Antarctic Science 14 (3): 244–252 (2002) © Antarctic Science Ltd Printed in the UK DOI: 10.1017/S0954102002000093

Sterols and linear alkylbenzenes in marine sediments from Admiralty Bay, King George Island, South Shetland Islands

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Abstract: Selected sterols (coprostanol, epicoprostanol, cholesterol, cholestanol), stanone (5ß-coprostanone) and linear alkylbenzenes (LABs) were measured in the surface sediments near Ferraz station sewage outfalls, in Admiralty Bay, King George Island, South Shetland Islands. during the summer of 1997/98 using GC-FID and GC-MS. Total sterol concentrations varied between 0.21 and 10.4 $\mu g\ g^{-1}$ dry sediment. Cholesterol was the major sterol at all sites, except at the sewage outfall, where coprostanol predominated. The concentration of coprostanol varied between 0.03 and 6.14 $\mu g\ g^{-1}$ dry sediment, but the majority of the samples contained levels below 0.13 $\mu g\ g^{-1}$ dry sediment. The parameters coprostanol+epicoprostanol in total sterols, coprostanol/epicoprostanol ratio versus % of cholesterol in total sterols and versus 5ß-coprostanone concentration were used to identify the sewage impacted locations in the study area. Only sites extending to 50m from the sewage outfall exhibited a sterol signal indicating sewage input. Total LABs varied from < 0.60 to 11.8 $ng.g^{-1}$ dry sediment with the maximum level at the sewage outfall. Faeces from different species of seals all contained large amounts of cholesterol and some 5ß-coprostanone. Relatively low levels of coprostanol and high levels of cholesterol observed in distant sites could be attributed to natural sources such as marine mammals.

Received 6 August 2001, accepted 20 May 2002

Key words: Antarctica, pollution, sediments, sewage, sterols, South Shetland Islands

Introduction

The increasing scientific interest in the Antarctic region has resulted in an increase in human activities contributing to localized pollution, for example through sewage discharge from the research stations. There are four earlier reports on faecal sterols as chemical markers of sewage contamination in the Antarctic, from McMurdo and Davis research stations and the adjoining coastlines (Venkatesan & Mirsadeghi 1992, Green *et al.* 1992, Green & Nichols 1995, Edwards *et al.* 1998). Faecal sterols, such as coprostanol and epicoprostanol, present in human faeces, have previously been used as a tracer for human waste along coastal areas of industrial and population centers in temperate regions (Venkatesan & Kaplan 1990, Grimalt *et al.* 1990, Jeng & Han 1994, Nguyen *et al.* 1995, Writer *et al.* 1995, Fattore *et al.* 1996, Jeng *et al.* 1996, Mudge & Bebianno 1997, Maldonado *et al.* 2000).

In the Antarctic environment, marine mammals must also be considered as a possible source of coprostanol, epicoprostanol and cholesterol (Venkatesan *et al.* 1986, Venkatesan & Santiago 1989). The ratios of these sterols can be used to indicate if a study area has been contaminated with sewage effluent and identify the contribution from marine mammals to this contamination (Venkatesan & Santiago 1989).

In temperate areas, linear alkylbenzenes (LABs) have also been used as molecular markers for sewage (Eganhouse *et al.* 1983, 1988, Raymundo & Preston 1992, Phillips *et al.* 1997, Zeng *et al.* 1997). There is no report which suggests that LABs are hazardous to marine flora or fauna. However, they have been detected in domestic sewage because LABs are present at levels of 1 to 3% in surfactants and detergents as linear alkylbenzene sulphonates and are discharged via sewage outfalls together with faecal matter. Since they are highly hydrophobic, they are adsorbed onto particles and could thus serve as sewage tracers in the aquatic environments.

We present sterol data for sediments collected in Admiralty Bay, located on the south-western part of King George Island, South Shetland Islands. There are three research stations in this bay. The Antarctic Brazilian station Estação Antártica Comandante Ferraz (EACF) is a medium size research station and is normally occupied by 10–15 personnel. During summer (November–March) the population may reach up to 45 which includes seasonal scientific groups, members of Brazilian navy and construction personnel. Ferraz which was established in 1984, is near biological communities. The presence of these communities warrants consideration of pollution control measures for sewage and other pollutants (Fig. 1). The Polish research station Arctowski, on the western end of the bay, near Thomas Point, and the Peruvian research station Macchu Picchu located near Crepin Point are the two other research stations in Admiralty Bay.

