Hydrocarbons and faecal material in urban stormwater and estuarine sediments: source characterisation and quantification

by

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For my parents

The saying that a little knowledge is a dangerous thing is, to my mind, a very dangerous adage. If knowledge is real and genuine, I do not believe that it is other than a very valuable possession however infinitesimal its quantity may be. Indeed, if a little knowledge is dangerous, where is the man who has so much as to be out of danger?

Thomas Huxley, 1877

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by

GRAHAM JAMES GREEN

ABSTRACT

Hydrocarbons from road runoff and faecal matter from sewage overflows have previously been implicated as major contributors to urban stormwater contamination, but little source identification or quantitative data exist. In this study chemical marker techniques were utilised to identify specific sources of these contaminants in selected stormwater catchments of Hobart, Tasmania. The mean concentration of hydrocarbons in stormwater during this study was found to be 2.88 mg/l with an estimated total annual discharge to the Derwent Estuary in the order of 164,000 kg/year. Assessment of the major hydrocarbon inputs to the Derwent estuary demonstrated that stormwater is the largest single contributor. Source elucidation of hydrocarbons demonstrated inputs to stormwater from automotive oils, diesel fuel, and plant waxes. Analysis of polycyclic aromatic hydrocarbon (PAH) profiles by gas chromatography-mass spectrometry and multivariate analysis confirmed, in most cases, that automobile sump oil, rather than unused lubricating oils were the major component of oil in stormwater. Cluster analysis, based on PAH composition, was used for grouping stormwater samples relative to potential source materials. Other techniques such as the use of PAH isomer pair ratios proved useful for determining the input of combustion derived PAH. In sheltered embayments of the Derwent Estuary a clear link was demonstrated between urban stormwater and the build-up of hydrocarbon contaminants in sediments. Localised extreme hydrocarbon concentrations were found associated with stormwater discharge and boat mooring areas. Aliphatic hydrocarbons (10,100 μg/g) and PAHs (27μg/g) in sediments at Prince of Wales Bay were the highest yet recorded levels for estuarine sediments in Australia. Stormwater in Hobart was found to

be highly contaminated with faecal pollution. Sterol and bacterial analysis of stormwater samples showed that dog faeces is potentially the most significant contributor to the faecal contamination. This finding was demonstrated primarily by the similarity between sterol profiles of dog faeces and stormwater samples and the low levels or absence of sterol markers for other sources of faeces. Human faecal material was detected in urban stormwater by tracing the faecal sterol coprostanol. During flood conditions, human faeces, attributed to cross contamination from the sewerage system, became a major contaminant in stormwater. During dry weather, urban runoff contained low levels of human faecal material possibly derived from illegal sewer connections. On an annual basis in Hobart, stormwater was calculated to represent an estimated 80-91% of faecal input to the Derwent estuary. A study of hydrocarbons and sterols in marine and shoreline sediments undertaken at Davis Station in Antarctica has been included in this project. This comparatively simple system, largely devoid of external pollution influences, provided an ideal test case for the determination of hydrocarbon and sewage impacts from a known human population.

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CHAPTER ONE

INTRODUCTION

Who that well his warke beginneth, the rather a good end he winneth.

J. Gower, 1395

1. INTRODUCTION

1.1 THESIS OUTLINE

This study arose from a demonstrated need for local information regarding sources and fates of organic contaminants in urban catchments in Australia. The thesis is comprised of six chapters. Chapter One, the introduction, provides a background to the major research topics including stormwater, hydrocarbons and sterols. Included in the introduction is a literature review in regard to source elucidation of hydrocarbons and faecal material in stormwater, and the impacts of stormwater on receiving waters.

Chapter Two describes the analytical methods used in the study. In Chapter Three the findings of hydrocarbon loads, sources and fate are presented and discussed. In chapter four the results of sterol content and composition and bacterial counts for stormwaters, faeces, storm drain sediments and estuarine receiving sediments are presented. The source of stormwater faecal contamination and the relative contribution of stormwater to the estuarine faecal load in Hobart are also discussed.

Chapter Five is a case study of hydrocarbons and sterols in marine sediments and soils at Davis Station in Antarctica. The overall study is concluded in Chapter Six with recommendations given for future work.

The central theme of the thesis is the use of organic biomarker techniques (molecular markers) for source elucidation of hydrocarbons and faecal material, and to assess the contribution these contaminants make, through urban runoff, to pollutant loads in receiving waters.

1.2 MOLECULAR MARKERS

Molecular markers were chosen as the primary investigative tool in this study as their chemical structures may be linked to specific origins. The molecular marker concept

has been developed by organic geochemists to understand the sources, transport, and fate of organic compounds. Because of their unique structures, molecular markers can provide highly specific information that cannot be obtained by other approaches (Takada & Eganhouse, 1997). Some molecular markers are produced by humans, whereas others are strictly biosynthetic (Eganhouse, 1997). The observation of a molecular marker in the environment signals the presence of a specific source material. When several markers are found in an environmental sample, contributions made by several potential sources may be investigated.

In this study the primary molecular markers used are the faecal sterol coprostanol and the hydrocarbon assemblage collectively called the PAHs (polycyclic aromatic hydrocarbons). Coprostanol is a natural organic compound which is not considered to be a pollutant *per se* but whose presence in the environment is almost exclusively related to human activity. On the other hand, several PAHs are known to be toxic and their presence in the environment is primarily attributable to industrial activity (Eganhouse, 1997). The use of PAHs and coprostanol as molecular markers for source derivation of particular environmental contaminants is discussed in detail in sections 1.7 and 1.10 respectively.

1.3 GENERAL

Several reviews and studies in Australia have identified the need for further work in the field of source derivation of organic contaminants in urban runoff. These include work by Aitken (1973 & 1975) who reviewed urban stormwater design and research. The reviews noted the almost complete absence of local water-quality information, and recommended further data collection and analysis be undertaken. Such information is a key part of any stormwater remediation process. A recent review of urban stormwater research in Australia by Scott (1996) identified as a research priority that tracer studies and mass balance calculations should be undertaken to determine the sources of major and toxic pollutants in urban catchments, including oil. In a bibliography of urban stormwater quality (Duncan (1995), a similar conclusion

was reached in identifying the need to determine the sources, fates and effects of particular contaminants in urban catchments.

More specifically, it has been identified that limited data exists on the sterols of stormwater, and there is considerable debate as to whether faecal pollution is derived from sewage, stormwater or other sources for specific locations in Australia (Nichols *et al.*, 1996). Indeed, assessment of microbial loadings has demonstrated that stormwater contributes substantially greater faecal pollution during storm events than from a city's sanitary waste system (Sartor *et al.*, 1974). Other studies have demonstrated a similarity between the microbial densities in urban stormwater runoff and dilute raw wastewaters (Qureshi & Dutka, 1979). In fact, the problem of sewage material in stormwater was recognised over 100 years ago by Wardle (1893) who stated '...the first storm washings contain quantities of putrescible organic matter ... they are very foul and often contain as much as the sewage itself'. It appears, however, that in spite of this early knowledge there was very little attention to stormwater quality for nearly sixty years after this (Duncan, 1995). In recent times, despite the proliferation of stormwater research, reviews and data collection, only a small number of papers deal specifically with stormwater microbiology (Duncan, 1995).

There have been many studies that have focussed on coprostanol as a tracer of sewage contamination in estuarine, coastal, lacustrine and marine sediments and waters e.g., (Goodfellow *et al.*, 1977; Kanazawa & Teshima, 1978; Brown & Wade, 1984; Dureth *et al.*, 1986; Venkatesan & Kaplan, 1990; Nichols & Leeming, 1991; LeBlanc *et al.*, 1992). However, characterisation of faecal sterols to determine the contribution by stormwater to faecal contamination in waterways of or near urban areas has largely been neglected. The contribution from stormwater is potentially considerable due to illegal sewer connections to the stormwater system, septic leachate in un-sewered areas, and sewer overflows. In fact, in Australia, many investigations of sewer overflows have been undertaken in Sydney (Ngo *et al.*, 1992; O'Loughlin, 1990; Scott, 1996). Of the 3000 sewer overflow points in the Sydney region, 200 were reported to overflow even during smaller storms (Sydney Water, 1994). Sewer

overflow events in Sydney typically occur after 5 to 10 mm of rain. This leads to 20 to 50 separate overflow events per year each of which lasts for an average of 12 hours (Carleton, 1987 & 1990). Despite sewer overflows being well known as a widespread phenomenon, the relative contribution of this process to the total sewage contamination in stormwater has, as yet, not been determined.

Dry weather studies of sewage contamination in Sydney stormwater, based on the presence of coprostanol in samples, have shown the presence of only low levels of human faecal contamination (0.006 - 1.0 µg/l of coprostanol)(Nichols *et al.*, 1993; Nichols *et al.*, 1996). However, based on microbiological data, water quality in stormwater discharge during dry weather from five catchments in Sydney exceeded the Clean Waters Act limit of 1000 faecal coliforms/100 ml in more than 98% of samples (Rowlands *et al.*, 1992). These results further stress the need for source derivation work in urban catchments where high bacterial levels appear to outweigh the potential input of human faecal material to stormwater.

In terms of hydrocarbons, dramatic oil spill events such as the *Exxon Valdez* tend to overshadow the low-level, chronic discharges of hydrocarbons from stormwater into estuarine and coastal waters. These discharges, on a mass balance, are believed to contribute more oil to near-shore environments than widely publicised oil spills (Hoffman *et al.*, 1980). The contribution of urban stormwater to total hydrocarbon loads entering coastal or estuarine receiving waters has not been extensively studied, however, of the estimated 3.2 million tonnes of petroleum hydrocarbons that enter the global marine environment annually (NRC, 1985), 30% is believed to be from municipal and industrial discharges (Fig. 1).

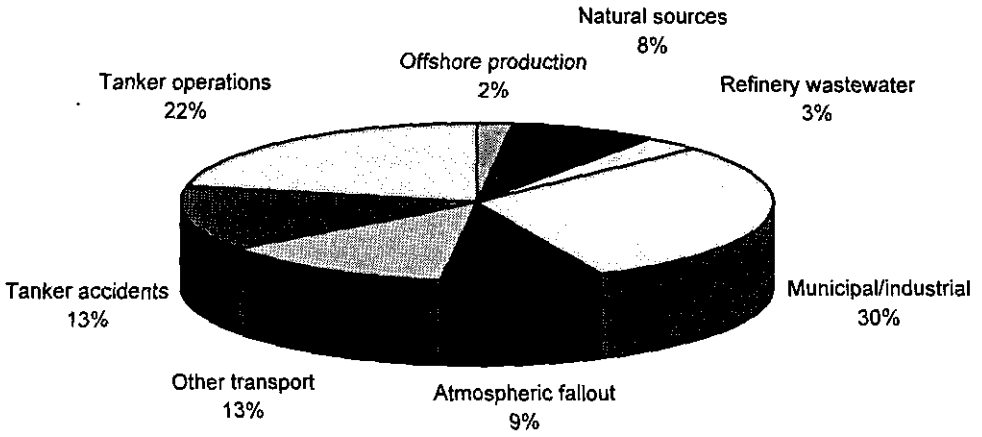


Fig. 1: Sources of hydrocarbons in the global marine environment from data compiled by the US National Research Council.

Little data exists on sources or quantity of hydrocarbons flushed from urban catchments in Australia. The discharge of hydrocarbons to the marine environment from municipal and industrial waste and runoff in Australia has previously been estimated at between 12,000 and 32,000 tonnes per annum (Volkman *et al.*, 1994). The contribution from urban runoff was estimated as 5,000 tonnes per annum. These estimates were, however, based on extrapolation from overseas information due to the lack of any specific studies of hydrocarbon budgets in Australia. Of the Australian studies of hydrocarbons in stormwater (Rowlands *et al.* 1992; Nichols *et al.*, 1993), none have addressed the magnitude of hydrocarbon discharge to receiving waters on an annual basis. It is, however, recognised that lubricating oils are a major cause of hydrocarbon pollution in many estuaries and coastal areas around Australia (Volkman *et al.*, 1992) from sources such as shipping, industry and urban inputs (Burns & Smith, 1982). This recognition perhaps lends greater urgency to the need to examine the extent to which urban runoff contributes to the hydrocarbon contamination of coastal waters in Australia.

1.4 STORMWATER - BACKGROUND

Stormwater runoff is increasingly being recognised as a major source of pollutants entering urban waterways and estuaries. As rainfall events are often intense and infrequent in Australia, it is believed that large fluxes of pollutants are mobilised from urban catchments into receiving waters during storm events. In highly urbanised areas, particularly industrial areas, up to 90% of rainfall may flow into the drainage system as stormwater (Scott, 1996; Commonwealth of Australia, 1996).

Urban stormwater characteristically carries high concentrations of suspended solids (dust and soil) with adsorbed nutrients, fertilisers, heavy metals, hydrocarbons, pesticides, bacteria, litter and other contaminants derived from human activities (CEPA, 1993). These contaminants originate from a diffuse array of sources within urban catchments and are linked to demonstrable impacts in receiving estuarine or coastal waters. These may include implications for primary contact uses, acute and chronic toxicity on biota, oxygen depletion, turbidity, siltation, nutrient enrichment and eutrophication (Scott, 1996; CEPA, 1993). To date in Australia there have only been a limited number of studies on water quality changes caused by stormwater contaminants (Scott, 1996). The study, and subsequent management, of pollutants in urban runoff is important because there is currently very little discharge control or treatment of stormwater in Australia.

The pollutant load carried by stormwater is unpredictable and is driven largely by the nature of meteorological events and watershed hydrology. It is however believed that up to 60% of the insoluble pollutant load including suspended solids, faecal coliforms and nutrients, is concentrated in the initial flush or less than 25% of the storm event water volume (Vorreiter & Hickey, 1994). Stormwater systems have traditionally been constructed with the aim of collecting and removing excess runoff as quickly as possible to avoid flooding during heavy rain. Despite this, flooding continues to be a problem due to increased peak flows which are a result of continued increase in development within urban catchments (Scott, 1996).

1.4.1 Historical perspective

The first specific studies related to the microbiological quality (Davis *et al.*, 1977; Olivieri, 1980; Qureshi & Dutka, 1979) and occurrence of hydrocarbons in stormwater (Wakeham, 1977; MacKenzie & Hunter, 1979; Whipple & Hunter, 1979). were published during the 1970's. In the 1980's interest in hydrocarbons in urban runoff increased with studies conducted in London (Gavens *et al.*, 1982), New York, (Connell, 1982), Rhode Island, USA (Hoffman *et al.*, 1982; Hoffman *et al.*, 1983, Hoffman *et al.*, 1984), Los Angeles (Eganhouse *et al.*, 1982); Bayreuth, Germany (Herrmann, 1984); Richmond, California (Stenstrom *et al.*, 1984); and San Francisco, California (Fam *et al.*, 1987). Other studies in the USA analysed hydrocarbons as part of a broader study of extractable organic matter in urban runoff (Eganhouse & Kaplan 1981; Eganhouse *et al.*, 1981; Ammon & Field , 1981). In Australia, a study of hydrocarbons in Victorian coastal ecosystems (Burns & Smith, 1982) brought attention to the collective importance of chronic low-level inputs of hydrocarbons to the coastal environment from urban and industrial sources.

During the 1990's there have been several contributions to research into hydrocarbons in stormwater including; Marsalek (1990), who studied specific PAH compounds in atmospheric deposition and runoff in Ontario, USA; Bomboi & Hernandez (1991) conducted a study of hydrocarbons in urban runoff in Madrid; Xanthopoulos & Augustin (1992) measured particulate pollution in street runoff and sewers in Karlsruhe-Waldstadt, Germany; and Jones *et al.*(1993) investigated the extent and ecological impacts of hydrocarbon contamination in waters and sediments in London.

1.4.2 Recent stormwater studies in Australia

Stormwater research in recent years in Australia has been undertaken by many organisations including CSIRO, Cooperative Research Centres, universities, state water agencies and private companies and is reviewed by Scott (1996). There has been a great deal of effort to develop improved stormwater systems with emphasis moving

away from concrete lined drains to systems that enhance infiltration, reduce peak flows and reduce pollutant levels (Scott, 1996). Also in recent years there have been a number of computer modeling studies that have been developed to aid in the design of urban water networks by predicting runoff volumes from catchments and the resulting flow rates in drainage systems. Many of the models have the capacity to predict water quality by estimating the amount of pollutants washed into drains by surface runoff (Scott, 1996). Despite this development there remains a lack of specific work on hydrocarbons in urban runoff in Australia.

