegetable-derived oils in the Freshwater Beach grease; many vegetable oils show a similar ratio of these two sterols.

Beach sands contained a number of marine (algal) derived sterols such as dinosterol (from dinoflagellates), brassicasterol (diatoms), and 22-dehydrocholesterol (diatoms). Differences in the relative levels of these sterols between locations indicate differing levels of the source organisms at the beaches [11,20]. Beach greases contained lower relative levels of these three sterols. Of interest was the observation that high levels of degraded and undegraded petroleum hydrocarbons were present in several beach sands (Fig. 5); the estimation of fecal contamination using coprostanol and the GC methodology allows simultaneous measurement of hydrocarbon input.

3.6. The sea-surface microlayer

Of concern with sewage outfalls is the enrichment of pollutant materials at the sea-surface interface and the resulting effects this may have on biota, including larvae. To better characterize the composition of the surface microlayer from Sydney waters, the sterol profiles were determined for samples collected prior to the commissioning of the deep ocean outfalls. Sterol content

of the microlayer and subsurface particulate matter from sites adjacent to the North Head sites ranged from 0.2–124 μg/l (Table 2). Particulates from the Manly Beach 1 site contained the highest concentration of microlayer total sterols (124 μg/l). Apart from the plume samples where higher amounts of sterols were present in the subsurface, other sites contained higher sterol concentrations in the microlayer. Sterol abundance for the microlayer samples showed: Manly 1 > plume > Manly 2 > control. For the subsurface, plume > Manly 1 and 2 > control.

Coprostanol was present in all plume and Manly Beach microlayer and subsurface samples. Coprostanol was present at a lower concentration in the Manly Beach 2 sampling. Coprostanol was detected in all control microlayer samples (Table 2). As noted earlier, unpolluted waters do not contain coprostanol. As coprostanol is generally not present in particulate matter from pristine waters [7,19], the amount and relative proportion of coprostanol provides a measure of sewage contamination. The results obtained for the plume and Manly Beach particulate samples are therefore indicative of gross sewage pollution. Coprostanol was the major sterol detected in both the microlayer and subsurface samples at the plume and Manly Beach sites; the ratio of coprostanol to cholesterol in these samples was between 1 and 2. The detection of low concentrations of coprostanol in control microlayer samples shows that sewage effluent was also impacting these more remote locations at the time of sampling.

The ratio coprostanol/cholesterol in microlayer and subsurface particulate matter at the control site was less than 0.1 or lower. As cholesterol and other alkylated sterols are abundant in marine organisms, the higher relative abundance of these sterols is an indication of a greater marine contribution to the microlayer and subsurface particulate matter at the control site.

Coprostanol concentrations in Sydney sewage effluent are typically 200–400 μg/l. Using these data, the following dilution factors (relative to effluent) based on coprostanol concentrations can be calculated: 2–4 for the plume microlayer and subsurface and Manly Beach 1 microlayer samples; 10^1–10^2 for Manly Beach 1 subsurface and both Manly Beach 2 samples; for the controls, 10^3 or greater for microlayer and subsurface samples. The detection limit was approximately 10 ng/l. For the subsurface control samples where coprostanol was not detected, the dilution factor is greater than 10^4. In other studies, we have measured water column coprostanol concentrations at an order of magnitude lower than in this study (due to larger water volumes). This corresponds to dilution factors of 10^5. Enrichment factors for the microlayer relative to the underlying bulk water can be calculated (Table 2). The estimation of enrichment factors provides a measure of the degree of enrichment of a range of lipophilic compounds, including toxicants such as polyaromatic hydrocarbons (PAHs).

Other 5β-stanols, e.g. epicoprostanol and 24-ethylcoprostanol, were present in particulate matter. The stanol/sterol ratio for 24-ethylcoprostanol was similar to that observed for coprostanol/cholesterol, indicating that sewage is also the predominant source of 24-ethylcoprostanol. This sterol is often not separated from 24-methylcholesterol and is therefore not always used as a biomarker in environmental studies. It is evident that quantitation of coprostanol provides an estimate of sewage contribution to microlayer particulate matter. The ratio of coprostanol to total sterols and other individual sterols should prove useful in future investigations examining sewage distribution.