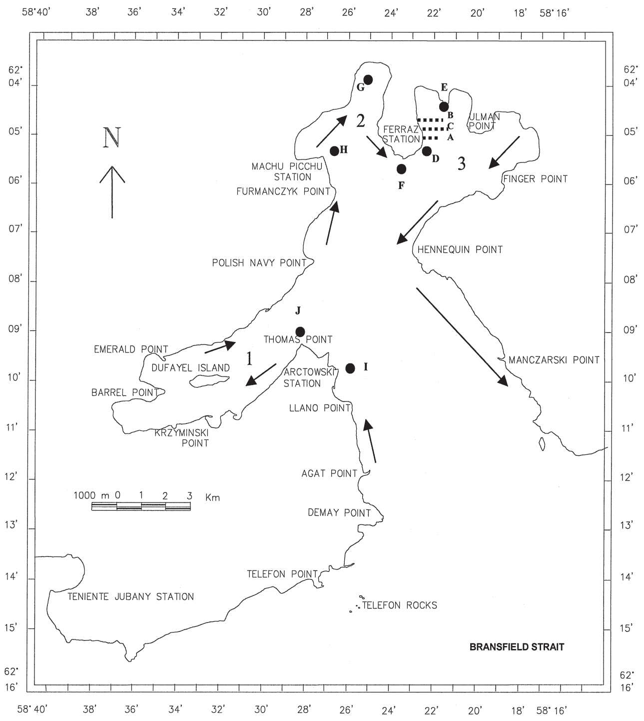

Fig. 1. Sampling stations at Admiralty Bay, King George Island. 1 = Ezcurra Inlet, 2 = Mackelar Inlet, 3 = Martel Inlet, For A–J seeTable I. The arrows indicate the general direction of water currents (Rakusa-Suszczewski 1980).

Table I. Sediment sampling stations and grain-size in Admiralty Bay.

Site	Sampling locations and distance from the coast (m)	Approximate distance (km) and direction from sewage outfall	Sample abbreviation	Grain-size of sediment
A	Ferraz 0 m (sewage outfall)	0	Fz 0	gravel
	Ferraz 5 m	0.005	Fz 5	gravel coarse sand
	Ferraz 10 m	0.01	Fz 10	medium sand
	Ferraz 50 m	0.05	Fz 50	fine sand mud
	Ferraz Cafangoria	0.05 (N)	Fz Cf	gravel coarse sand
B	Whale 90 m	0.4 (N)	Wh 90	medium–fine sand
	Whale 124 m	0.4 (N)	Wh 124	fine sand mud
C	Tanks 20 m	0.2 (N)	Tk 20	medium sand
	Tanks 50 m	0.2 (N)	Tk 50	fine sand
	Tanks 124 m	0.2 (N)	Tk 124	fine sand mud
D	Refugee–1 90 m	0.5 (S)	Rf 90	gravel mud
E	Stenhouse 50 m	0.7 (N)	Sten	fine sand mud
F	Plaza Point 144 m	1.0 (S)	Pz Pt	fine sand mud
G	Mackelar Glacier 35 m	5.5	Mack	mud
H	Crepin Point 124 m	3.5	Cr Pt	mud
I	Thomas Point 30 m	9.0	Th Pt	mud
J	Arctowski 50 m	10.0	Arct	coarse sand

Of all the stations in Admiralty Bay, only the Brazilian one has a sewage system to treat the raw effluent before disposal. All human waste and wastewater from this station receives intermediate primary and secondary treatment before discharge through a short pipe into the sea. The sewage treatment plant was designed in the summer of 1995/96 to serve a population of 50. In spite of the continued efficient operation of the treatment plant, mechanical breakdowns or accidental spills may happen and potentially contaminate the adjoining shoreline. It is therefore relevant to monitor the extent of sewage plume near-shore from the sewage outfall.

Previous studies on anthropogenic chemical compounds in the study area investigated PCBs (Montone *et al.* 2001a, 2001b), hydrocarbons and polycyclic aromatic hydrocarbons (Bicego *et al.* 1996, 1998), but none of the earlier studies in Admiralty Bay addressed sewage input. This research focuses on the faecal sterol contributions from Ferraz to Admiralty Bay. The aim of this study is to identify, by use of sterol biomarkers in the sediments, the extent of present contamination in the vicinity of the sewage outfall and to assess the degree of sewage addition in the area adjacent to Ferraz. Samples of faeces from Weddell seal (*Leptonychotes weddelli*), crabeater seal (*Lobodon carcinophagus*), fur seal (*Arctocephalus gazella*), leopard seal (*Hydrurga leptonyx*) and elephant seal (*Mirounga leonina*) were analysed to distinguish the source of sterols in the bay. LABs were also analysed in the sediments to elucidate if the faecal sterols derive from sewage input and/or from natural (marine animal) sources.

Sampling and methods

Study locations and sediment collection

Sediments were collected in the summer of 1997–98 from sites near Ferraz station (Fig. 1 & Table I). Sites A, B and C included multiple sample location at increasing distances from the coast along transects.

Hydrology and hydrodynamics in Admiralty Bay are influenced by exchange of waters with Bransfield Strait, by the inflow of fresh waters from the land and by local mixing processes. Waters from Bransfield Strait can be recognized by salinity. These waters enter Ezcurra Inlet where they upwell at the surface in the region of a central sill. Frequent and strong winds in this area cause intensive mixing of surface waters. The circulation of waters in the bay and their mixing with the waters from Bransfield Strait are influenced mainly by tides. Since tides in this region are irregular, the episodic reversal in the direction of water current in the bay could occur every 5 to 14 hours. Although study of currents in Admiralty Bay has been fragmented, the general circulation pattern is that waters from Bransfield Strait enters Admiralty Bay from the south and moves first north-west towards Ezcurra Inlet. Water leaving Admiralty Bay for Bransfield Strait flow at the surface mainly on the eastern side of the bay (Fig. 1). The exchange of waters in Admiralty Bay occurs over a period of one to two weeks