In Sydney there has been growing awareness in the last 5-10 years that stormwater and associated sewer overflows are a major cause of pollution in urban waterways and ocean beaches. This has lead to the implementation of many stormwater projects by the Sydney Water Corporation (Scott, 1996). Rowlands *et al.* (1992) studied water quality in five coastal catchments in the Sydney region. In this study PAHs were measured as one of the water quality parameters. Linforth *et al.* (1994) conducted a comprehensive monitoring program of stormwater runoff and sewer overflows in the Upper Parramatta River catchment of Sydney including determination of the faecal coliform load.

1.4.3 Stormwater studies in Tasmania

There have been very few stormwater studies conducted in Tasmania, with no data specific to hydrocarbons or sterols. Jenkins (1991) conducted an investigation of urban stormwater quality in Hobart using pH, temperature, conductivity, TSS (total suspended solids), zinc, lead, nitrate and phosphate as parameters. An increase in pollutants towards the city centre was noted, however no pollution sources were described. A microbiological study of Hobart's major urban stream, the Hobart Rivulet, was undertaken in 1995 (Blacklow, 1995). The major conclusions of the study were that human sewage contaminated soils were assumed to be the primary contributor to faecal contamination of the rivulet during dry weather. During wet weather, faecal coliform levels were significantly higher and attributed to surface runoff of dispersed animal faeces.

Aside from these university-based studies, there have been no major stormwater research or management projects undertaken in Hobart. The lack of data in the area of stormwater pollution contribution to the Derwent Estuary has been recognised (Tasmanian Government, 1996). A study of nutrient input to the Derwent River estuary (Hammerschmid, 1994) did, however, incorporate a nutrient budget for stormwater flow from a small residential catchment over the period of a year. The conclusions of this study were that stormwater contributes high levels of oxidised nitrogen and orthophosphate to the estuary with relatively low levels of ammonia.

1.5 HYDROCARBONS - GENERAL BACKGROUND

Hydrocarbons may be broadly divided into two chemical classes; aliphatic and aromatic. Aliphatic hydrocarbons contain either no double bonds (alkanes or saturates) or non-conjugated double bonds (alkenes or unsaturates)(Volkman *et al.*, 1994). Alkanes may occur as a straight chain (e.g. *n*-C_{16}), a straight chain with side branches (e.g. the isoprenoids pristane and phytane), or cyclic (e.g. the petroleum biomarker compounds steranes and hopanes)(Volkman *et al.*, 1994). Aromatic hydrocarbons usually contain from 1 to 6 carbon cyclic rings, each with alternating double and single bonds (conjugated bonds). The simplest aromatic hydrocarbon, in terms of chemical structure, is benzene (C_6H_6)(Volkman *et al.*, 1994). Polycyclic aromatic hydrocarbons (PAHs), some of which are carcinogenic and/or mutagenic, contain three or more fused aromatic rings. Structural formulas of some hydrocarbon types are shown in Fig. 2.

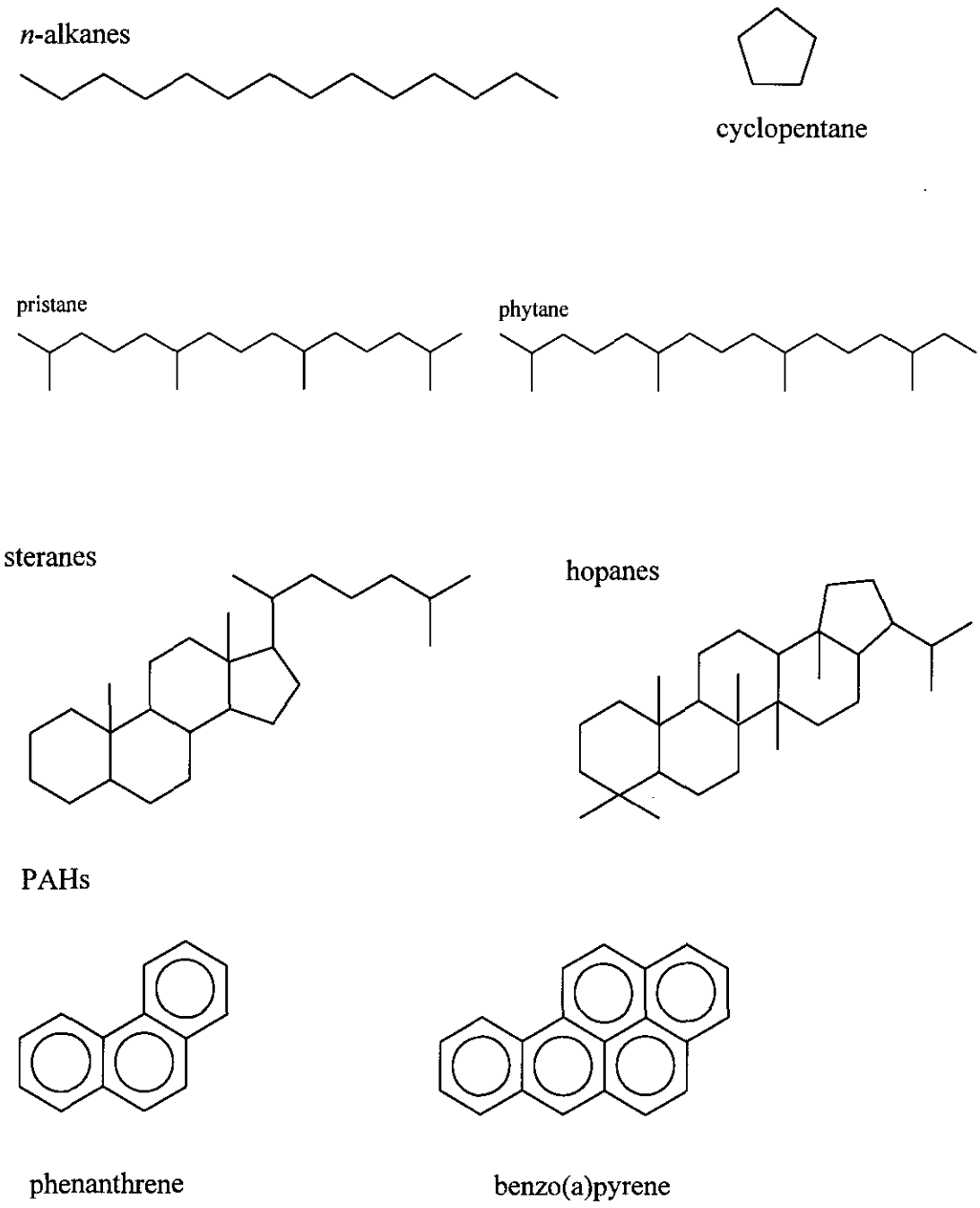


Fig. 2: Chemical structures representing some of the different hydrocarbon groups

Petroleum products are a complex mixture of linear, cyclic, branched, saturated, unsaturated and aromatic compounds. PAHs occur in relatively low concentrations in most petroleum substances (Connell, 1982). Presented in Table 1 are some common petroleum products and their characteristics. Each distillation fraction contains

aliphatic and aromatic hydrocarbons and any fraction can occur as a pollutant in the environment.

Table 1: Characteristics of petroleum products

Product	Boiling Point Range (°C)	Main Carbon No. Range
Natural gas	0-25	C_1-C_4
Petrol	25-100	C_5-C_8
White spirit	130-220	C_9-C_{11}
Kerosene	150-230	C_9-C_{13}
Diesel (light gas oil)	230-340	C_{12}-C_{20}
Heavy gas oil	340-360	C_{19}-C_{22}
Lubricating oil	345-540	C_{18}-C_{45}
Asphalt	>540	>C_{40}

1.6 SOURCES OF HYDROCARBONS IN URBAN CATCHMENTS

Petroleum hydrocarbons have previously been recognised as a major contributor to the pollution in urban stormwater (Hoffman *et al.*, 1983). The major individual source of the hydrocarbon pollution has been found to vary between study sites. Vehicle exhaust emission was found to be the major source in urban runoff from Madrid (Bomboi & Hernandez, 1991); used crankcase (sump) oil in urban runoff at Rhode Island (Hoffman *et al.*, 1983; Latimer *et al.*, 1990); and motor oil and diesel fuel in San Francisco (Fam *et al.*, 1987). Automotive crankcase oil was the major source of hydrocarbons in runoff from a commercial land-use area (Hoffman *et al.*, 1982). These authors also observed elevated levels of hydrocarbons in the first flush and that hydrocarbons were typically associated with particulate material in storm runoff.

Other contributions to hydrocarbon pollution in urban runoff are believed to be derived from pavement attrition (Eganhouse *et al.*, 1981; Hoffman *et al.*, 1984), tar-based pavement sealants, and industrial discharges (Marsalek, 1990), fuel oils (Eganhouse *et al.*, 1981), and from wood smoke and other atmospherically transported particulates (Eganhouse *et al.*, 1981; Webber, 1983 & 1986). Hydrocarbons believed to be derived from plant wax components, which are not considered as a contaminant, are sometimes detected in urban stormwater (Eganhouse *et al.*,1981) and are common in undeveloped areas (Fam *et al.*, 1987).

PAHs are present in fresh oil, and to a greater extent in used oil, and are produced during pyrolysis of fossil fuels or organic-rich materials (Lipiatou & Saliot, 1991). PAHs are a minor component (less than 2 wt%) of fresh oil (Bence *et al.*, 1996). However, as PAHs are formed during motor operation and can also be accumulated into oil from petrol, the PAH content of used motor oil from petrol motors can be considerably higher than that of new crankcase oil (Vazquez-Duhalt, 1989). The major sources of PAHs in urban runoff are: those adsorbed to atmospheric dust; industrial wastewater; disposal of used oils; automotive oils and greases; and road wear particles (Bomboi & Hernandez, 1991; Hoffman *et al.*, 1984). Combustion-derived PAHs may be deposited directly onto the surface of water bodies and subsequently incorporated into sediments or enter natural waters through continental runoff and erosion (Gschwend & Hites, 1981). Marsalek (1990) found a city-wide (Sault Ste. Marie, Ontario) mean deposition rate of PAHs from atmospheric fallout of 25 $\mu g/m^2$/week. High concentrations of PAHs are often found in waters and sediments near urban centres (Lee & Ryan, 1983). This is of great concern regarding the health of humans and aquatic life, as several PAHs have demonstrated carcinogenic and mutagenic properties (Aboul-Kassim & Simoneit, 1995; Vazquez-Duhalt, 1989).

Aside from oil and combustion sources, several PAHs, such as perylene and retene, are naturally occurring (Hoffman *et al.*, 1984). Perylene (peri-dinaphthalene) is widespread primarily in reducing marine sediments and originates in sediments fed by both terrestrial and aquatic organic debris (Venkatesan, 1988). Retene is abundant in resins of conifers in temperate climates (Lipiatou & Saliot, 1991). However,

environmental concentrations of PAHs due to natural processes are usually low compared to PAHs originating from anthropogenic sources (Witt, 1995).

1.7 HYDROCARBON IDENTIFICATION AND FINGERPRINTING

To successfully determine hydrocarbon sources in environmental samples a combination of techniques is required. In this study three main techniques were utilised: (i) GC (gas chromatography) for determination of aliphatic hydrocarbon profiles, including pristane and phytane; (ii) analysis of biomarkers such as hopanes, steranes and diasteranes conducted by gas chromatography-mass spectrometry (GC-MS); and (iii) analysis of di-aromatics and PAHs by GC-MS provided additional source information, particularly for combustion derived hydrocarbons. The three techniques used are described in detail below:

(i) Hydrocarbons derived from the epicuticular waxes of higher plants are identifiable in environmental samples by a strong predominance of odd-over-even carbon numbers in the range (n-C_{25} to n-C_{35})(Wakeham, 1996)(Fig. 3). The predominance of molecules with an odd number of carbon atoms can be measured by the 'carbon preference index' (CPI), i.e., the ratio, by weight, of odd to even molecules (Tissot & Welte, 1978). Hydrocarbons from epicuticular plant waxes have a CPI > 1.

The presence in chromatograms of an UCM (unresolved complex mixture) is one of the more convincing indications of petroleum contamination in environmental samples and is a common feature of chromatograms of biodegraded oils (Volkman *et al.*, 1994)(Fig. 3). The UCM is generally considered as a mixture of many structurally complex isomers and homologues of branched and cyclic hydrocarbons (Gough & Rowland, 1990). The UCM GC-profile usually rises significantly above the chromatogram baseline. Resolution of a substantial proportion of the hydrocarbons within the UCM into individual components is not possible by use of even the best capillary columns (Gough & Rowland, 1990).

Further evidence of hydrocarbon contamination in stormwater samples may be achieved by identification of compounds which are unique to petroleum and absent or below detection in unpolluted samples. The common isoprenoid hydrocarbons pristane (C_{19}) and phytane (C_{20}) are present in most petroleums and are often considered as good indicators of petroleum contamination (Volkman *et al.*, 1992) (Fig. 3). The abundance of pristane (Pr) and phytane (Ph) in most petroleums allows their direct measurement from GC traces without the necessity for identification by GC-MS. Pr/Ph ratios of petroleum reflect the nature of the contributing organic matter and are commonly used for oil/oil correlation (Peters & Moldowan, 1993). Isoprenoid/*n*-alkane ratios such as pristane/*n*-C_{17} are sometimes used in petroleum correlation studies. As *n*-alkanes are generally attacked by aerobic bacteria prior to isoprenoids (Peters & Moldowan, 1993), these ratios may be used as an index for biodegradation in environmental samples.

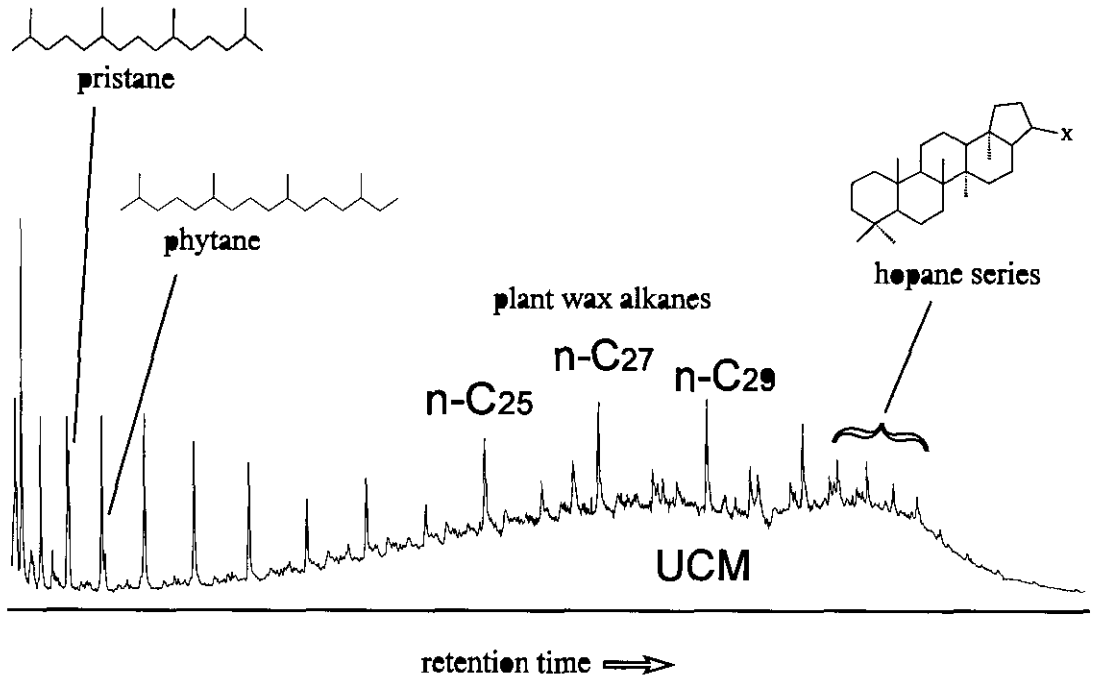


Fig. 3: GC-FID chromatogram of aliphatic petroleum-derived hydrocarbons showing some source identification characteristics.