3.7. Regional studies

Coprostanol concentrations (ng/g) were determined for sediments collected offshore from Malabar, Sydney in November 1989, May 1991, January 1992 and December 1993 (Fig. 6). The latter three samplings were after commissioning of the deep water outfalls. The aims of these regional surveys were to map the distribution of sewage-derived material and to examine spatial and temporal variations that may occur.

During 1992 and 1993, high levels of sewage-derived organic matter were detected in inner-

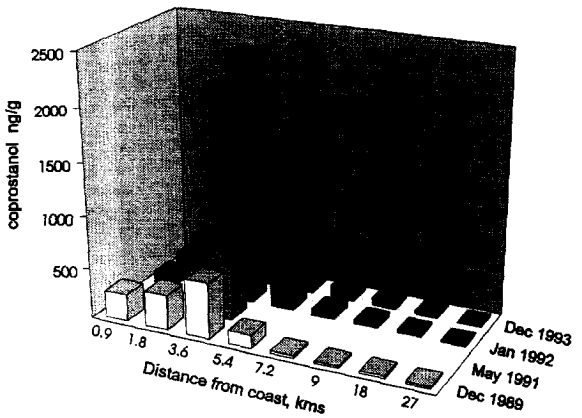

Fig. 6. Plot of sedimentary coprostanol concentration (ng/g) for the Malabar transect: November 1989, May 1991, January 1992 and December 1993. The Malabar deep ocean outfall is located approximately 3.5 km offshore (Fig. 1) and was commissioned in September 1990.

shelf sediments from north of Sydney Harbour to south of Botany Bay. The areas most affected were within 2–4 km of the deep water outfalls. The 1992 and 1993 results indicate that since the commissioning of the outfalls, most inner-shelf sediments (1–10 km) have shown an increase in the concentration of coprostanol; the areas of highest concentration were located in sediment adjacent to the deep water outfalls (Fig. 6); dispersion of sewage-derived matter further offshore (and on-shore to beaches) is minimal, rather this material may be moving in a north–south direction [7]. Based on coprostanol results for the Malabar transect, no further accumulation of sewage-derived material occurred in this region, as indicated by comparing the 1993 results to those of 1992. Of interest is that during the 1993 sampling, the highest concentration of coprostanol (1850 ng/g) in sediment was 1.8 km further inshore of the outfall than in 1992 (Fig. 6). This finding is consistent with the occurrence of westward bottom currents in this zone during or prior to the 1993 sampling.

The distribution of coprostanol in sediments provides an integrated picture of the variation of currents over longer periods in comparison to water column sampling. This variability may explain the changes between the 1992 and 1993 samplings. Over the nine days that RV Franklin was in the Sydney region during December 1993, surface (and midwater) currents were variable; currents ranged from 1 knot southward, to weaker on-shore currents, then turning to northward at up to 0.7 knots during the final days of the cruise. Directional variability of the currents off Sydney suggest that sewage-derived material is not always swept from this region; rather this material may remain in the vicinity of the outfalls (or may move towards shore; see 1993 results, Fig. 6) being deposited in sediments, as shown by the coprostanol distribution.

For comparison, coprostanol concentrations of 100 and 1100 ng/g were determined for sediments collected in 1989, prior to the commissioning of the deep water outfalls, from sites in a small bay immediately south of Malabar (Long Bay) and adjacent to the Malabar cliff face outfall [21]. Coprostanol concentrations greater than 500 ng/g are considered to be indicative of significant sewage pollution. Sewage sludge contains around 10^6 ng/g coprostanol. Using these data, the concentration of coprostanol in sediments within 0.5 km from the Malabar deep water outfall (1992, 2350 ng/g; 1993, 1300 ng/g) correspond to 1 in 400 (0.25% of sludge) and 1 in 750 (0.13%) dilutions of sludge.

Coprostanol is mainly associated with fine sediments, as are generally most microbes, metals and organic molecules, including toxicants. This is the case for sediments in the Sydney region; in the 1992 and 1993 Sydney surveys, sites with the highest levels of fines and organic carbon generally contained the highest concentrations of coprostanol ([7] and unpublished data).