Table II. Sterol content (µg g^{-1}) and total LABs (ng.g^{-1}) and other parameters in the dry sediment samples, from Admiralty Bay.

		sterols							selected parameters		
Sites	sample locations	coprostanol	epi-coprostanol	5ß-coprostanone	cholesterol	cholestanol	total sterols	cop/e-cop	%cholesterol in in total sterols	%(cop+e-cop) in total sterols	Total LABs
A	Fz 0	6.14	0.28	1.44	3.57	0.42	10.4	21.9	34.3	61.7	11.8
	Fz 5	0.12	0.04	0.15	0.31	0.05	0.52	3.00	59.6	30.8	*
	Fz 10	0.12	0.08	0.06	0.24	0.08	0.52	1.50	46.2	38.5	*
	Fz 50	0.24	0.10	0.29	1.34	0.32	2.00	2.40	67.0	17.0	*
	Fz Cf	0.13	0.06	0.18	0.18	0.09	0.46	2.17	39.1	41.3	*
B	Wh 90	0.09	0.08	0.09	0.74	0.17	1.07	1.13	68.5	15.9	*
	Wh 124	0.11	0.09	0.10	1.88	0.25	2.33	1.22	80.7	8.58	*
C	Tk 20	0.07	< 0.004	0.04	0.40	0.08	0.56	∝	72.7	12.5	*
	Tk 50	0.10	< 0.004	0.13	1.61	0.13	1.83	∝	87.5	5.46	*
	Tk 124	0.13	0.12	0.17	5.74	0.32	5.03	1.08	91.0	4.97	*
D	Rf 90	0.11	0.08	0.09	0.66	0.19	1.04	1.38	63.5	18.3	*
E	Sten	0.13	0.08	0.11	0.44	0.15	0.80	1.63	55.0	26.3	0.70
F	Pz Pt	0.04	0.06	0.11	1.32	0.19	1.60	0.67	82.0	6.25	4.14
G	Mack	0.34	0.21	0.40	1.60	0.48	2.63	1.62	60.8	20.9	< 0.60
H	Cr Pt	0.09	0.08	0.06	1.09	0.14	1.39	1.13	77.9	12.2	< 0.60
I	Th Pt	0.03	0.05	0.10	1.15	0.21	1.44	0.60	79.9	5.56	*
J	Arct	0.03	0.05	0.06	0.13	< 0.013	0.21	0.60	61.9	38.1	< 0.60

* = not analysed, ∝ = not detected

(Rakusa-Suszczewski 1980).

Analytical procedures

Sediments were collected with a modified inox steel Van Veen sampler. Approximately 200 g of surface sediment was wrapped in aluminium foil, dried at 50°C in an oven for two days, homogenized in a mortar, stored in glass bottles and returned to Laboratório de Química Orgânica Marinha, São Paulo, Brazil. The sampling sites were chosen to track the expected effluent dispersion pathway in the Bay. Faeces of seals were collected from individual identified animals.

The study included the sterols: coprostanol (5ß-cholestan-3ß-ol), epicoprostanol (5ß-cholestan-3α-ol), cholesterol (cholest-5en-3ß-ol), cholestanol (5ß-cholestan-3ß-ol) and the stanone 5ß-coprostanone (5ß-cholestan-3-one).

25 g of sediment from each site were extracted using a Soxhlet system for 8 hours with 70 ml of ethanol. The internal standard, 5α-cholestane, was added to each extraction. The ethanol extract was reduced to *c.* 2 ml by rotoevaporation. The concentrated ethanol extract was submitted to a clean up by column chromatography using a 2 g of 5% deactivated alumina and elution with 15 ml of ethanol. The extracts were evaporated to dryness and derivatized to form trimethylsilyl ethers using BSTFA (bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylcholorsilane) for 90 min at 65°C. The sample work-up procedure was based on a method described by Kawakami & Montone (2002) and regular analyses of reference material for sterols from the International Atomic Energy Agency (MEL/IAEA) gave satisfactory results.

The TMS ethers of sterols were quantified using a HP 5890A Series II gas chromatograph, equipped with a 5% SE-54 (methyl-phenyl silicone) Ultra II HP capillary column, 25 m, 0.32 mm ID and flame ionization detector (FID).

For determination of LABs, Soxhlet extraction was carried out with a mixture of dichloromethane (DCM) and n-hexane (1:1). The internal standard nonadecyl-alkylbenzene (C_{19}-LAB) was added to each extraction. Extracts were subjected to the same column clean-up procedure as above, but eluted with 15 ml of n-hexane. The sample work-up procedure was based on the method described by UNEP (1991).

The identification of sterols and quantification of LABs were performed by GC-MS using a Series II HP 5890 coupled to a VG Mass Lab Fisons Model Trio 1000. The mass fragments (m/z) used to identify sterols and LABs were: 217 (5α-cholestane – IS), 215 (coprostanol, epicoprostanol and cholestanol), 213 (cholesterol), 231 (5ß-coprostanone), 91 and 105 (LABs).