(ii) Polycyclic alkanes such as hopanes (pentacyclic triterpanes), steranes and diasteranes have distinctive structures and are resistant to biodegradation (Volkman *et al.*, 1994). Hence they are useful biomarker compounds which may be identified by monitoring their characteristic fragment ions using the selected ion monitoring mode of the GC-MS. Ion chromatograms commonly monitored include *m/z* 191 for hopanes (Fig. 4) and other triterpanes, *m/z* 217 for steranes (Fig. 4) and diasteranes, and additionally *m/z* 259 for diasteranes (Volkman *et al.*, 1994). Additionally, biomarker analysis has the potential to provide detailed information, not only on source, but of the depositional environment and thermal maturity of the hydrocarbons (Peters & Moldowan, 1993).

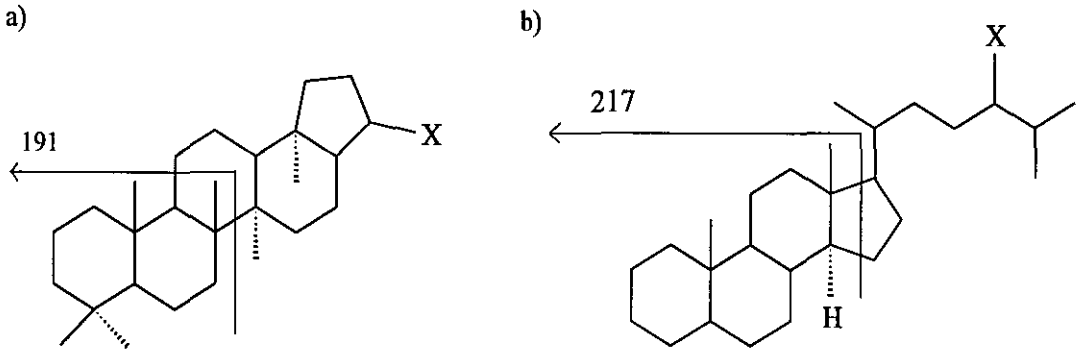
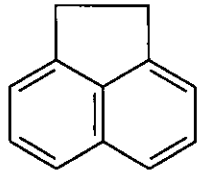
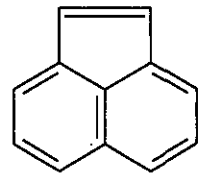


Fig. 4: Diagnostic mass spectrometric fragmentation of a) hopane biomarkers (m/z 191) and b) 14α(H)-sterane biomarkers (m/z 217). Fragmentation arrows indicate carbon-carbon bond cleavage.

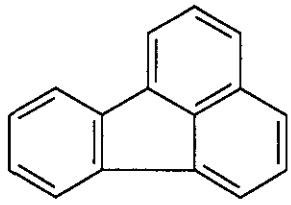
(iii) Analysis of PAHs can provide a useful tool for the determination of relative input from combustion-derived hydrocarbons to environmental samples. PAHs formed by combustion are distinguished from those in oils by the dominance of 4 to 6 ring compounds over the lower molecular weight di-aromatic and tri-aromatic compounds and by the dominance of the unsubstituted compounds over their corresponding alkylated homologues (Bence *et al.*, 1996). Additionally, particular PAH compounds are specific to a combustion source. These include acenaphthene and acenaphthylene (Yunker & MacDonald, 1995) and fluoranthene and pyrene (Fig. 5) which share a similar pyrolytic origin (Kayal & Connell, 1989).



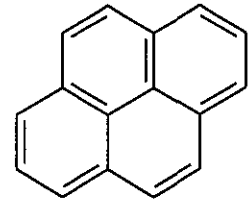
Acenaphthene



Acenaphthylene



Fluoranthene



Pyrene

Fig. 5: Some combustion-source specific PAHs

Combustion *vs* anthropogenic source of PAHs may also be inferred by the relative amounts of unstable ("kinetic") PAHs compared with the more stable ("thermodynamic") isomer (Yunker & MacDonald, 1995). For example, when the primary PAH source is combustion, the less stable PAH isomer(s) tend to be enhanced relative to the more stable PAH isomers of the same molecular mass. The less stable isomers are linear or predominantly linear (anthracene, benz(a)anthracene, benzo(a)pyrene, and dibenz(a,h)anthracene) or contain a five-membered ring (fluoranthene, benzo(b/j/k)fluoranthene, and indeno(1,2,3-cd)pyrene)(Yunker & MacDonald, 1995). Hence, the use of ratios such as; phenanthrene / anthracene and fluoranthene / pyrene; can be useful in PAH source elucidation.

The distribution of phenanthrene and its alkylated homologues can provide useful information on PAH sources in environmental samples. Generally, the proportion of alkylated to parent PAHs in samples depends on temperature of combustion of the organic material. Coal and wood smoke contains a phenanthrene mixture maximising at the parent PAH, vehicular emissions have low amounts of the parent compound

with a maximum at the C_1 homologue, and petroleum is characterised by a distribution increasing from less to more alkylated homologues (usually showing a C_2 or C_3 maximum)(Aboul-Kassim & Simoneit, 1995).

Comparison of PAH profiles in environmental samples with those of source materials can thus be used as an identification criteria. However conclusions may only be drawn under the assumption that little modification of the PAHs has occurred from their emission to their deposition. Processes contributing to the degradation of PAH include microbial degradation, photo-oxidation, and chemical oxidation (Witt, 1995). The rate and degree of PAH degradation is influenced by factors such as temperature, nutrients, presence of petroleum degrading bacteria, ultraviolet light and depth in sediments (Lee & Ryan, 1983). These factors are important considerations when designing a monitoring program with the aim of linking the composition of PAHs in source materials to those in environmental samples.

An additional consideration in such monitoring programs is PAH phase associations. PAHs are readily associated with particulate matter and deposited in the sediment. The solubility of PAHs is low and decreases with increasing molecular weight (Witt, 1995). Due to their hydrophobic nature (log K_{ow}=3-8), the concentrations of dissolved PAHs in seawater are very low (Witt, 1995).

1.8 ECOTOXICOLOGY OF HYDROCARBONS

Petroleum hydrocarbon inputs to urban waterways do not produce the dramatic toxic effects normally associated with large oil spills (Snelder & Trueman, 1995), however, continual flushing of hydrocarbons into receiving waters from stormwater systems leads to a range of problems including a decline in water quality, stained shorelines, unpleasant odours and colouration, health risk to humans, loss of recreational usage, and accumulation of toxicants in the food chain.

The impact of urban stormwater discharges on biological communities has been addressed in a limited number of studies. A series of extensive studies has been carried out on rivers in the Netherlands where marked changes in benthic invertebrate and planktonic communities were observed both during and immediately following stormwater discharge events (Gast *et al.*, 1990; Willemsen *et al.*, 1990).

Urban runoff can contain high levels of PAHs (Marsalek, 1990), some of which are highly toxic to aquatic animals and are known animal carcinogens (Vazquez-Duhalt, 1989). Crude oil and its products (petroleum, lubricating oils and bitumen) contains abundant 1 - 3 ring aromatic hydrocarbons, however, higher molecular weight PAHs are formed during combustion, with subsequent release to the environment in smoke, exhaust fumes and used lubricating oils (Vazquez-Duhalt, 1989). The direct toxicity of PAHs in stormwater may be reduced by the fact that adsorbed pollutants are mostly biologically unavailable (Scholze *et al.*, 1993).

Used oil can become highly enriched in PAHs and hence is a particularly hazardous environmental pollutant. Used oil can provoke change in microbial communities, decrease the primary production of phytoplankton, and can badly damage shellfish. It is one of the primary mutagenic agents in the aquatic environment (Vazquez-Duhalt, 1989).

1.9 BACTERIA AND STEROLS - BACKGROUND

The use of hydrocarbons as markers for determining sources of anthropogenic pollution in urban catchments was introduced in sections 1.3 - 1.5. In this and the following section, biological markers (faecal bacteria), and chemical markers (sterols) for the determination of faecal contamination and sources in environmental samples are introduced.

1.9.1 Bacteria and pathogenic microorganisms

The predominant concern with microbiological contamination of waterways is public health. Stormwater has been shown to contain significant concentrations of pathogenic microorganisms, viruses and protozoa that exceed Australian and New Zealand Environment and Conservation Council (ANZECC, 1992) guidelines for recreational purposes (Geldreich *et al.*, 1968; Wade, 1983; Olivieri *et al.*, 1989; Jacobs & Ellis, 1991; Williamson, 1991). These organisms include *Pseudomonas aeruginosa, Salmonella sp., Staphylococcus aureus, Aeromonas hydrophyla, Shigella sp., Klebsiella sp., Vibrio cholerae, Escherichia coli*, hepatitus A & B, polio virus, giardia and cryptosporidium (Blacklow, 1995). As a result, there is exposure risk and implications for human health at virtually all stormwater outfalls, even at high dilution ratios (House *et al.*, 1993).

Total coliforms, faecal coliforms and faecal streptococci also consistently occur at high levels in urban runoff. These bacteria appear to increase with increasing urbanisation and typically show highest levels at the time of first flush (Duncan, 1995). During the first flush period of storm events the mandatory bacterial levels in urban receiving waters are often violated (Ellis, 1991).

In sediments of urban waterways and receiving waters, bacteria may become encapsulated in sediments where survival times become considerably extended. Typical levels of *Escherichia coli* (*E. coli*), faecal streptococci and faecal coliforms in sediments occur in the range 10^5 to 10^6 cfu/g (Lijklema *et al.*, 1987) with *Pseudomonas* in the range of 500 to 1000 colony forming units (cfu)/g (House *et al.*, 1993). House *et al.* (1993) indicated that due to the survival times of these bacteria in stormwater receiving sediments, the sediments are potentially permanently contaminated. This feature may result in prolonged health risk in the overlying waters through continual inoculation by resuspension of sediments.

In order for an organism to be a reliable indicator of the presence of enteric pathogens in environmental samples it must be exclusively of faecal origin, consistently present

in fresh faecal material and reliably and easily detected and enumerated (ANZECC, 1992). Commonly used indicators of faecal pollution include the coliform bacteria, faecal coliform bacteria, faecal streptococci and *Clostridium perfringens* spores.

Faecal coliforms include three genera of bacteria (*Escherichia, Citrobacter and Enterobacter*). They are found in the gut and faeces of all warm blooded animals (Blacklow, 1995). *E. coli* is a bacterium that normally inhabits the intestines of warm blooded animals, including humans (Eyles & Davey, 1989). For many years *E. coli* has been used as the main indicator of recent faecal contamination. It tends not to persist in the environment for more than two weeks unless recontamination has occurred (Eyles & Davey, 1989). *E. coli* strains isolated from the intestinal tract of humans are generally regarded as harmless, however, in rare cases, *E. coli* strains from the gut of humans and other animals can become pathogenic (Padye & Doyle, 1992).

The occurrence of *E. coli* in environmental samples implies the concurrent presence of pathogens (Melbourne Water, 1993). Pathogens are microscopic organisms which include viruses, bacteria, fungi and parasites. Some pathogens occur naturally in soil and water and many are present in faeces. Pathogens can cause disease in plants and animals including hepatitis and gastroenteritis in humans (Melbourne Water, 1993). Compared with many pathogens, *E. coli* is relatively easy to detect and count, hence observed levels of *E. coli* are used as the primary indicator of the presence of pathogens in water (Melbourne Water, 1993). Although *E. coli* is not a harmful organism in most circumstances, its presence indicates that water has been contaminated by faecal material.

In urban catchments *E. coli* is found in the faeces of dogs, cats, poultry, horses, cows, pigs, birds, rats, wildlife, and in garden fertilisers. *E. coli* contributions from human faeces may enter stormwater drainage systems through septic systems that are not operating optimally or through direct illegal connection of household sewage to stormwater drains. Leachate of faecal material from septic tanks, sewer overflow points and damaged sewers may enter stormwater drains through cracks, poor joins,

holes, or by infiltration. It is believed that the most significant cause of elevated *E. coli* levels in urban waterways can be attributed to street runoff (Melbourne Water, 1993). For example, a study of urban stormwater runoff quality (EPA, 1981) showed that in one Melbourne drain the *E. coli* count rose from 36,000/100 ml to 720,000/100 ml within an hour following the onset of storm runoff.

Clostridium perfringens is a Gram positive, spore-forming, heat-resistant, rod shaped bacterium which grows only in anaerobic conditions and can survive in water and sediments for months or even years (Garland *et al.*, unpublished). Its presence in environmental samples is evidence that contamination has occurred from the faeces of warm blooded animals. *C. perfringens* has been recognised as a foodborne pathogen for many years and is now one of the principal agents of foodborne disease (Gilbert, 1987). Following wound infection, *C. perfringens* can cause gas gangrene, septicaemia, and other serious illnesses (Jay, 1986).

1.9.2 Sterols - chemical / physical properties and chemical structures

Sterols are a component of the lipid fraction of all plant and animal cells and are present in bacteria. The basic sterol skeleton is shown in Fig. 6. Sterols have great structural diversity and usually contain from 26 to 30 carbon atoms. The different sterol structures can change the cell's plasticity and general properties and can also be used to regulate the loss or gain of electrolytes through membrane permeability and the transfer of mobile cell constituents (Boutry *et al.*, 1976). Sterols may contain one or more double bonds, often between carbon atoms 5 and 6, and have an alkyl side chain of C_7 to C_{11}.

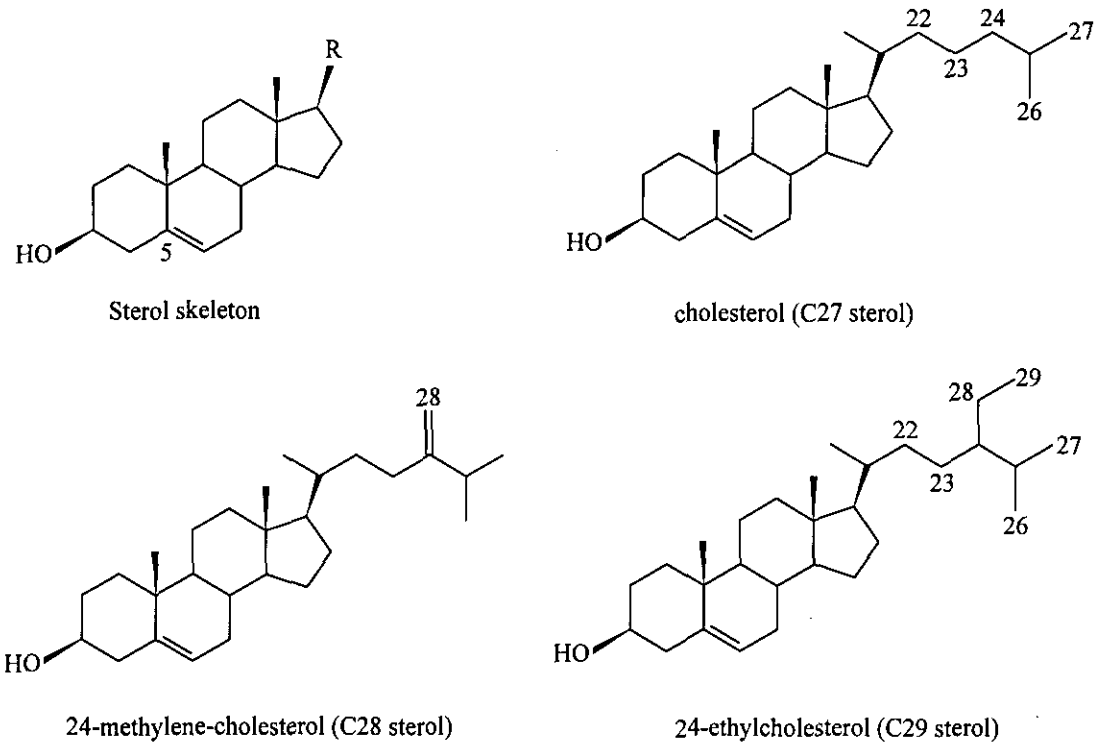


Fig. 6: Sterol chemical structures

Cholesterol is one of the better known sterols as it is the principal sterol of animals. Sterols act as a precursor to other steroids required in metabolism such as bile acids and sex and adrenocortical hormones in animals (Goodwin, 1974). Sterols are also significant components of lipids in planktonic organisms (Volkman, 1986), in marine and estuarine suspended particles (Wakeham & Lee, 1989; Laureillard & Saliot, 1993) and in terrestrial higher plants (Laureillard & Saliot, 1993).