Sedimentary material (predominately fine particles) was observed on filter samples collected from bottom waters under the weather (mainly fine, wind speeds less than 20 knots) and current conditions encountered in December 1993. This finding indicates that sediment transport, at least of fine particles, also occurs during periods of less intensity than suggested earlier [22], including the possible influence of westerly currents based on coprostanol results (Fig. 6). A better knowledge of the consequences of storm events with respect to transport of sewage-derived sedi-

mentary matter will be useful to understanding the ultimate fate of Sydney's sewage effluent.

Recommendations for future studies include that sediment cores be analyzed to determine historical inputs; the use of parallel dating and other assays (e.g. sediment trapping, particle size, and degradation, including half life, measurements) would increase our understanding of sedimentary processes. Comparison of the distribution of the fecal sterol coprostanol with algal-derived sterols (e.g. dinosterol from dinoflagellates) in sediments and water may provide information on the effect of sewage effluent on possible bloom-forming organisms.

3.8. Rapid assay of fecal sterols

We have compared recovery of coprostanol and cholesterol from environmental samples for supercritical fluid extraction (SFE) and conventional solvent extraction. Preliminary results showed that SFE can be used as a rapid, quantitative and reproducible extraction method for fecal sterols [23]. SFE provided recoveries of coprostanol and other sterols comparable with solvent extraction; an extraction time of approximately 20 min was sufficient. SFE offers the capability of direct interfacing to GC and/or GC–MS. In addition, development of sequential extraction procedures may allow other lipid classes and compounds of interest to be obtained from the same sample (e.g. phospholipids, hydrocarbons, PAHs, tocopherol). GC assays of fecal sterols, as described in earlier sections, required ca. 70–90 min to resolve earlier eluting components present in the non-saponifiable neutral lipid fraction. The use of a rapid SFE method encouraged the use of a shorter GC assay time. Using the same GC column and gas chromatograph, a shorter assay was developed; sterols eluted between approximately 10–15 min (Fig. 7). The development of rapid SFE-based methods for analyzing fecal sterols and other components will enable such compounds to have wider application in urban-effluent studies and wastewater management.

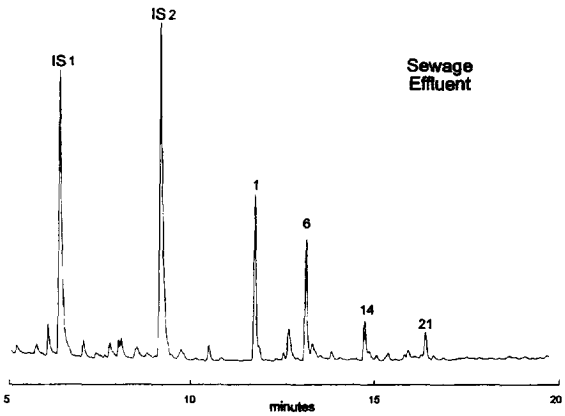

Fig. 7. Partial gas chromatogram (short temperature program) of sterols from SFE of sewage effluent. Peak numbers refer to Table 1. HP1 capillary column. IS1 and IS2 denote internal standards (C_{23} fatty acid methyl ester and 5α-cholestane).

4. Conclusions

The present work establishes a benchmark for future regional studies in Sydney and other coastal waters and provides reference data for sterols from a range of environments. In particular, the fecal biomarker coprostanol is shown to be a suitable biomarker for detection of sewage pollution in the environment, and further validates the use of the technique for field applications. Development of appropriate component ratios both within and between compound classes may be a useful mechanism to further exploit such data. These ratios possibly could be used, for example, to distinguish stormwater and sewage inputs. Collectively, sterol data can complement other physical, chemical and biological measurements obtained in studies of urban and regional catchments.