Procedural blanks contained a few minor contaminant peaks (phthalates from plasticizers) which did not interfere with the analyses of target compounds. Recoveries ranged from 70–120%.

Results and discussion

Sterols

The highest concentration (10.4 µg g^{-1}) of total sterols in sediments was found at Fz 0, the sewage outfall (Table II). The lowest concentration of total sterols (0.21 µg g^{-1}) was at the Arct, the most distant sample location, approximately 10 km from Ferraz. The site Arct is near Arctowski station. However, at this station all domestic wastes including

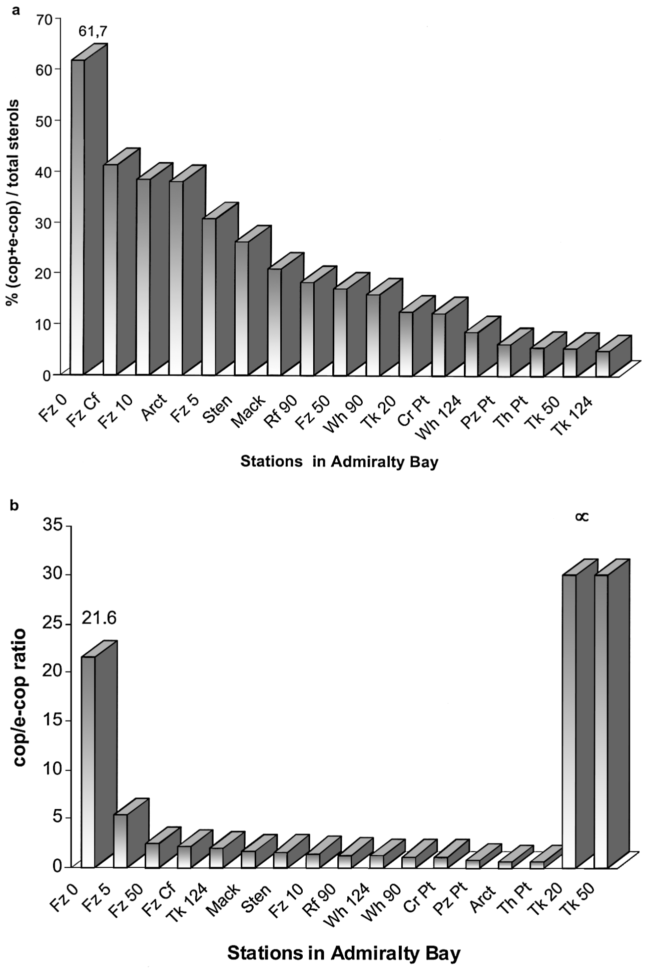

Fig. 2. a. % (coprostanol + epicoprostanol)/total sterols, and **b.** coprostanol/epi-coprostanol (cop/e-cop) ratio vs sampling stations in Admiralty Bay. ∝ = epicoprostanol not detected. For sample abbreviations refer to Table I.

sewage are discharged into a septic tank farther away from the bay.

Coprostanol, epicoprostanol and 5ß-coprostanone, which are indicators of sewage pollution, were detected at elevated levels only in samples collected at the sewage outfall. Their concentrations respectively were 6.14 μg g^{-1}, 0.28 μg g^{-1} and 1.44 μg g^{-1}. The maximum concentration of these three compounds at other sites occurred at Mack at levels of 0.34 μg g^{-1}, 0.21 μg g^{-1} and 0.40 μg g^{-1}, respectively.

Using the proportion of coprostanols (coprostanol+ epicoprostanol) in total sterols has been considered the way to eliminate the influence of grain size on the coprostanols content and to infer the degree of sewage contamination in marine sediments (Venkatesan & Kaplan 1990). Assuming that the coprostanols are derived from human faeces, the percentage of coprostanols in total sterols (Table II) indicates the degree of sewage contamination in Admiralty Bay especially at locations A, B, C and D which are within 0–500 m from the outfall, Fz 0. Sewage input decreases in the following order: Fz 0 >Fz Cf >Fz 10 >Fz 5 >Fz 50 >Rf 90 >Wh 90 >Tk 20 >Wh 124 >Tk 50 >Tk 124 (Fig. 2a). The station, Sten and Mack also exhibit relatively high coprostanol percentage. However, they are much farther (700 and 5500 m) from Fz 0 and input at these sites may originate exclusively and/or largely from marine mammals (see below).

Arct sediment contains a high percentage of coprostanols (38.1%), but the levels of individual sterols as well as total sterols at this site are generally too low (lowest of all the stations) to attach any significance to this data. Cr Pt with relatively higher sterol content and 12.2% of coprostanols suggests that marine mammals contribution is a probable source of these sterols. No sewage or domestic wastes are discharged to the ocean from these stations which suggests that sterols in Arct and Cr Pt are from natural sources.

Cholesterol was the dominant sterol in all samples, including faeces, except in the sewer outfall, where coprostanol was found in higher quantities. Human and Antarctic animals (seals, penguins) are known to contribute large amounts of cholesterol (Venkatesan & Santiago 1989, Green & Nichols 1995). The cholesterol concentration ranged from 0.13 to 5.74 μg g^{-1} and appears to be associated with grain sizes of sediments in that fine-grained sediments contain higher values of cholesterol than the sandy/gravel sediments.