Sterols have several features that enable them to be used as biological markers. They have a long geological record, and possess structural features, (such as positions of double bonds and patterns of side-chain alkylation), which may be diagnostic of a particular group of organisms (Volkman, 1986). Also, sterols are stable enough to be used to discriminate between sources of organic matter inputs in the water column (Wakeham & Lee, 1989). However, because some sterols are widely distributed in biological systems, their value for assigning unambiguous sources of organic material can be limited. Although higher plants typically contain higher relative and absolute

concentrations of C_{29} sterols (Nichols *et al.*, 1993), many of the sterols found in higher plants also occur in marine algae, sometimes as major constituents. Hence, it is not always possible to distinguish between marine and terrigenous organic matter by using sterol profiles alone (Volkman, 1986).

1.10 USE OF BACTERIAL INDICATORS AND STEROL BIOMARKERS IN FINGERPRINTING FAECAL MATERIAL

1.10.1 Traditional techniques

Sewage contamination has traditionally been determined by use of faecal coliform bacteria counts, in particular *E. coli*. The use of this test as an indicator of sewage pollution is however questionable due to die-off of the bacteria when exposed to; i) UV light (Leeming *et al.*, 1994); ii) the toxic effects of water chlorination or industrial effluents (Dureth *et al.*, 1986); and iii) poor survival of the bacteria in saline surface waters or loss through grazing by microzooplankton (Servais & Menon, 1991). Also, the use of this measure for the determination of water quality relies on the assumption that faecal coliforms behave in a way similar to that of other sewage derived pathogenic bacteria and viruses (LeBlanc *et al.*, 1992). In addition, bacterial indicators may overstate the amount of human derived faecal contamination present in an environmental sample as they are not source specific. Ideally, more than one technique of sewage identification should be used to achieve a more reliable guide to the levels and sources of faecal contamination present.

The degree of potential health risk in urban waterways has conventionally been measured by the ratio of faecal coliforms (FC) to faecal streptococci (FS) to indicate whether bacterial sources are of human origin (FC/FS > 4), or derived from other warm-blooded animals (FC/FS < 1). Typical indicator FC/FS ratio values for urban surface runoff fall predominantly in the range of 0.1 to 4 (House *et al.*,1993). The reliability of this indicator ratio as an index of impact risk has been questioned as the indicator species used are not the aetiological agents of disease and have much shorter

environmental survival times than the pathogens for which they act as surrogates (House *et al.*,1993).

Another biological indicator that has been utilised in tracing sewage pollution in waterways is the sulphate-reducing sporing anaerobe *Clostridium perfringens* (Emerson & Cabelli, 1985).These bacteria are commonly found in nature, particularly in soils and sediments as well as in warm blooded animals, including humans (Bergey, 1974; Bitton, 1994). As they are spore producing, they are able to resist many environmental stresses such as salinity, temperature extremes, UV light, and chlorination. The organism appears to be reliable as an indicator for tracing faecal pollution in the marine environment and for checking the efficiency of UV and chlorination treatment in wastewater plants (Bitton, 1994). The relationship between the occurrence of this organism and the presence of human viruses in water samples is also more reliable than with normal faecal coliforms and *E. coli* (Bitton, 1994). *C. perfringens* spores and coprostanol have been shown to correlate well in tracing human sewage particles (Nichols *et al.*, 1993a; and references cited within LeBlanc *et al.*, 1992).

1.10.2 Coprostanol and other sterols

Since the late 1960's many researchers, including (Murtaugh & Bunch, 1967; Dutka *et al.*, 1974; Walker *et al.*, 1982), have proposed coprostanol (5β-cholestan-3β-ol) as a measure of faecal pollution. Coprostanol is the principal sterol in human faeces (Walker *et al.*, 1982). It is formed in the digestive tract in high relative and absolute amounts by the microbial biohydrogenation of cholesterol (Rosenfield & Gallagher, 1964)(Fig. 6). The structural difference between cholesterol and coprostanol and two other common saturated isomeric stanols are shown in Fig. 7. The orientation of the hydrogen atom at the carbon-5 position and the hydroxyl group at the carbon-3 position result in different compounds (stereo isomers) which can be utilised as biomarkers (Leeming *et al.*, 1996).

Coprostanol has also been detected in the sterol profile from the faeces of pigs, cats, cows, horses, sheep and possums and as a very minor component in hen and dog faeces (Leeming *et al.*, 1997). The concentration of coprostanol in human faeces is generally between 1 and 3 orders of magnitude greater than that observed for other animals (Leeming *et al.*, 1997)(**Table 2**), hence it may be used as a useful marker of human faecal material in urban stormwater catchments.

Table 2: Coprostanol concentrations in human and animal faeces. From Leeming *et al.*, (1996)

Animal	Coprostanol (ng/g)
Rosella	1
Magpie	7
Dog	8±3
Hen	12±7
Seagull	13±12
Duck	29
Horse	43±6
Sheep	170±27
Cow	213±72
Brushtail possum	219±59
Ringtail possum	249±125
Pig	353±76
Cat	397±167
Human	3432±636

Although coprostanol will degrade under certain aerobic conditions (McCalley *et al.*, 1981), it has been shown experimentally to be stable and persistent under anaerobic conditions in sediments to time spans estimated at 160 years (LeBlanc *et al.*,1992). Hence, as coprostanol has the potential for aerobic degradation in water, its detection in urban stormwater samples may only be used as an indicator of recent and/or continuing discharge of faecal material into the urban drainage system. The relative stability of coprostanol in sediments may be used to advantage for the detection of a history of human sewage accumulation in sediments in the vicinity of stormwater discharge points.

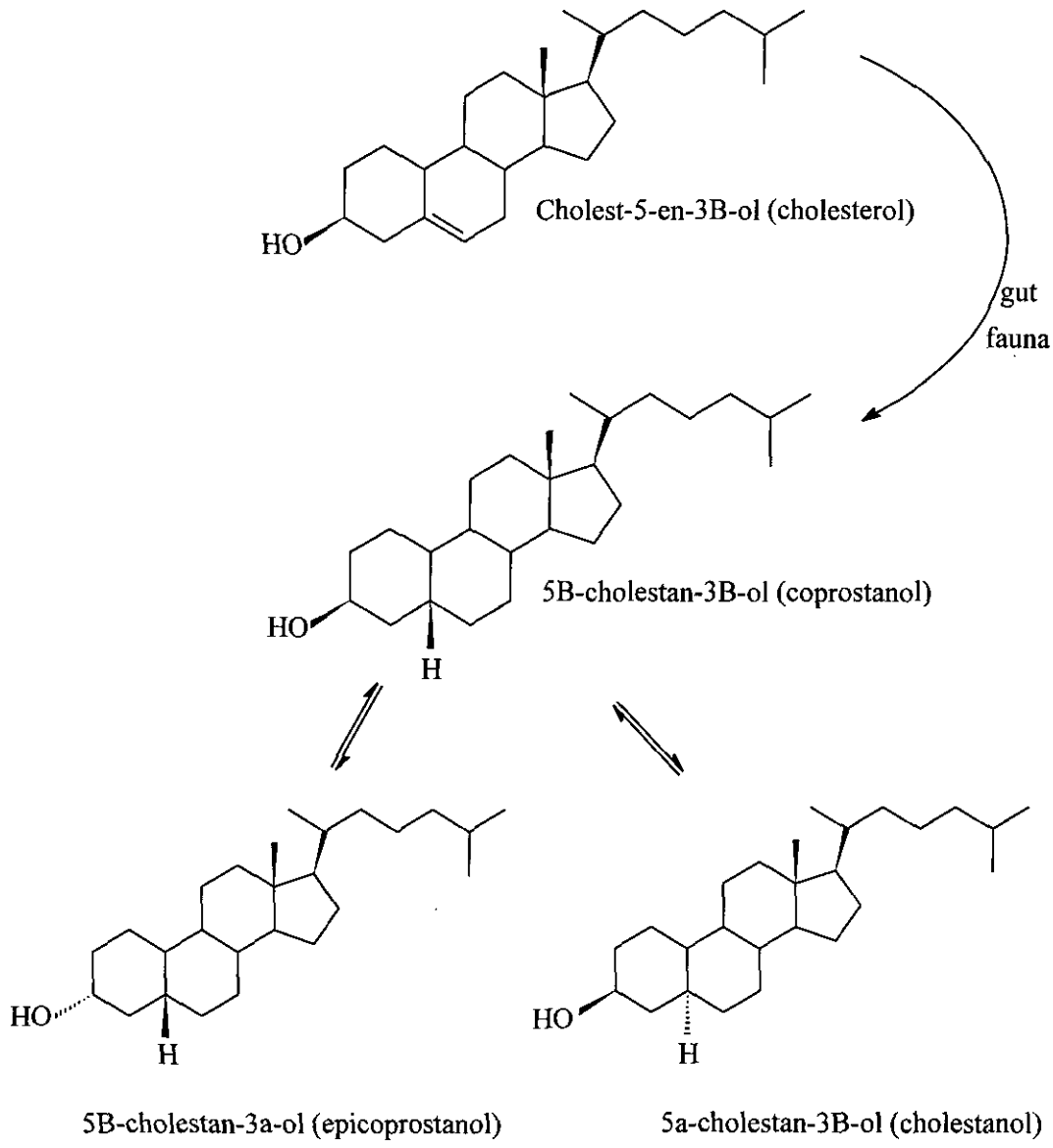


Fig. 7: Sterol chemical structure relationships between cholesterol and coprostanol and its other isomeric stanols epicoprostanol and cholestanol.

Isomers of coprostanol include epicoprostanol (5β-cholestan-3α-ol) and cholestanol (5α-cholestan-3β-ol)(Fig. 7). Epicoprostanol is formed during the activated sludge digestion process of secondary sewage treatment (LeBlanc *et al.*, 1992) and has been proposed as a marker for sewage discharge (McCalley *et al.*, 1981; LeBlanc *et al.*, 1992). Cholestanol is a naturally occurring compound derived from cholesterol (Walker *et al.*, 1982).

Coprostanol has been used successfully to trace sewage derived material in a range of environments, however, due to lack of epidemiological evidence relating coprostanol concentration to health risk, it has not yet been adopted as a water quality indicator (Leeming & Nichols, 1996). Coprostanol has been shown to correlate well with traditional bacterial indicators under some circumstances (Nichols *et al.*, 1993a), however, results reported in the literature have not been consistent enough to determine a concrete relationship between coprostanol concentrations and bacterial levels in environmental samples (Takada & Eganhouse, 1997).

Despite the fact that coprostanol is widely used to trace sewage derived material, it has been found in some ecosystems where no known sewage impacts were thought to exist (Eganhouse & Takada, 1997). *In-situ* production of coprostanol from cholesterol has been demonstrated by some laboratory incubation experiments under anaerobic conditions (Nishimura, 1982). The contribution of coprostanol from *in-situ* reduction may be calculated by using the ratio 5β/(5α+5β) where 5β: (5β-cholestan-3β-ol) and 5α: (5α-cholestan-3β-ol)(Eganhouse & Takada, 1997).

To determine other non-human faecal inputs to the urban drainage system, analysis of sterols in dog, bird, cow or other faeces may give a characteristic profile, or show unique sterol compounds, which can then be examined in environmental samples to help elucidate the source(s) of the faecal material. An indication of faecal input from herbivorous animals, for example, may be gained by tracing the sterols 24-ethylcoprostanol and 24-ethylepicoprostanol which are found in herbivore faeces in much higher amounts than in human faeces (Leeming *et al.*, 1994). Further, it is possible to estimate the relative contributions of faecal pollution from herbivorous animals by a technique developed by Leeming *et al.* (1997) which is based on a relationship between the faecal sterols, coprostanol and 24-ethylcoprostanol. This technique is described in section 4.3.1.

1.10.3 Bacterial and sterol source identification techniques used in conjunction

A more detailed insight into the nature of faecal pollution can be gained when bacterial counts are used in conjunction with sterol biomarkers. Leeming & Nichols (1996) have proposed a relationship between the concentration of coprostanol and the abundance of faecal coliforms in order to establish more reliable guidelines for the assessment of water quality. They have suggested that 60 and 400 ng/l of coprostanol correspond to the current primary and secondary contact limits for bacteria in Australia of 150 cfu/100 ml and 1,000 cfu/100 ml respectively. These guidelines, whilst potentially applicable where there are high levels of human faecal pollution, need to be reassessed if they are to be applied in urban environments where faecal material from a diverse range of sources, and not necessarily containing coprostanol, is potentially deposited.

1.11 AIMS AND OBJECTIVES OF THE PRESENT STUDY

The recently completed Tasmanian State of the Environment Report (Tasmanian Government, 1996) arrived at the conclusion that a full assessment of the environmental quality of the Derwent estuary is severely constrained by lack of information in some critical areas, including stormwater, which was defined as a "serious data gap". A need was also identified for data on organic contaminants in waters and sediments of the Derwent estuary. A major aim of the current study is to address some of these data gaps by providing information on abundance of hydrocarbon and faecal pollutants from their source(s) within catchments to their points of deposition in the estuary. This is the first study of its kind conducted in Australia and will provide data crucial for the management of local stormwater issues.

In addition to the provision of local information essential to the management of urban runoff and receiving waters, this study will compliment recent research projects on the distribution of organic matter in the Derwent estuary (**Fig. 8**). A study by Leeming & Nichols (1996) utilised sterol biomarkers and identified pulp fibre as the major

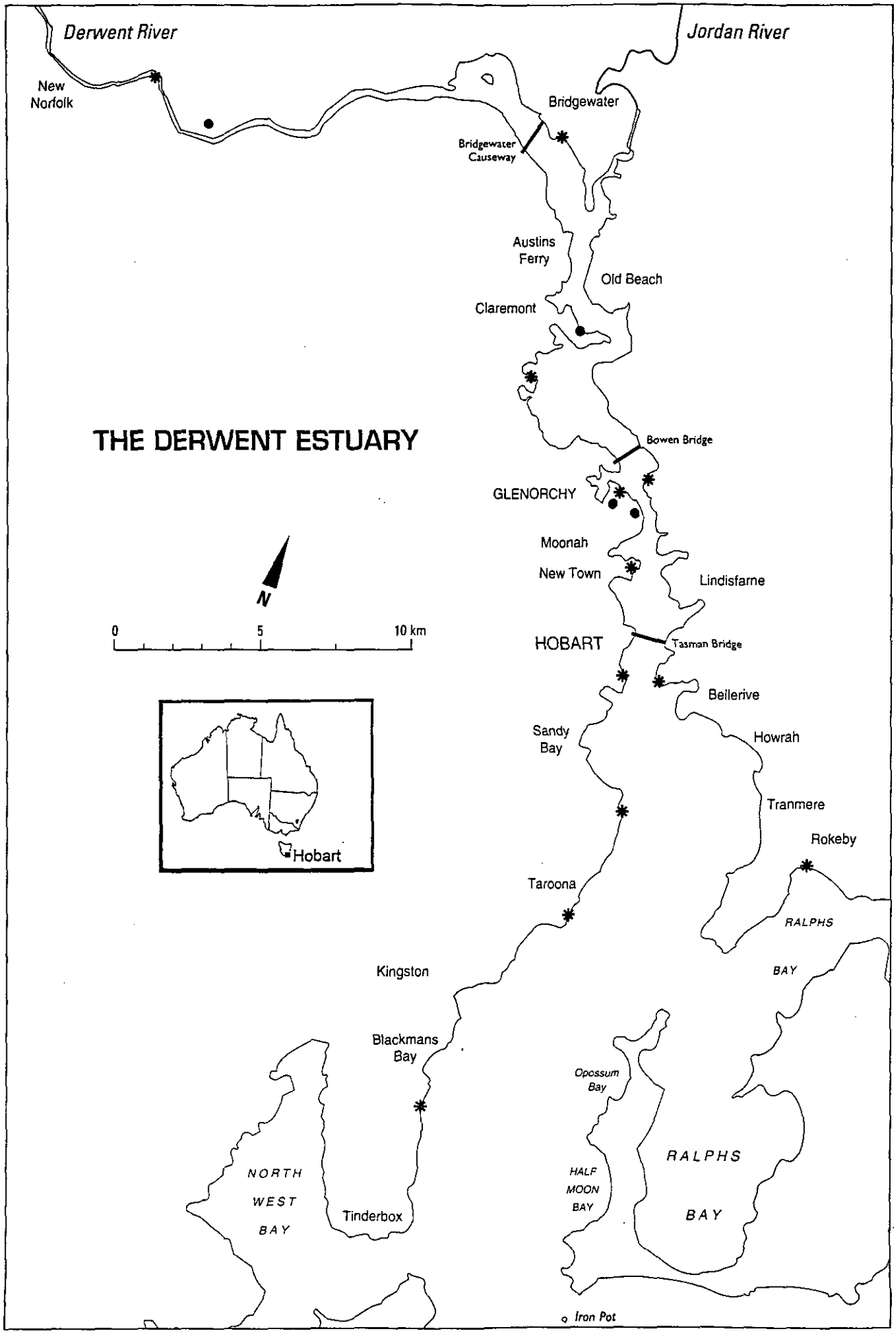


Fig. 8: Location map of Hobart, major suburbs and the Derwent estuary.

pollutant in the upper Derwent estuary with the middle estuary identified as being heavily polluted by sewage. Studies by Volkman *et al.* (1988) and Volkman *et al.* (1989) demonstrated a gradation from extremely high hydrocarbon concentrations in sediments collected from the upper estuary (1,100-4,600 μg/g) to much lower concentrations of primarily biogenic hydrocarbons in the lower estuary sediments (0.8-6.4 μg/g). Integration of data from these previous investigations and the current study will help ascertain the relative contribution of stormwater to sewage and hydrocarbon contamination in the Derwent estuary.