Acknowledgements

Our thanks to: the master and crew of RV Franklin; fellow CSIRO colleagues, in particular the ORV Hydrochemistry group; AWT-Science and Environment (Nick Ashbolt and Andrew Ball), NSW EPA, ANSTO and University of

Western Sydney during field programs; George Cresswell for ongoing support; Stephanie Barrett, Ed Butler, John Church, Peter Craig, Quentin Espey, Peter Franzmann, Christobel Ferguson, Gary Jones, Adrian Heggie, Carol Mancuso, Sarah Nespolo, Teresa O'Leary, Jan Peterson, Paul Rendell, Andy Revill, Martin Riddle, Jennifer Skerratt, John Volkman, and others. Part of this work was funded by AWT-Science and Environment and NSW EPA.

References

[1] C.M.G. Vivian, Sci. Total Environ., 53 (1986) 5.
[2] J.W. Readman, M.R. Preston and R.F.C. Mantoura, Mar. Pollut. Bull., 17 (1986) 298.
[3] P.D. Nichols and R. Leeming, Chem. Aust., 58 (1991) 274.
[4] M.R. Sherwin, E.S. Van Vleet, V.U. Fossato and F. Dolci, Mar. Pollut. Bull., 26 (1993) 501.
[5] H. Takada, J.W. Farrington, M.H. Bothner, C.G. Johnson and B. Tripp, Environ. Sci. Tech., 28 (1994) 1062.
[6] L.A. LeBlanc, J.S. Latimer, J.T. Ellis and J.G. Quinn, Estuarine Coastal Shelf Sci., 34 (1992) 439.
[7] P.D. Nichols, R. Leeming, M.S. Rayner, V. Latham, N.J. Ashbolt and C. Turner, J. Chromatogr., 643 (1993) 189.
[8] P. Rendell and Q. Espey, Environmental Monitoring Program Precommissioning Phase, Vol. 8, Microlayer, 1993.
[9] E.G. Bligh and W.M. Dyer, Can. J. Biochem. Physiol., 35 (1959) 911.
[10] D.C. White, R.J. Bobbie, J.S. Herron, J.D. King and S.J. Morrison, in J.W. Costerton and R.R. Colwell (Editors), Native Aquatic Bacteria: Enumeration, Activity and Ecology, ASTM STP 695, Am. Soc. Test. Mater., Philadelphia, 1979, pp. 69–81.
[11] G.J. Jones, P.D. Nichols and P.M. Shaw, in M. Goodfellow and A.G. O'Donnell (Editors), Chemical Methods in Prokaryotic Systematics, John Wiley, 1994, pp. 163–195.
[12] D.S. Nichols, T.A. McMeekin and P.D. Nichols, Microbiology, 140 (1994) 577.
[13] S.M. Barrett, J.K. Volkman, G.A. Dunstan and J.-M. Leroi, J. Phycol., 31 (1995) 360.
[14] R. Leeming, A. Ball, N.J. Ashbolt, G.J. Jones and P.D. Nichols, Chem. Aust., 61 (1994) 434; R. Leeming and P.D. Nichols, Water Res., in press.
[15] G. Hallegraff, P.D. Nichols, J.K. Volkman, S. Blackburn and D. Everitt, J. Phycol., 27 (1991) 591.
[16] J.K. Volkman, S.M. Barrett, G.A. Dunstan and S.J. Jeffrey, Org. Geochem., 18 (1992) 131.
[17] D.V. McCalley, M. Cooke and G. Nickless, Water Res., 15 (1981) 1019.
[18] L.J. Goad and T.W. Goodwin, Prog. Phytochem., 3 (1972) 113.
[19] P.G. Hatcher and P.A. Mcgillivary, Environ. Sci. Technol., 13 (1979) 1225.
[20] J.K. Volkman, Org. Geochem., 9 (1986) 83.
[21] P.D. Nichols and Q. Espey, Aust. J. Mar. Freshwater Res., 42 (1991) 327.
[22] A.D. Gordon and J.G. Hoffman, Public Works Department, NSW, Technical memo No. 85/2, 1985.
[23] M.S. Rayner, D.E. Nivens, P.D. Nichols and D.C. White, Rapid extraction of the fecal sterol coprostanol using SFE, in Abstracts Third International Symposium on the Interface between Analytical Chemistry in Environmental Microbiology, Knoxville, TN, USA, 1995, p. 17.