The occurrence of cholesterol at high concentrations in the shoreline sediments can probably be attributed to faeces of seals and also probably penguins as reported elsewhere (Venkatesan & Santiago 1989, Green & Nichols 1995). This

Table III. Percentage of selected sterol in total sterols in the faeces of seal s from Admiralty Bay.

	percentage in total sterols				
Seal faeces	%coprostanol	%epicoprostanol	%5ß-coprostanone	%cholesterol	cholestanol
Crabbeater seal (*Lobodon carcinophagus*)	33.2	5.4	7.1	54.3	n.d
Elephant seal (*Mirounga leonina*)	0.8	n.d	0.8	98.4	n.d
Fur seal (*Arctocephalus gazella*)	n.d	n.d	0.4	96.7	2.9
Weddell seal (*Leptonychotes weddelli*)	1.1	n.d	7.8	91.1	n.d
Leopard seal (*Hydrurga leptonyx*)	n.d	n.d	2.3	97.7	n.d

n.d = not detected

Table IV. Total sterols and relevant parameters from sediments from different Antarctic study areas.

Sample location	Reference	total sterols (μg g^{-1})	coprostanol (μg g^{-1})	cop + e-cop (μg g^{-1})	cop/e-cop
Davis station	Green *et al*. (1992)	8.53$^{\$}$ 20.8*	0.07–1.28 0.25*	0.21–1.32	3.33–4.00 6.67–20.0
McMurdo station					
surface sediments	Venkatesan & Mirsadeghi (1992)	18–1600	tr–1500*	tr–1500*	38–∝
sediment core, 2cm sections from 0–22cm at the outfall	Extracted from Venkatesan & Mirsadeghi (1992) & Venkatesan, (unpublished data)	300–4200	160–2800	200–2900	5–38
Davis station	Green *et al*. (1995)	0.46–119 21.8*	tr–13.2*	nr	nr
McMurdo station	Edwards *et al*. (1998)	nr	n.d.	tr–0.20*	∝ (e-cop n.d.)
Ferraz station	(this paper)	0.21–10.4*	0.03–6.14*	0.07–6.43*	0.59–21.6*

∝ = not detected, nr = not reported, * = sewage outfall, $^{\$}$ = maximum value, tr= trace amounts

is further corroborated by our sterol analysis of seal faeces. Faeces of these mammals have >90 % of cholesterol except crabeater seal which has 55% of cholesterol (Table III).

Table IV presents data from recent studies on faecal sterols in the Antarctic environment. Coprostanol data from the Ferraz sewage outfall lie between the values reported for samples at two different periods (1992 and 1995) from Davis. Coprostanols (coprastanol+epicoprostanol) content at the Ferraz outfall was much less than at McMurdo (1992) and higher than at Davis (1992). All human waste and wastewater from Davis receive primary and secondary treatment before discharge through an outfall pipe into the sea close to the shoreline. The optimum population size served by the installation is c. 60 persons, but during summer the population may reach 100 (Green & Nichols 1995). McMurdo has a population of *c.* 800 to 1000 people and only primary sewage treatment by a macerator was in effect at the time of the study (Edwards *et al.* 1998). The faecal sterol values from these three stations are thus commensurate with their type of sewage treatment and the population capacity during the active season.

There is no known consensus concerning the general minimum value to be used to indicate a sewage impacted area. Studies in temperate areas show that values more than 0.10 μg g^{-1} of coprostanol indicate faecal pollution (Grimalt *et al.* 1990). However, if there are no other known sources of faecal sterols it is reasonable to attribute it to human sources. In the Antarctic environment, marine mammals such as whales and seals can contribute to epicoprostanol and coprostanol and/or 5ß-coprostanone (Venkatesan & Santiago 1989, Green & Nichols 1995). One needs to determine a background value for faecal sterols in pristine environments to assess the true contribution of sewage from Ferraz to other areas.

Conversely, a representative background value is difficult to determine because natural sources will not be the same in all stations. To evaluate the anthropogenic contribution and level of sewage input based on faecal sterols, many parameters have been computed to delineate sewage pollution and to estimate the contribution from marine mammals. For example, the coprostanol/epicoprostanol ratio was proposed by Venkatesan & Santiago (1989), and was later also used by Green *et al.* (1992), to estimate the sewage input versus that from marine mammals.