Many stormwater studies have noted the first flush effect which is characterised by a high concentration of contaminants, however, aside from Eganhouse *et al.,* (1981), there has been little emphasis on the variation of pollutant type and load with time during storm events. Hence, another aim of this study is to determine the relative proportions of the various hydrocarbon components (including fuels, oils and biogenic compounds) in stormwater throughout the duration of storm events.

Previous studies of hydrocarbon inputs to estuarine waters (Wakeham, 1977; Connell, 1982; Hoffman *et al.*, 1983; Hoffman *et al.*, 1984) have implicated urban runoff as one of the major contributors to the total flux of hydrocarbons entering receiving waters. In this study, calculations of total discharge of hydrocarbons by urban runoff will be estimated relative to other known hydrocarbon inputs to the Derwent Estuary. This will allow the first hydrocarbon budget regional assessment in Australia.

The Derwent estuary at Hobart has had a history of water quality problems which have traditionally been attributed mostly to sewage and industrial effluent. Due to public pressure and media coverage of beach closures due to bacterial contamination, a great deal of attention has been given to improving methods of sewage disposal in Hobart. However, there remains little knowledge of the nature of pollutant sources from the many stormwater drains that enter the estuary. To address this, another aim of this study is to assess the faecal pollution load of stormwater in relation to sewage plant inputs into the Derwent estuary.

Gannon & Busse (1989) found that faecal coliform to faecal streptococci ratios suggested animal rather than human origin of faeces in stormwater in Ann Arbor, Michigan. Geldreich *et al.* (1968) suggested from the proportions of bacterial species present in stormwater that dogs, cats and rodents were the source of the faecal contamination. Additionally, a Melbourne study demonstrated that feral and domestic animals and ineffective septic tanks appear to be the main contributors of bacteria to waterways during dry weather conditions with sewage overflows contributing during wet weather (Melbourne Water, 1993). In light of this work, another major aim of the current study is to verify or discount the growing body of evidence that sources of faecal contamination other than humans are major contributors to faecal pollution in urban waterways.

Leeming *et al.* (1997) used a combination of bacterial indicators and sterol markers to demonstrate that faecal contamination in runoff collected in the Wyong (NSW) region was most likely to be predominantly from native birds (79-90%) and domestic pets (8-19%). This was the first study to assign more than speculative source identification to faecal material in creek waters in Australia. Nichols *et al.* (1996) used sterol biomarkers to confirm sewage derived material in stormwater samples and a predominance of terrestrial vegetation inputs to urban creek samples. A final aim of this study is to build on the body of research that uses both traditional faecal indicators and faecal sterol techniques for the elucidation of the source of faecal material in catchments.

CHAPTER TWO

ANALYTICAL METHODS

A theory can be proved by experiment; but no path leads from experiment to the birth of a theory.

Einstein

2. ANALYTICAL METHODS

2.1 STUDY AREA DETAILS

Hobart, population 170,000, is the capital of the state of Tasmania, Australia. The city is situated approximately 20 kilometres up-river from the mouth of the Derwent Estuary (Fig. 8) and lies at latitude 42°53'S, longitude 147°21'E.

2.1.1 Climate and rainfall

Hobart's climate is generally cool to mild with an average annual rainfall of 626 mm (1882-1992 data) which is distributed fairly evenly throughout the year with a minimum of 40 mm in February and a maximum of 64 mm in October (Table 3). Hobart has an average of 159 rain days per year. The most common or frequently recurring rainfall event is one hour or less of rainfall with a maximum intensity of 0.2 mm/hr or less. High intensity rain (>5.0 mm/hour) falls infrequently in Hobart (Hammerschmid, 1994).

Precipitation is routinely measured by the Bureau of Meteorology at a number of sites in the vicinity of the Derwent estuary, including Bellerive, Glenorchy, Hobart (Battery Point), Kingston, Rokeby and Taroona (DELM, 1995). Table 3 summarises the long-term rainfall statistics for Hobart's Battery Point station and is compared to the rainfall which fell during the study period.

Table 3: Rainfall statistics for the Derwent estuary region

Year/Month	Rainfall (mm) 1994-95	Rain days (> 0.2 mm) 1994-95	Mean Rainfall (mm) (1882-1992)	Rain days (mean) (> 0.2 mm) (1882-1992)
1994				
December	0.4	1	57	13
1995				
January	60	18	48	11
February	28	7	40	9
March	9	11	47	11
April	78	16	52	12
May	8	8	49	14
June	39	16	56	14
July	46	14	54	15
August	100	18	52	15
September	33	15	52	15
October	45	14	64	16
November	64	18	55	14
December	131	13	57	13
Totals (1995 only)	*641*	*168*	*626*	*159*

2.1.2 Derwent Estuary Hydrology

The circulation of the Derwent estuary has been described by Davies & Kalish (1994), Hunter & Andrewartha (1992) and DELM (1995). The estuary is described as a highly stratified salt wedge/partially mixed estuarine system. The lower estuary is dominated by wind-driven and tidal mixing, with relatively large vertical mass movements within the water column (DELM, 1995). The upper estuary is highly stratified with a distinct salt wedge, the toe of which is normally situated in the vicinity of New Norfolk. During high river flows (approximately 150 m^3/s), the toe of the salt wedge migrates downstream as far as Bridgewater. During low river flows, the waters of the upper estuary are poorly mixed, predominantly through the action of sluggish tidal exchange (DELM, 1995).

The average tidal range of the Derwent estuary is slightly greater than one meter. ranging from a minimum of 0.3 m to a maximum of 1.6 m. Tidal currents are

relatively weak, typically in the order of 0.1 to 0.2 m/s (Davies & Kalish, 1994; DELM, 1995). Recent investigations and modeling conducted by the CSIRO Division of Marine Research indicate that, on average, surface currents flow to the south at velocities of 0.1 to 0.2 m/s, while bottom currents flow northwards at velocities of 0.1 to 0.5 m/s (DELM, 1995).

The average flushing period for the estuary is estimated to be approximately 15 days (J. Hunter, CSIRO, pers. comm.), although the relatively isolated deep waters of the upper estuary may be retained for a much longer period (20 to 35 days) during low river flows (Davies & Kalish, 1994). The main focus of this study was at Prince of Wales Bay, which is situated on the middle Derwent estuary (Fig. 9). Bays on the Derwent estuary are believed to have a flushing period in the order of a few days (J. Hunter, pers. comm.), however, no specific hydrological studies have been undertaken to verify this. Low sediment scouring rates in sheltered bays such as Prince of Wales Bay mean that sediments can remain within the bays indefinitely (J. Hunter, pers. comm.).

2.1.3 Discharges to the Derwent estuary

The Derwent Estuary has received discharge from sewage, stormwater and industry for more than 175 years (Tasmanian Government, 1996). In 1843 the Hobart Rivulet was sanctioned by legislation to be used as a public sewer. In the 1880's, 533 water closets emptied directly into the rivulet (Petrow, 1994). Two major industrial complexes, a paper mill and an electrolytic zinc refinery, are currently located on the shoreline of the Derwent estuary (Fig. 10). The volumes of liquid effluent discharged into the Derwent Estuary are 56.8 million litres/day for domestic effluent from municipal wastewater plants and 200 million litres/day for industrial effluent (1994 values, Tasmanian Government, 1996)(Fig. 10)(Table 4). These volumes have changed little in the last two decades. Additionally, two hundred and seventy stormwater drains (greater than 300 mm diameter) and several large rivulets discharge, largely unmonitored and untreated into the Derwent Estuary from Hobart's urban area.

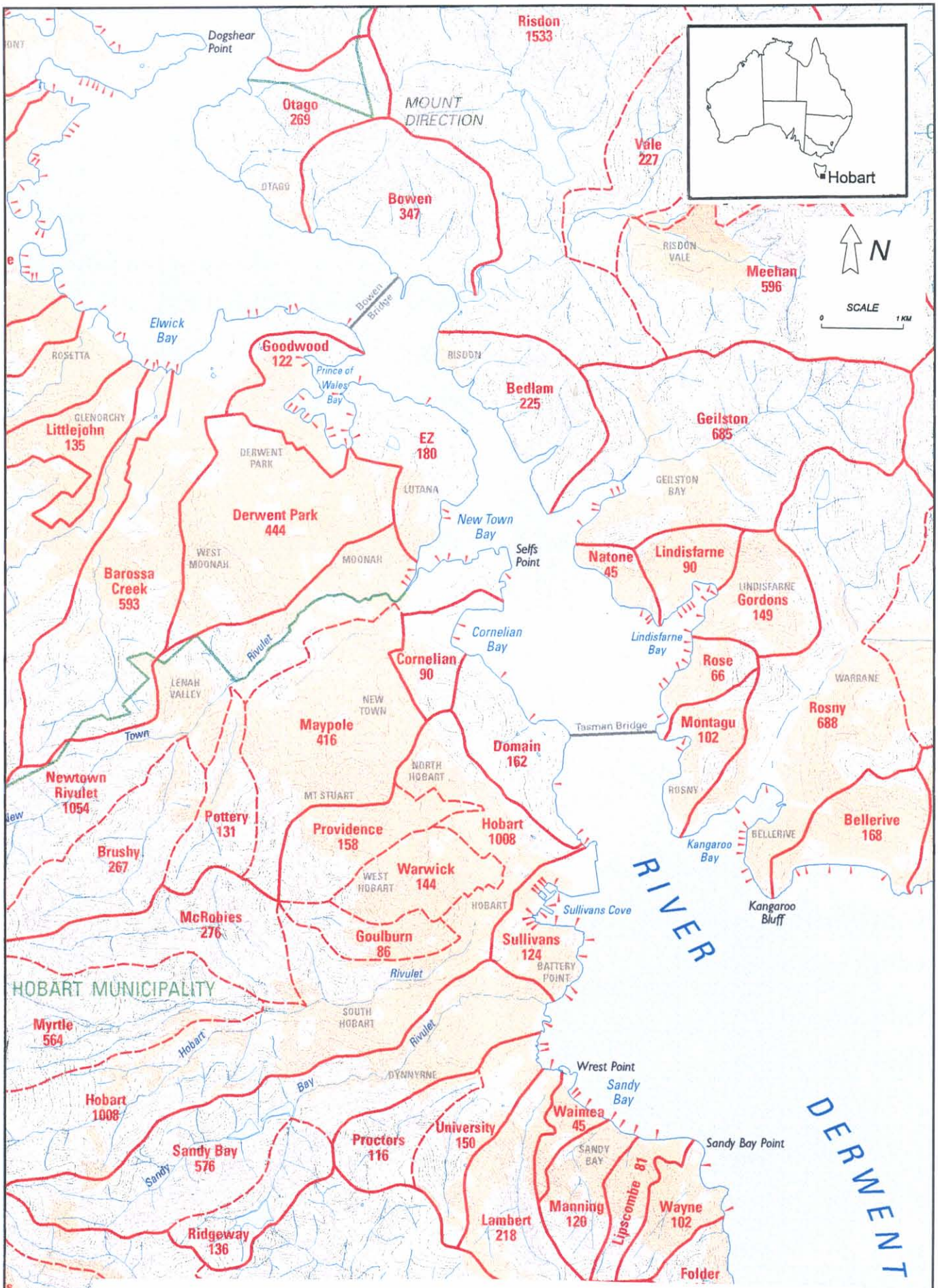


Fig. 9: Hobart metropolitan area and the Derwent estuary. Stormwater catchment boundaries are delineated by solid red lines with sub-catchments in dashed lines. Catchment names and areas in hectares are shown. Stormwater outfall points are shown with red arrows.

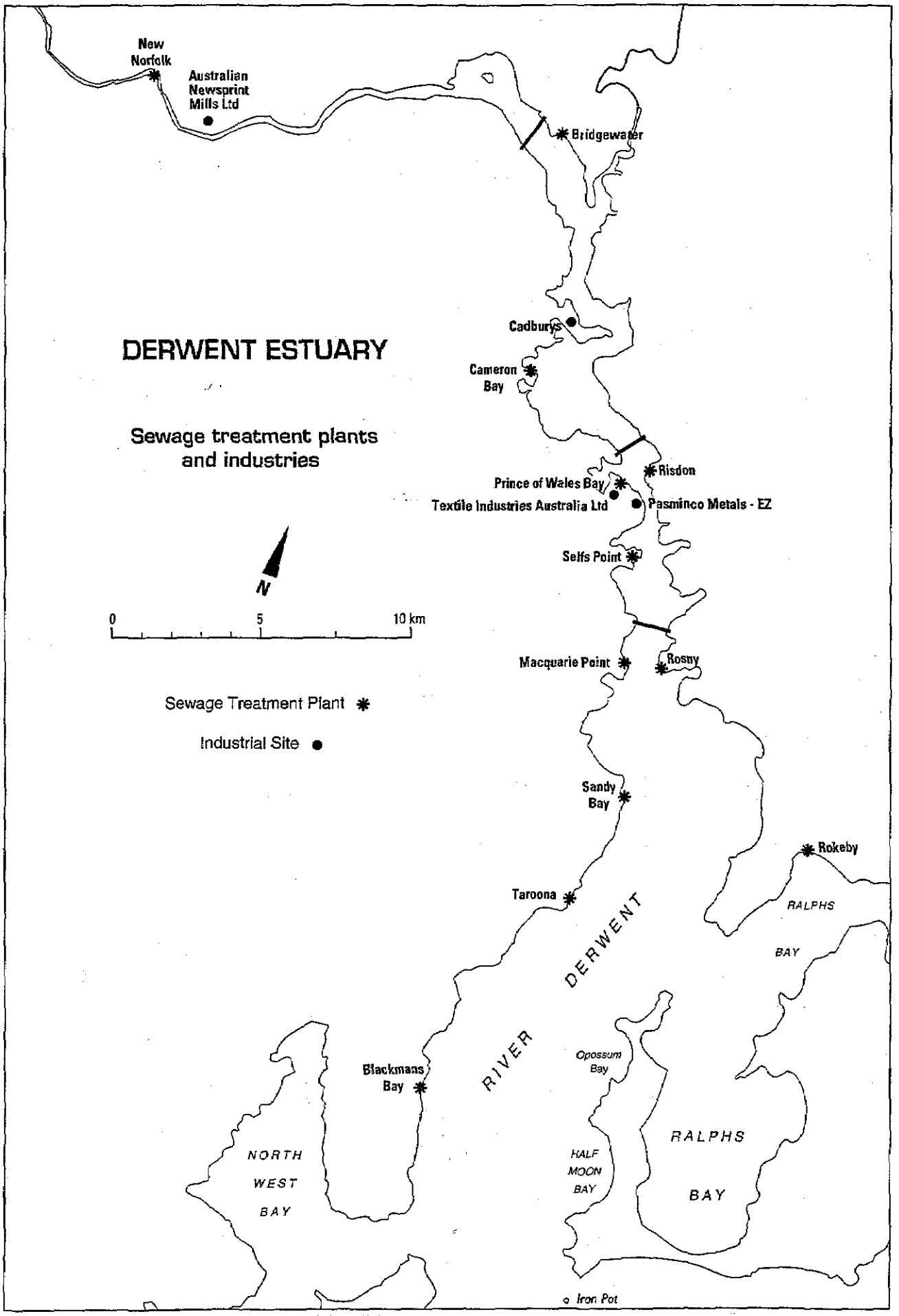


Fig. 10: Location map of sewage treatment plants and major industries on the Derwent estuary.