Figure 2b shows that the sites can be separated into three distinct groups based on the ratio of coprostanol/epicoprostanol (cop/e-cop):

a) cop/e-cop > 2.5 (Fz 0 sewage outfall, and Fz 5 - high sterols contribution from sewage) - Fz 0 exhibits the highest values of coprostanol (6.14 μg g^{-1} dry sediment), epicoprostanol (0.28 μg g^{-1} dry sediment) and ratio (21.9) of all samples. This is despite the fact that the sediment comprises mostly gravel which is not conducive for accumulation of organic matter. This site has been highly impacted with faecal pollution as expected for adjacent to an outfall. At Fz 5m, which is 5 m away from the outfall, the concentration of coprostanol has decreased. Total sterols is less than at some other sites, probably because of the prevalence of gravel.

b) cop/e-cop >1.5 but < 2.5 (for Fz 10, Fz 50, Fz Cf, Mack and Sten - moderate sterols contribution from sewage, except Mack). All these stations contain both coprostanol and epicoprostanol. Higher coprostanol content at Fz 50 relative to Fz 10 is consistent with the finer grain sediment at the former site. Fz Cf is a point located 50 m north of sewage outfall. This location was sampled when there was clean up of the septic tank of the treatment plant and the sewage sterols were most probably derived during this period. Sten showed similar data to Fz 10. Sten is 700 m from the sewage outfall and comprises largely of mud. It is possible that the faecal material can be transported to and accumulated at Sten but the level of faecal input seems much less than at Fz 0, Fz 10 and Fz 50. Mack is 5.5 km from the outfall in the other inlet. It is a partially closed area where mud is the major

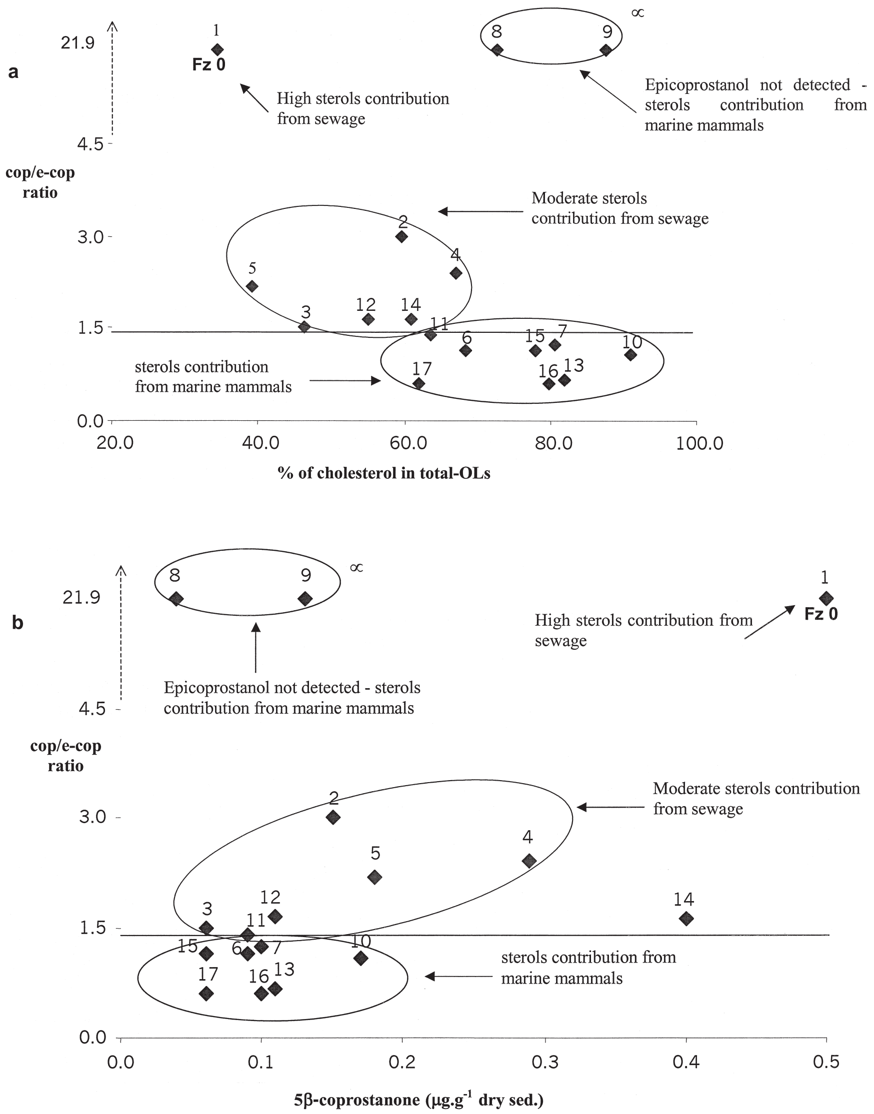

Fig. 3. **a.** coprostanol/epicoprostanol (cop/e-cop) ratio vs % of cholesterol in total sterols, and **b.** cop/e-cop vs 5ß-coprostanone concentration (µg/g dry sediment). Value 1.50 to cop/e-cop ratio has been arbitrary proposed in this report as the limit indicating significant human sewage contribution, in Admiralty Bay. 1 = Fz 0, 2 = Fz 5, 3 = Fz 10, 4 = Fz 50, 5 = Fz Cf, 6 = Wh 90, 7 = Wh 124, 8 = Tk 20, 9 = Tk 50, 10 = Tk 124, 11 = Rf 90, 12 = Sten, 13 = Pt Pz, 14 = Mack, 15 = Pt Cr, 16 = Pt Th, 17 = Arct.

component in the sediment. The concentration of coprostanol and epicoprostanol were higher in Mack (cop =0.34 µg g^{-1} and e-cop = 0.21 µg g^{-1}) than in Fz 10, Fz 50. As it is far from human activity, it is possible that Mack received contributions largely from whales (because epicoprostanol is higher than in other sites). The relatively higher proportions of coprostanone and coprostanol also suggest inputs from seals rather than from sewage. Mack, therefore, could reflect mixed inputs from whales and seals.

c) cop/e-cop < 1.5 or cop/e-cop = ∝ (for other sites - sterols contribution from marine mammals). These locations contain coprostanol and epicoprostanol below of 0.12 and 0.10 µg g^{-1} respectively, except Tk 124 (cop =0.13 µg g^{-1} and e-cop = 0.12 µg g^{-1}). Mud is a major sediment constituent at these locations and they are all 400 m or more from the outfall. The presence of epicoprostanol would suggest, sewage contribution and/or whale and seal contribution at these points. Specifically Th Pt and Arct, which are much farther away from the outfall, contain the least amount of coprostanol with epicoprostanol predominating and therefore, the lowest ratios of coprostanol to epicoprostanol of the samples studied. It is likely that these two locations have little sewage impact, and receive sterol inputs from marine mammals (Venkatesan & Santiago 1989). Tk 20 and Tk 50 were about 200 m north of the sewage outfall. Coprostanol concentration is 0.07 and 0.10 µg g^{-1} at the two sites, which is much lower than at other sites and epicoprostanol was not detected in these sediments. This is probably because of dilution effects with increasing distance from the outfall, since it is

> normally found in relatively smaller amounts than coprostanol in sewage. Alternatively, a more likely contribution to these sediments could be from seals depleted in epicoprostanol as documented in Table III.

Further, based on plots of cop/e-cop ratio versus % of cholesterol in total sterols (Venkatesan & Santiago 1989, Venkatesan & Kaplan 1990) and cop/e-cop ratio versus 5ß-coprostanone concentration, it is possible to identify high, medium or low sewage inputs from the Ferraz station outfall and to identify natural contributions.

The plot of cop/e-cop versus % of cholesterol shows two distinct groups of sites (Fig. 3a):

1) cop/e-cop < 1.5 or cop/e-cop = ∝ and % of cholesterol > 65%. These sites can be classified as having sterols contributed mostly from marine mammals rather than from a low sewage influx. All sites are more than 300 m from the outfall where the sediment was mainly mud. Epicoprostanol was detected, implying contribution from whales and seals. The high cholesterol percentage indicates probably a large contribution from penguins and seals.

2) cop/e-cop > 1.5 and % cholesterol < 70%. The value of cop/e-cop, absolute amount of coprostanol and % of cholesterol < 70% show that sewage contribution was higher than the contribution from Antarctic animals. High levels of coprostanol and epicoprostanol decrease the relative proportion of cholesterol in the total sterols. These sites can be classified as moderate or high (only Fz 0) sewage impacted areas. Although Mack exhibited cop/e-cop ratio and % of cholesterol similar to sites with moderate sterols contribution from sewage, it was an exception because it is distant from the sewage outfall. It can be classified as having a very significant contribution from marine mammals as described previously.

The plot cop/e-cop vs [5ß-coprostanone] (Fig. 3b) shows similar groupings of locations consistent with Fig. 3a. High coprostanol values are generally accompanied by high coprostanone levels (i.e. Fz 0, Fz 50, Mack). Relatively higher coprostanone concentration in Tk 124 and Mack suggests that the sterols could be derived from seals. In this plot, Mack appears as a distinct site from the two mains groups because the 5ß-coprostanone was higher. The 5ß-coprostanone, coprostanol and epicoprostanol levels suggests that this site is receiving sterol contributions predominantly from marine mammals in contrast to the other sites.

A similar correlation occurs between coprostanol, % of cholesterol and 5ß-coprostanone concentration (Fig. 3). The sites classified as receiving medium or high sewage input are within 50 m of the sewage outfall (e.g. Fz 0, Fz 5, Fz 10, Fz 50 and Fz Cf). Except for Fz 0, coprostanol was less than 0.25 µg g^{-1} at all other sites; such levels are low when compared to sites from industrial and high population centres, e.g. lagoon of Venice, Italy (> 5.00 µg g^{-1}) (Sherwin *et al.* 1993), Tan-Shui estuary, Taiwan (33.3 µg g^{-1}, 2 km from outfall) (Jeng & Han 1994), San Pedro Shelf, USA (> 1.00 µg g^{-1} in stations near outfall) (Maldonado *et al.* 2000). Nichols & Leeming (1991) showed that concentrations of coprostanol in grossly contaminated sediment can be as high as 9 µg g^{-1}.

Since Mack sediment contains relatively high coprostanol and epicoprostanol, it also plots near stations affected by sewage input. However, its remote location and other sterol parameters, when considered collectively, strongly suggest that all or most of its faecal sterols are from the marine mammals.