Table 4: Sewage treatment plants on the Derwent estuary: plant type, discharges and proposed improvements*.

Location	Discharge kL/day	Discharge %	Treatment	Treatment type	Recent/planned improvements
Bridgewater	2,600	5%	2°	activated sludge (biofiltration)	reuse proposed 1995/96
East Risdon	2,200	4%	2°	oxidation ditch	
Rokeby	1,200	2%	2°	activated sludge plus lagoons	extensive improvements proposed in 1998
Rosny	7,000	13%	2°	biofiltration, solids contact tank	extensive improvement completed
Cameron Bay	4,000	8%	2°	activated sludge	extensive improvement completed
Prince of Wales Bay	8,000	15%	2°	activated sludge	extensive improvement completed
Sandy Bay	4,000	8%	none	maceration only, no disinfection	tertiary treatment planned at Selfs Point in 1997
Macquarie Point	13,000	25%	2°	biofiltration	
Selfs Point	4,000	8%	2°	biofiltration	tertiary treatment proposed in 1997
Blackman's Bay	3,200	6%	2°	extended aeration	secondary clarifier to be completed 1995
Taroona	7,50	1%	2°	biofiltration	improvements completed
New Norfolk	2,500	5%	2°	biofiltration	wetland system constructed
Total	52,450				

*Adapted from the Derwent Estuary Nutrient Program technical report (DELM, 1995)

2.1.4 Stormwater catchment study sites and land-use characteristics

The sampling program for this study was conducted over a period of eighteen months and focused predominantly at the Prince of Wales Bay outfall which is the discharge point for the Derwent Park catchment (Figs. 9 & 11). There are no remaining natural drainage channels in the Derwent Park catchment and stormwater flow is conducted entirely through pipes. The stormwater catchment area of Prince of Wales Bay is 444 hectares (1 hectare is equivalent to 10,000 m^2) and contains the most intensive light industrial area of Hobart (Fig. 12). The catchment, which also includes commercial and residential areas, is comprised largely of impermeable surfaces which facilitate high and rapid rates of rainfall runoff. The impact of the major stormwater outfall at Prince of Wales Bay may be seen in Fig. 13 by the presence of a coloured plume visible in the southern reaches of the bay.

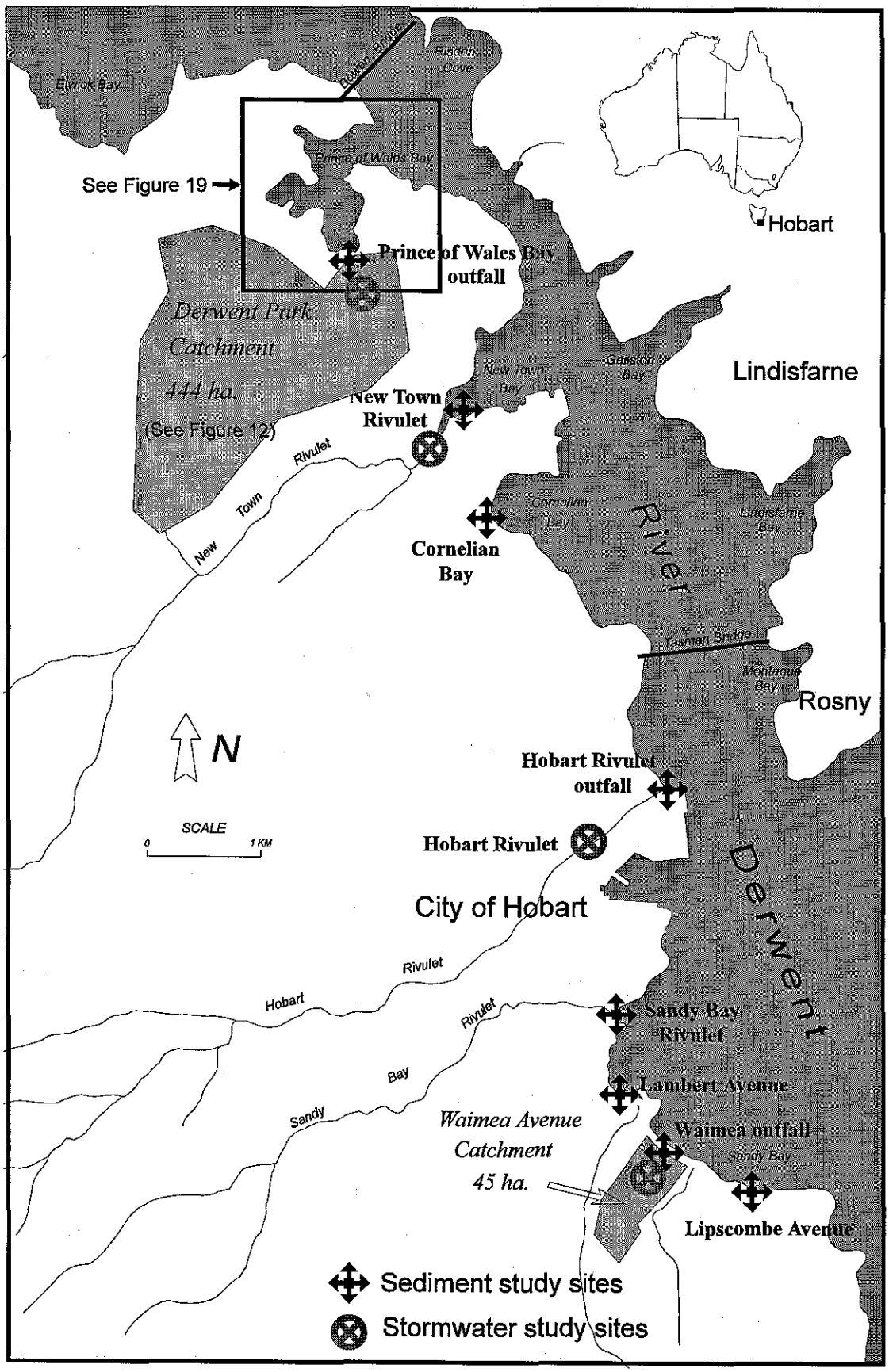


Fig. 11: Stormwater catchments and stormwater outfall sediment study sites.

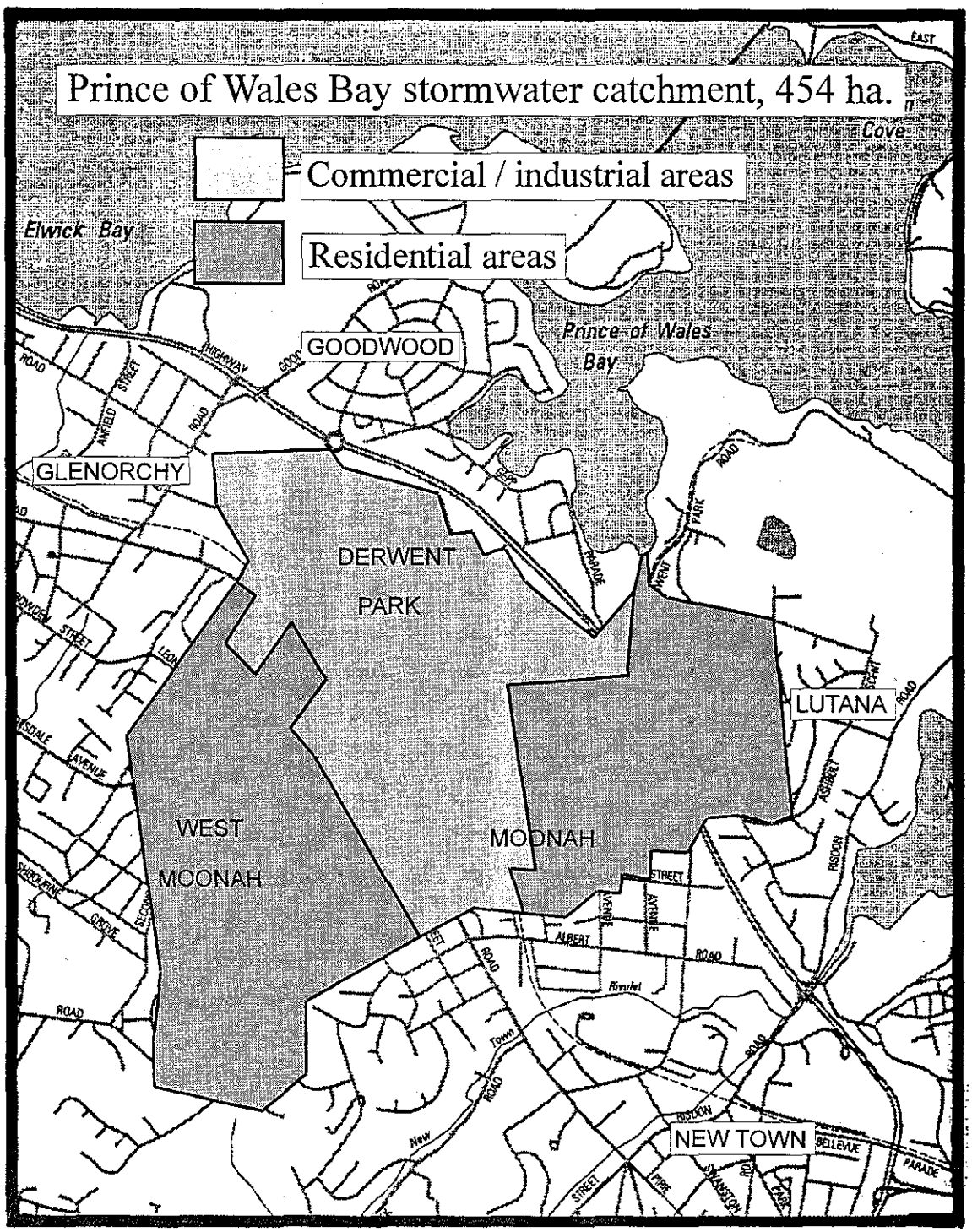


Figure 12: Map of the Derwent Park stormwater catchment showing the major land use types.

Fig 13: Aerial photograph of Prince of Wales Bay and surrounding area. A coloured plume of stormwater discharge is evident in the southern reach of the bay. Photo © TASMAP, 11/3/95.

Other stormwater study sites were at Waimea (Figs. 9 & 11) which is a fully piped catchment with only one land-use characteristic; medium density residential. Two rivulet study sites were chosen, Hobart Rivulet and Newtown Rivulet (Fig. 11), in order to provide a range of catchment types and land-use characteristics in the study.

The land-use characteristics of the entire Derwent estuary stormwater catchment are residential, 3,030 ha (8%); commercial, 163 ha (0.5%); industrial, 494 ha (1.5%); park, 974 ha (2.5%); agricultural, 10,165 ha (27%); bare soil, 931 ha (2.5%); and forest, 21,743 ha (58%).

2.1.5 Hobart's sewerage system

An important consideration for any study of faecal material in urban areas is the nature of the sewage conveyance system and the potential for cross contamination to the stormwater system to occur. Some of the sewerage system in Hobart is very old, being first constructed in 1912. At present no damaged pipework has been identified, however, the possibility of cracks, breakages or weakened joins cannot be excluded (Blacklow, 1995). Some sewage discharge direct to the estuary has been known to occur due to overload at sewage plants during wet weather and blockages also occur, however, the sewerage system is believed to be well provided for in terms of excess capacity (Tasmanian Dept. Environment & Planning, 1992). An additional means by which raw sewage may be delivered in an untreated form to the estuary is through sewage pump-station malfunctions or during pump station maintenance. At these times sewage is directed to a stormwater drain.

The sewerage system of Hobart is extensive, with only a very small number of homes using a separate septic system. The only unconnected area according to the Hobart City Council Sewage Catchment and Pump Zones Map (1993) includes a few houses in the vicinity of Old Farm road in South Hobart (Blacklow, 1995).

2.2 SAMPLING

2.2.1 Stormwater

Many stormwater studies have reported significant variations in pollutant concentrations within storms, between storms and between areas; e.g., Rowlands *et al.*, (1992). These variations occur for a number of reasons including;

- Variability in sources of pollution between different locations and between different storms.
- The time since the previous storm and catchment management practices during this period.
- The intensity and duration of rainfall is variable both temporally and spatially.

These factors were taken into account in designing the stormwater sampling program described here.

Stormwater samples were collected manually from drain outfalls and creeks in pre-cleaned (solvent rinsed x3 with hexane) 2 litre glass jars at both dry weather (base flow) conditions and during rainfall events. Glass jars were lowered into storm channels by use of a specifically constructed aluminium sampling arm. Samples were taken from the centre of the channel. Glass containers were used for collection of both water and sediment samples. Samples were filtered as soon as possible after collection, usually within two hours. Stormwater sampling points at Prince of Wales Bay and Hobart Rivulet are shown in Fig. 14.

Stormwater flow was estimated using a hand-held, factory calibrated 'Global Flow Probe' computerised flow meter which is capable of providing instantaneous (±0.15 m/s) and average (±0.03 m/s) velocity measurements for a specified time period. For each stormwater sample collected an average flow for the duration of 10 seconds was recorded. The flow meter was moved across the channel and back during this time period to allow for variation in flow rates within the channel. Water depth in outfall

a)

b)

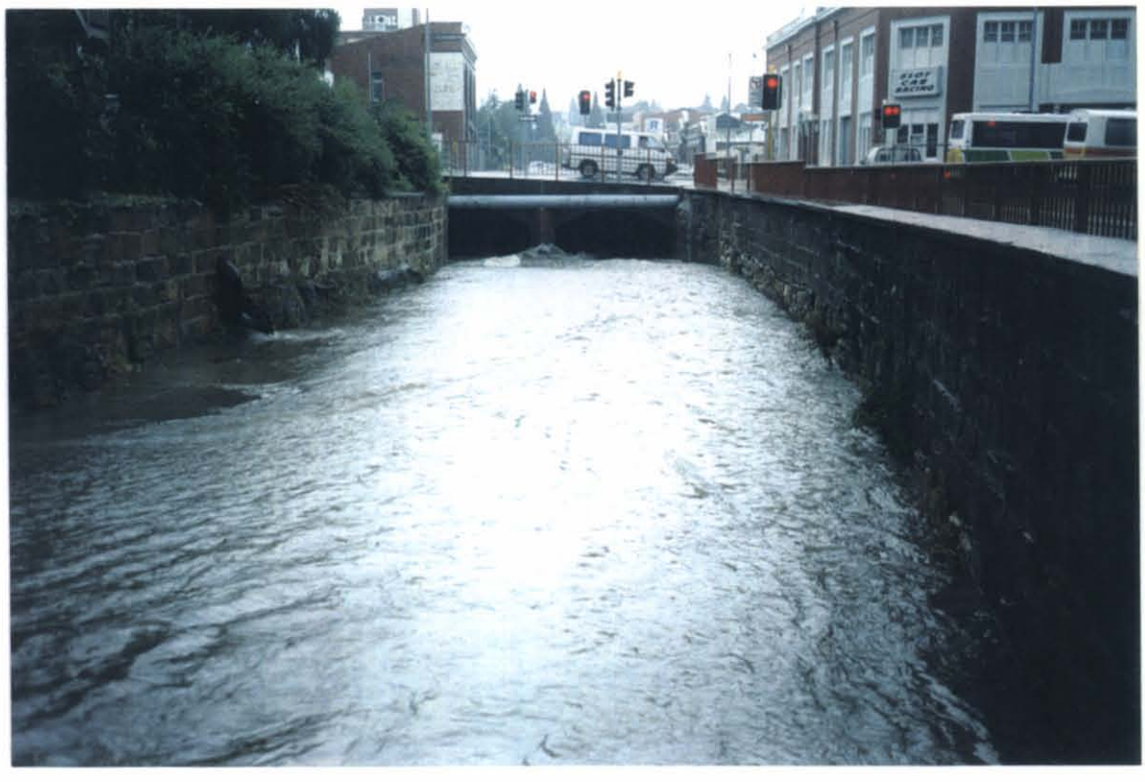


Fig. 14: a) Prince of Wales Bay and **b)** Hobart Rivulet stormwater sampling points.

pipes was recorded manually (using a graduated pole) with each sample to enable estimated water discharge volumes to be calculated. A summary of the stormwater sampling program conducted at Prince of Wales Bay is shown in Table 5.