Linear Alkylbenzenes (LABs)

LABs were found in only three of the six sediments analysed (Table II). Data for LAB show that contributions from sewage effluent can be detected at a distant site such as Sten. The relatively significant level of LAB at Pz Pt could indicate the episodic reversal of water current in the Bay. This is consistent with the hydrocarbon data of Bícego *et al.* (1998) who found highest normal alkanes content in their stations 1 and 3 (corresponding to the current study stations Fz 0 and Pz Pt respectively) reflecting petroleum input from Ferraz station. From the alkane and polycyclic aromatic hydrocarbon data, they concluded that other sites several kilometres away were nearly pristine. Further, total LABs found at sites from the current study, in general, are very low compared to levels at Davis station (0.51 µg g^{-1}) (Green & Nichols 1995) and levels from urban coastal regions such as Santos Bay, Brazil (16.94–430.6 ng.g^{-1}) or São Sebastião Channel, Brazil (12.65–27.76 ng.g^{-1}) (Medeiros 2000), the Wash and Humber estuaries (2.5–84.8 ng.g^{-1}) and Thames estuary (0.10–2.3 µg g^{-1}) (Raymond & Preston 1992).

A consideration of the parameters, (1) cop/epi ratio, (2) cop/epi ratio vs % cholesterol, (3) cop/epi ratio vs 5ß-coprostanone, (4) % coprostanols/total sterols and (5) LABs concentration collectively, helps assess sewage input at each site. For example, Fz 0, (at the sewage outfall) is a severely contaminated site as documented by the content and composition of sterols as well as total LABs. Fz 5, Fz 10, Fz Cf and Fz 50 are points near to the sewage outfall. The parameters show that these sites receive some effluent discharge from Ferraz, however, their levels of faecal sterols compared with Fz 0 are much less because of the dilution effect concomitant with their distance from the outfall. Mack and Sten are sites that exhibit coprostanol levels similar to the sites near sewage outfall. However, the low concentration of total LABs and a low value of % coprostanol (compared to Fz 0) implies that Sten could receive minimal faecal material from outfall while Mack, which is 5.5 km from Ferraz, receives almost all of its faecal sterols from mammalian sources. Pz Pt shows low level of coprostanol, and the sterol ratios suggest this to be a pristine site. However, the presence of LABs at moderate levels at this site compared to at Fz 0 indicate that some sewage material may have been transported to this site.

The other sites (Arct, Rf 90, Tk 20, Tk 50, Tk 124, Wh 90, Wh 124, Cr Pt and Th Pt) show consistently low concentrations of coprostanol (0.09–0.13 µg g^{-1}). However, the sterol ratios indicate that Arct and Th Pt receive sterols largely from natural sources with possibly low levels of sewage input from Ferraz. According to Rakusa-Suszczewski (1980) the water enters in Ezcurra Inlet, traverses through Mackelar Inlet, and then towards Martel Inlet, (always along the coast) and leaves Admiralty Bay from the opposite side towards Bransfield Strait. The study sites located along this path from Ferraz station (A) are Wh 90, Wh 124 (B), Tk 20, Tk 50, Tk 124 (C) and Sten (E). This would explain the detection of faecal (sewage) material north of Ferraz. However, episodic reversal of water current in the Bay explains the possibility of faecal material migrating south-west towards sites D and F (Rf 90 and Pz Pt).

Conclusions

Analysis of coprostanol, in combination with all sterols and LABs, in the sediments from Admiralty Bay indicates that sewage contamination has accumulated to a relatively high level only in the vicinity of the sewage outfall (Fz 0). The grain-size of sediment found at the sites near the sewage outfall is not generally conducive for accumulation of sterols or organics. The levels of coprostanol were significantly lower in sediments 5m farther from the outfall. The percentage of coprostanol in total sterols serves as a measure of sewage contamination from the outfalls at all the locations, except where potential contribution from marine mammals are indicated from other parameters. The cop/e-cop ratio vs % of cholesterol and vs the 5ß-coprostanone concentration indicates that, although the concentration of coprostanol is lower in stations beyond 5 m, the chemical signal of human sewage can be detected up to 50 m from the sewage outfall.

Other sites in the bay beyond 50 m from the outfall were found to contain coprostanol and epicoprostanol at much lower levels compared to the outfall sites (Fz 0, Fz 5, Fz 10, Fz 50 and Fz Cf). The sterols in these sediments are derived from natural sources such as marine mammals. High relative levels of cholesterol found in sediments far from the sewage outfall indicates probable contribution from penguins and/or seals.

Although high levels of coprostanol occur near the Brazilian station outfall, it is still lower than that found in other study areas in the Antarctic and temperate regions of the world. This finding documents the importance of sewage treatment in reducing the anthropogenic impact in the pristine Antarctic environment.

Acknowledgements

Financial support was obtained from the Antarctic Brazilian Program (PROANTAR) by a grant from the Conselho Nacional de Pesquisa (CNPq) with logistical support from the Secretaria da Comissão Interministerial para os Recursos do Mar (SECIRM). Cesar de Castro Martins acknowledges financial support from CAPES MSc Scholarship. The authors would like to thank the Ferraz station staff for the collection of samples and members from Laboratório de Química Orgânica Marinha for suggestions, especially Dr Satie Taniguchi in improving this report. We thank Alison Reeves and an anonymous referee for their constructive and helpful comments. Partial financial support to MIV from DPP8816292 (NSF) is acknowledged.

This is contribution no 5675 of Institute of Geophysics and Planetary Physics, University of California, Los Angeles, CA 90095-1567.

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