Table 5: Summary details of stormwater sampling program at Prince of Wales Bay.

Storm event #	Rainfall (mm)	Samples collected	Sampling duration	Sample names	Max. recorded flow (l/s)	Comments
1	0.5 mm	3	15 min	POWB 2-4	2100	Steady shower
2	13 mm	4	14 hrs	POWB 5-8	4030	Light rain
3	10 mm	4	11 hrs	POWB 10-13	2850	Light rain
4	6 mm	5	50 min	POWB 17-21	7720	Short summer downpour
4a	23 mm	4		POWB 22	9280	Heavy rain
5	18 mm	4	1.5 hrs	POWB 23-26	2510	Steady light rain
6	41 mm	7	7 hrs	POWB 29-35	4970	Heavy rain
7	3 mm	12	45 min	POWB 36-47	2290	Light rain
8	10 mm	5	30 min	POWB 48-52	1600	Light rain
9	146 mm	10	29 hrs	POWB 53-62	10500	Flooding rain

As many storm events as practicable were sampled during the year. It was not possible to sample every rain event as it was discovered that rainfall doesn't always occur at times that are amenable to stormwater sampling. Despite this, it is believed that the stormwater samples obtained encompass many different rainfall intensities and seasonal variation, which may lead to changes in contaminant loadings and characteristics within the catchment. Aside from the storm events shown in Table 5, 10 other base flow samples were collected at regular intervals throughout the year from the Prince of Wales Bay stormwater outfall. These were collected and analysed in order to gain an appreciation of background contaminant concentrations in the stormwater.

Due to the difficulty of sampling at more than one stormwater catchment at any given time without the aid of automatic sampling equipment, the other selected catchment study sites (Waimea, Hobart Rivulet and Newtown Rivulet) were sampled only twice during the year. These sampling times were chosen to coincide with periods of steady rain so that samples collected would be representative of consistent flushing across the

whole Hobart catchment. Samples from all four catchments were collected on 7/04/95 and 7/08/95.

2.2.2 Sediments and faeces

Sediment samples were collected from within Prince of Wales Bay and at selected locations on the Derwent estuary near stormwater outfalls (Fig. 11). Sediments were collected at low tide using a hand-operated polycarbonate push corer (7 cm diameter). The top 1 cm of the sediment core was then transferred to pre-cleaned (solvent rinsed x3 with hexane) glass jars. Sediment samples were frozen immediately after collection in order to minimise alteration of analytes due to chemical reaction or microbial activity.

Animal (dog and rat) faecal samples were collected from street surfaces and from within stormwater drains from the Hobart catchment (Fig. 9). Faeces was collected with sterilised forceps and placed in solvent rinsed (hexane x3) glass jars.

2.3 EXTRACTION AND FRACTIONATION OF SAMPLES

Stormwater particulates were separated and extracted for subsequent analysis of hydrocarbons and sterols. Stormwater samples were first homogenised by shaking and then filtered through pre-weighed Whatman glass microfibre filters (GF/F 9 cm diameter) with an average pore size of 0.8 μm. Filters were then freeze dried and re-weighed (microbalance to 10^{-4} g) in order to determine the mass of particulate matter in each sample. Nanograde solvents (Mallinckrodt) and reagents were used throughout the procedures and glassware was solvent rinsed prior to use.

Stormwater filters, sediment and faecal (dog and rat) samples were extracted by the modified one-phase chloroform/methanol Bligh and Dyer method (Bligh & Dyer, 1959). Samples were placed in a 250 ml separatory funnel to which $CHCl_3$ (37.5 ml), MeOH (75 ml) and H_2O (milliQ - 30 ml) were added. No water was added for wet

sediments. Samples were shaken at regular intervals and left to extract for at least 24 hours. The next step was phase separation by addition to the separatory funnel of $CHCl_3$ (37.5 ml) and H_2O (milliQ - 37.5 ml).

After phase separation, the lower layer (chloroform fraction) was drained into a round-bottomed flask. Lipids (the fatty fraction containing hydrocarbons and sterols) were recovered from the chloroform fraction by rotary evaporation under vacuum. The lipid fraction was then transferred to glass vials (1800 µl) using a glass pipette. The round bottomed flask was rinsed four times to assure quantitative transfer. Total lipid samples were made up to a known volume (usually 500 µl) in the vials and stored under nitrogen at -20°C.

Total sterols were obtained by alkaline saponification of a measured aliquot of the total lipids. This procedure involved reducing the lipid aliquot to dryness under a stream of nitrogen followed by the addition of 2 ml of 5% w/v potassium hydroxide in 80:20 methanol : milliQ H_2O. Samples were then heated at 60°C for 3 hours. Following the addition of 1 ml of milliQ H_2O, the neutral lipid fraction containing sterols, hydrocarbons and alcohols, was extracted (x3) with 4:1 hexane : chloroform (2 ml). Each extract was transferred to a round bottomed flask containing 0.15 g of alumina (aluminium oxide - Brockman grade 1 neutral) and then rotary evaporated to dryness under vacuum. The alumina, with adsorbed organic extract, was then added to the top of a chromatography column in preparation for separation of individual compound classes (hydrocarbons and sterols).

Column chromatography was performed using 3.0 g of activated alumina in a 5 mm internal diameter glass column. Alumina was pre-cleaned by a 24 hour soxhlet extraction using 50:50 chloroform : methanol. Chromatography columns were packed by slow addition of the alumina to columns containing hexane. Solvent elution sequence was 35 ml hexane (aliphatic hydrocarbons) followed by 40 ml of 20% dichloromethane in hexane (PAH) and finally 20 ml of chloroform to obtain the fraction containing sterols (Nichols *et al.*, 1989).

Prior to analysis, sterols were converted to their corresponding trimethylsilyl (TMSi) ethers by treatment at 60°C for 60 minutes with 50 µl of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)(Nichols *et al.*, 1989).

For determination of total hydrocarbons, a portion (usually 10%) of the total organic extract (obtained by the Bligh/Dyer extraction explained above) was set aside for analysis by thin layer chromatography with flame ionisation detection (TLC-FID). A second portion of the total organic extract was fractionated by column chromatography as described above. The PAH fraction contained a range of compounds from the two-ring aromatics (naphthalenes) to six ring PAH compounds such as benzo(ghi)perylene.

2.4 ANALYSIS

2.4.1 TLC-FID (thin layer chromatography-flame ionisation detection)

Detailed discussion of the application of TLC-FID for the analysis of lipid classes in environmental samples is given by (Volkman *et al.*, 1986). In this study TLC-FID was utilised to quantify total aliphatic hydrocarbons in all samples. This also proved to be a useful way of screening samples in order to determine the most appropriate concentration for subsequent analysis by gas chromatography. A portion (1µl) of the total organic extract was applied to silica coated quartz rods and developed in a solvent system of 98 ml hexane/2 ml ether for 25 minutes. This proved to be the most appropriate solvent system for adequate separation of aliphatic hydrocarbons from PAHs and from other lipid classes present in the samples. After development, the silica rods were dried at 100°C for 5 minutes and then scanned through the flame ionisation detector (FID) of the Iatroscan analyser (Iatron Laboratories, Japan). Each sample was analysed in triplicate. The FID response was calibrated using the hydrocarbon standards, nC$_{22}$ and 3,6-dimethylphenanthrene, which were used to quantify total aliphatic and total PAHs respectively. The standards produced a linear

response over the concentration range 1-10 μg with aliphatic hydrocarbons and PAHs quantified by the relationships:

$$HC\ (\mu g/\mu l) = 1.6837 \times 10^{-5}x - 3.702 \times 10^{-12}x^2$$

where x = the hydrocarbon peak area quantified by DAPA (Data Acquisition, Plotting and Analysis) chromatography software (Kalamunda, W.A.),

$$PAHs\ (\mu g/\mu l) = 1.9325 \times 10^{-5}x - 1.1216 \times 10^{-11}x^2$$

Accurate quantitation of total PAHs was not possible by TLC-FID as this compound class was often below the limit of detection. TLC-FID is 2 - 3 orders of magnitude less sensitive than gas chromatography-flame ionisation detection (GC-FID), and lipid masses less than 2 μg are difficult to measure accurately (Volkman & Nichols, 1991). However, the technique was useful for quantifying total aliphatic hydrocarbons in the heavily polluted stormwater and sediment samples of this study.

2.4.2 Gas chromatography (GC)

Gas chromatographic analyses of aliphatic hydrocarbon and sterol fractions were performed with a Hewlett Packard 5890 GC equipped with a 50 m x 0.32 mm internal diameter HP1 fused-silica capillary column (0.17 μm film thickness) using splitless injection and flame ionisation detection (Nichols *et al.*, 1989). The initial GC oven temperature was 50°C which, after 1 minute, was ramped to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1} and finally to 300°C at 5°C min^{-1}. Hydrogen was used as the carrier gas, and the injector and detector were maintained at 290°C and 310°C respectively. The internal injection standard used for hydrocarbons and sterols were squalane and methyltricosanoate respectively. Peak areas were quantified using DAPA chromatography software and compound concentrations were calculated using the following relationship;

$$\text{Concentration (ng/g)} = \frac{\text{a) Peak area of compound}}{\text{Peak area of standard}} \times \frac{\text{b) } 100}{2} \times \frac{\text{c) Standard (ng/g)}}{1} \times \frac{\text{d) } 100}{\text{Aliquot}} \times \frac{\text{e) } 1}{\text{Sample (g or l)}}$$

Where; **a)** Peak areas are as integrated by DAPA; **b)** sample volume in vial in relation to the volume injected onto the GC column; **c)** amount of internal standard injected (ng); **d)** % of total lipid extract used in sample workup; **e)** initial volume of sample filtered or mass of sample extracted.

Initial sterol identifications from GC chromatograms were based on relative retention time data (Jones *et al.*, 1994) obtained from laboratory standards. The GC flame ionisation detector was previously found to be linear over the concentration range of 0.5-150 ng for individual compounds (Nichols *et al.*, 1996).

2.4.3 Gas chromatography/mass spectrometry (GC-MS)

All PAH, hydrocarbon biomarker samples and selected sterol fractions were analysed on a Fisons MD 800 quadrupole mass spectrometer linked to a Carlo-Erba 8000 series gas chromatograph equipped with an HP5 Ultra 2 column (50 m, 0.32 mm i.d., 0.17μm film thickness). Sterols were analysed qualitatively and PAHs both qualitatively and quantitatively. The GC was temperature programmed from 45 to 140°C at 30°C/min., from140 to 300°C at 3°C/min and 5 min. at 300°C. Samples (0.5μl) were injected on-column, using a Fisons AS 800 autosampler, into a 0.53μm retention gap attached to the head of the column. Helium was used as carrier gas. The mass spectrometer was run in selected ion monitoring (SIM) mode at an electron energy of 70eV (Leeming *et al.*, 1997).

Sterols identified by GC were verified by GC/MS analysis by comparison of the mass spectra obtained for individual peaks and those of standards or spectra previously reported in the literature.

2.4.4 PAH compound identification and quantification

PAH compound identification was based on molecular ion monitoring by GC-MS and comparison of GC retention times with 24 PAH in Standard Reference Material (SRM) 1491 (National Institute of Standards and Technology - NIST)(see Appendix 1). Ions used to identify and quantify PAHs are shown in (Table 7). Quantification

was based on the addition of deuterated compounds to PAH fractions as internal injection standards. The quantification of naphthalenes, acenaphthene and acenaphthylene was based on D_8-naphthalene, whereas the quantification of tri-aromatic and larger compounds was based on D_{10}-phenanthrene and D_{12}-chrysene respectively. In order to correct compound concentrations for detector response, relative response factors were determined for all PAH in SRM 1491. This was done by co-injection of the deuterated standard mixture with SRM 1491 at different concentrations. Ten replicate analyses at each concentration were performed. PAH response factors, and an equation for quantification of individual PAH, are presented in Appendix 2. The detector was calibrated for each PAH measured and the linearity of the detector response determined over the concentration range expected. This is because structurally similar compounds may have response factors differing by up to a factor of two (Jones *et al.* 1994).

At peak instrument sensitivity, the limit of detection for individual PAH compounds was 0.01 ng/l for water samples and 0.01 ng/g for dry sediment samples. PAH compounds could be detected at 0.05 pg.

2.4.5 Compound identification / quantification uncertainties

The faecal sterol, 24-ethyl-5β-cholestan-3β-ol (24-ethylcoprostanol), which is a key compound in the elucidation of faecal inputs from herbivorous animals to environmental samples, was often difficult to accurately quantify in stormwater samples. This was due partly to the fact that this sterol was present as a very minor component or absent from the sterol profile, and partly due to the fact that it co-eluted with 24-methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol) on the HP5 chromatography column and with 24-methylcholest-5-en-3β-ol (campesterol) on the HP1 column. In some cases relative proportions of the two compounds could be assessed by comparing mass fragmentograms of the total ion current (TIC) with that of the *m/z* 129 ion (the base peak ion for 24-methylenecholesterol and campesterol). A change in the relative peak size for 24-methylenecholesterol in the TIC fragmentogram could be assumed to be due to the presence of the *m/z* 398 ion which

is the base peak for 24-ethylcoprostanol. Additionally, in stormwater samples containing only very low levels of sterols, definitive mass spectra for the minor sterol components were often impossible to obtain.

2.4.6 Assessments of procedural accuracy and precision

It has long been observed that organic matter in sediments is not uniformly distributed and may be highly variable in concentration and composition, even within small, uniform sampling sites (Downing & Rath, 1988). It is useful to make some assessment of the variability of the parameters being studied otherwise extrapolation, or results based on single core samples may yield greatly biased estimates of actual conditions. The magnitude of bias depends on the degree of spatial heterogeneity of the sediments and the relative size of analytical error and spatial variation (Downing & Rath, 1988). Heterogeneity among replicate sediment samples is many times greater than microscale or analytical error necessitating care in sampling design (Downing & Rath, 1988). To assess the spatial patchiness of sediment samples from the Derwent Estuary nine equally spaced sediment samples were collected from within a 300 mm^2 quadrat. One of these samples was homogenised and analysed in six separate aliquots to determine laboratory procedural precision. Aliphatic hydrocarbons, the bacterium *Clostridium perfringens*, and coprostanol were the constituents determined in these sediment samples. Results are presented in Table 6.

Table 6: Assessment of mean concentration and standard deviation between samples and for sample replicates.

a)Small scale sediment variability

Sediment sample	Hydrocarbon content mg/g	*Clostridium perfringens* /g
1a	8.3	11000
2a	16.2	20000
3a	53.2	20000
4a	11.2	
5a	26.4	57000
6a	5.9	
7a	15.4	32000
8a	24.0	
9a	19.8	38000
Mean	*20.0*	*29667*
Standard deviation	*20.9*	*16476*

b) Single sample (sed. 3a) replicate analysis

Sediment sample	Hydrocarbon content mg/g	Coprostanol content ng/g
3a.1	46.0	50.6
3a.2	57.1	158.0
3a.3	56.7	110.0
3a.4	62.1	88.4
3a.5	52.6	98.7
3a.6	58.4	54.4
Mean	*55.5*	*93.4*
Standard deviation	*5.6*	*39.6*

The results from Table 2 demonstrate a high degree of spatial heterogeneity for the estuarine sediments sampled. The standard deviation for aliphatic hydrocarbons (n=9) was 105% of the mean and for *C. perfringens* (n=6) 56% of the mean. Laboratory analytical precision, determined as standard deviation relative to the mean, was 10% for aliphatic hydrocarbons (n=6) and 42% for coprostanol (n=6). The large difference

in analytical precision between hydrocarbon and coprostanol analyses was possibly due to a combination of factors. Firstly, three procedural steps were undertaken to determine hydrocarbons by TLC-FID, whereas six steps were required to determine coprostanol by GC-FID. Additionally, hydrocarbons were present at mg/g levels compared to ng/g for coprostanol, and TLC-FID is 2 - 3 orders of magnitude less sensitive than GC-FID. Lipid masses below 2 μg are difficult to measure accurately by TLC-FID (Volkman & Nichols, 1991). Lastly, it must be acknowledged, that despite sample homogenisation, variability within a sample is still possible.

In previous studies similar to the one reported here, a coefficient of variation has been calculated for the determination of coprostanol. Environmental factors were the major contributor to variation (51.6%), followed by analytical (37.4%), and instrumental (11.0%)(Leeming & Nichols, 1996a).

Laboratory measures taken to ensure that precision was maximised throughout the study period included instrument housekeeping such as regular injector and detector maintenance, running of instrument blanks to check for column contamination, watching for column deterioration (stationary phase bleed), and monitoring instrument sensitivity. The running of an internal injection standard with every sample compensated for minor differences in injection volume or changes in instrument sensitivity between samples.

Procedural blanks were regularly analysed in order to establish whether contaminants were introduced during laboratory work-up of samples and also to establish the initial and ongoing purity of laboratory standards. The purity of solvents is of great importance due to the degree to which samples are concentrated work-up and the potential for magnification of any solvent contaminants present.

2.4.7 Microbiological analyses

All microbiological analyses for stormwater and sediment samples were conducted at Aquahealth laboratories in Hobart. Aquahealth is a NATA (National Association of

Testing Authorities, Australia) registered laboratory. Counts for faecal coliforms and *Escherichia coli* are reported as colony forming units (CFU) per 100 ml of stormwater. *Clostridium perfringens* are presented as spores per 100 ml of stormwater or as spores per 1 g of sediment.

The membrane filtration technique was used to determine *E. coli* counts. This method involves filtering a sample of appropriate volume through a membrane filter, placing the filter on an appropriate agar medium and incubating it. Prior to each filtration each sample (120 ml) was mixed thoroughly 15 times. For each sample, three volumes of water were filtered; 10 ml, 1 ml, 0.1. These volumes were extracted from the sample bottles with sterile pipettes and were filtered through 47 mm diameter, 0.45 μm pore filter papers (cellulose acetate - Gelman).The filter papers were then aseptically transferred to Membrane Lauryl Sulphate Agar (oxoid) plates and incubated at 30°C for 2-4 hr, followed by 14-18 hr at 44°C. Prior to filtering samples through a fresh (sterile) filter housing, a peptone water negative control was filtered and also incubated. Following filtering of one sample (three dilutions) the filter housing was removed and immersed in boiling water for at least 30 seconds to eliminate cross contamination between samples (Blacklow, 1995).

After incubation, each plate was checked for the presence of presumptive faecal coliform bacteria (yellow colonies) and counted. These counts were then converted to a count per 100 ml (counted colonies X 100 / no. ml filtered) and recorded. For each sample, a plate was chosen with a count between 10 and 100. The number of faecal coliform organisms present was confirmed by subculturing single colonies from the plate into separate test tubes containing Lauryl Tryptose Broth (Oxoid) and inverted Durham tubes. These were incubated at 44°C for a further 24 hr. and then checked for gas production (CO_2 bubble in inverted Durham tube). Durham tubes showing gas production confirmed the presence of faecal coliforms. Confirmed faecal coliform counts were recorded as colony forming units (cfu) per 100 ml. (Blacklow, 1995).

For enumeration of *C. perfringens* in samples, the method of Ball & Shipway (1993) was used. After filtration of samples and appropriate serial dilutions, 0.45 μm filters

were placed on perfringens agar (OPSP, Oxoid) supplemented with 85 mg'l MUP (4-methylumbelliferyl phosphate) which had been membrane-filter sterilised and added separately to cooled OPSP media after autoclaving. The plates were incubated for 24 hours at 35°C anaerobically. Typical presumptive colonies produced black fluorescence. Confirmatory tests included Gram stain and morphology, and characteristic reactions in nitrate motility medium and lactose-gelatine medium.

CHAPTER THREE

SOURCES, AMOUNT AND FATE OF HYDROCARBONS IN DERWENT ESTUARY STORMWATER CATCHMENTS

Our wasted oil unprofitably burns, like hidden lamps in old sepulchral urns.

W. Cowper, 1782

3. SOURCES, AMOUNT AND FATE OF HYDROCARBONS IN DERWENT ESTUARY STORMWATER CATCHMENTS

3.1 INTRODUCTION

In this chapter an assessment is made of hydrocarbon (HC) sources and mass discharge rates to the Derwent estuary. This is achieved from analyses of aliphatic and polycyclic aromatic hydrocarbons, the use of biomarker techniques, and multivariate statistical analysis. Estimates of total discharge of hydrocarbons by urban runoff are assessed relative to other hydrocarbon inputs to the Derwent Estuary. This provides the first hydrocarbon budget regional assessment in Australia.

Chapter Three is divided into three main sections. In the first section, the composition of hydrocarbon source materials is described. These source materials include fuels and oils and effluents collected from Hobart catchments. The composition of the hydrocarbon source materials is discussed throughout Chapter Three in relation to hydrocarbons detected in stormwater and estuarine sediment samples.

The second section of Chapter Three describes the content, composition and sources of aliphatic hydrocarbons in stormwater and estuarine sediments and proposes a total hydrocarbon discharge budget to the Derwent estuary from a variety of sources. Finally, section three describes the PAH content, composition and sources of Hobart stormwater samples and estuarine sediment samples.

SECTION ONE

HYDROCARBON SOURCE MATERIALS

3.2 COMPOSITION OF HYDROCARBON SOURCE MATERIALS

3.2.1 Aliphatic hydrocarbons

The aliphatic hydrocarbon composition of hydrocarbon source materials is shown by way of GC-FID chromatograms in Fig. 15. These chromatogram profiles are utilised in ensuing sections to aid in the identification of hydrocarbons in stormwater and estuarine sediment samples.

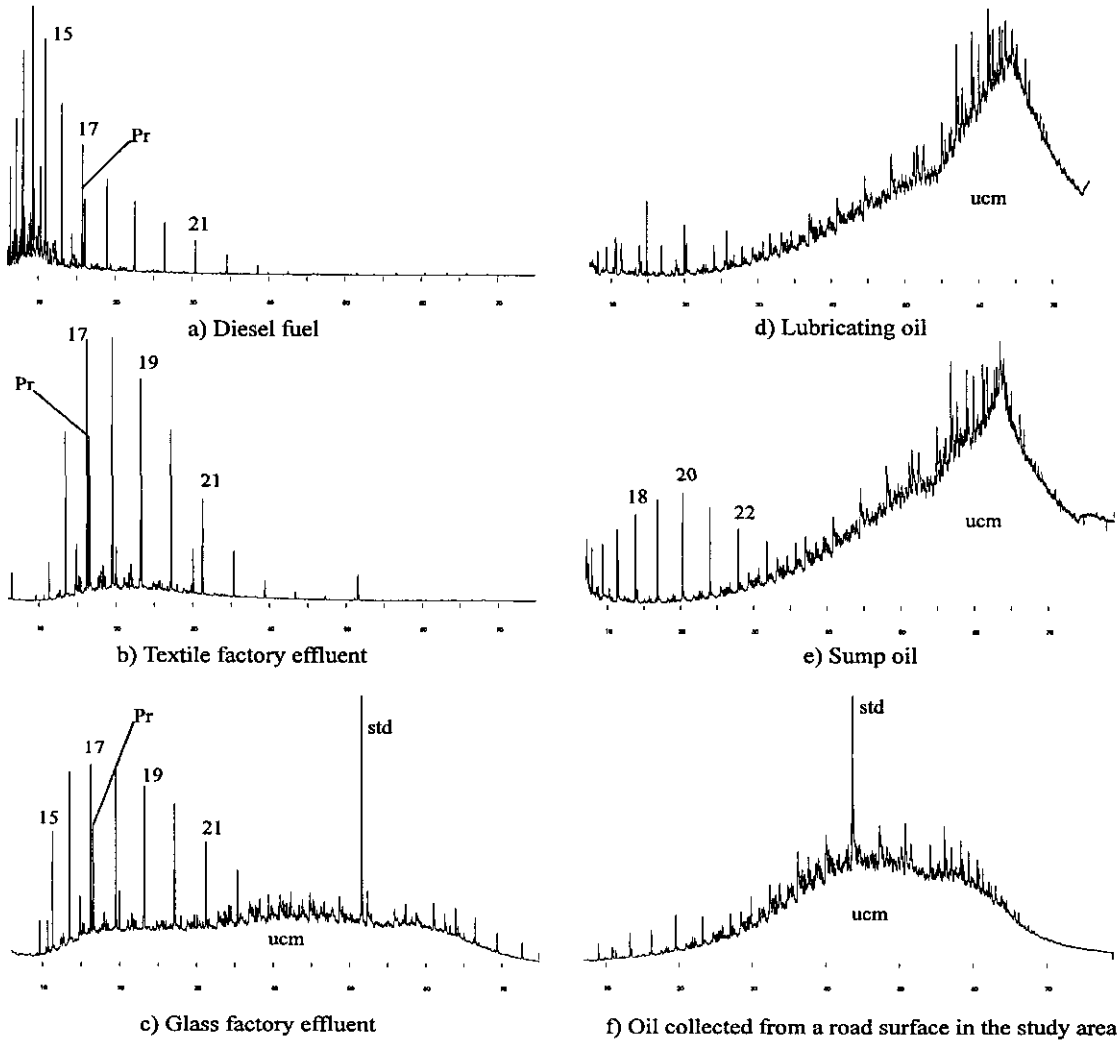


Fig. 15: GC-FID chromatograms of hydrocarbon source materials (aliphatic hydrocarbons). Factory effluents (x2) were collected from outfall pipes at there point of entry into stormwater drains in the Derwent Park catchment. Sample f) was collected by swabbing oil directly from the surface of a Hobart city street. Numbered peaks refer to *n*-alkane carbon chain length; Pr = pristane; ucm = unresolved complex mixture; std = standard.

3.2.2 PAH

The PAH composition of several oils and effluents is shown in Table 7. Boat exhaust fumes have been included in Table 7 as they are a potentially significant contributor to hydrocarbon contamination in sediments near marina complexes. Boat exhaust was collected by placing a filter paper over the exhaust outlet of an outboard (2-stroke fuel) motor.

Several major differences between the composition of used oils (sump oil/road oil) and lubricating oil were noticeable and may be exploited in this study to distinguish them in environmental samples. The compositional differences reflected the enrichment of lubricating oil with combustion derived PAHs formed during motor operation. Sump oil contained significantly lower levels of 2 and 3 ring compounds (47% *versus* 72%) and alkylation (50% *versus* 74%) than lubricating oil (Table 7). Additionally, several PAHs (acenaphthene, acenaphthylene and five and six membered ring compounds) were detected in sump oil and road oil but not in lubricating oil (Table 7).

Factory effluents were also readily distinguishable from other hydrocarbon source materials based on PAH composition. Textile (effluent 1) contained entirely 2 and 3 ring PAHs whereas glass factory (effluent 2) had a relatively high proportion of benz(a)anthracene and chrysene (Table 7).

Boat exhaust is readily identifiable from other potential PAH environmental contaminants by its low relative proportion of 2 and 3 ring compounds (23 %) and percentage of alkylated compounds (36%)(Table 7).

Table 7: Aromatic hydrocarbon and PAH composition of hydrocarbon source materials. Factory effluent 1 refers to textile factory effluent, factory effluent 2 refers to glass factory effluent. % alkylated compounds refers to combined percentage of all PAH containing alkylated substituents.

	m/z	sump oil	lub oil	road oil	sewage effluent	factory effluent 1	factory effluent 2	boat exhaust
(2 rings)		%	%	%	%	%	%	%
naphthalene	128	0.01	0.90	0.04	0.08	0.71	2.71	0.93
2-methylnaphthalene	142	0.01	0.96	0.01	0.04	0.29	1.88	0.72
1-methylnaphthalene	142	0.01	0.37		0.02	0.29	1.10	0.36
2,6-dimethylnaphthalene	156		0.75					
2,3,5-trimethylnaphthalene	155	1.43	0.41	0.04	0.03			
(3 rings)								
acenaphthylene	152	0.04						
acenaphthene	154	0.03						0.01
fluorene	166	1.07	0.88	0.12	0.26	1.48	0.62	0.26
phenanthrene	178	10.89	9.15	10.17	14.36	20.90	10.72	3.66
anthracene	178	2.24	0.26	0.99	0.89	0.44	0.24	0.98
methyl phenanthrenes	192	18.98	22.98	24.73	18.84	34.82	13.27	5.17
dimethyl phenanthrenes	206	12.03	35.61	17.13	8.81	41.08	16.07	11.07
total 2 & 3 rings		**46.75**	**72.27**	**53.22**	**43.31**	**100.00**	**46.60**	**23.16**
fluoranthene	202	7.89	2.16	8.41	18.41			6.50
pyrene	202	12.11	4.99	10.83	31.81			10.57
b-fluorenes/m-pyrenes	216	19.29	13.29	18.16	4.35			18.69
benz(a)anthracene	228	2.28	0.28	2.34	1.25		23.39	3.80
chrysene	228	3.19	5.35	2.48	0.86		30.01	11.35
(5 rings)								
benzo(b)fluoranthene	252	1.00	0.75	1.19				5.98
benzo(k)fluoranthene	252	1.33	0.75	0.23				
benzo(j)fluoranthene	252	0.28	0.17	0.26				2.22
benzo(e)pyrene	252	1.64		1.42				2.81
benzo(a)pyrene	252	1.50		0.91				3.09
perylene	252	0.40		0.18				0.84
dibenz(ah)anthracene	278	0.18		0.11				2.25
(6 rings)		0.05						
indeno(123-cd)pyrene	276			0.02				0.23
benzo(ghi)perylene	276	2.12		0.24				8.52
% alkylated compounds		**50.36**	**73.96**	**60.06**	**32.09**	**76.48**	**32.31**	**36.00**
phenanthrene/anthracene		4.9	34.8	10.3	16.2	48.0	44.3	3.7

SECTION TWO

ALIPHATIC HYDROCARBON IN STORMWATER AND SEDIMENT SAMPLES. DERWENT ESTUARY HYDROCARBON BUDGET ASSESSMENT.

3.3 ALIPHATIC HYDROCARBONS IN STORMWATER

3.3.1 Aliphatic hydrocarbon content of stormwater samples

Hydrocarbons were readily detectable in stormwater sampled in this study under all types of flow conditions and in all seasons. There was a noticeable 'first flush' effect during most storm events studied, depicted in Fig. 16 as a high concentration of hydrocarbons in stormwater in the initial stages of storm flow followed by a leveling out. The 'first flush' is a distinctive feature of urban runoff and is related to both storm and catchment characteristics (Duncan, 1995). These include duration and intensity of rain, type of land use and the proportion of impervious surfaces, topography, and the design and management of stormwater systems (Commonwealth of Australia, 1996).

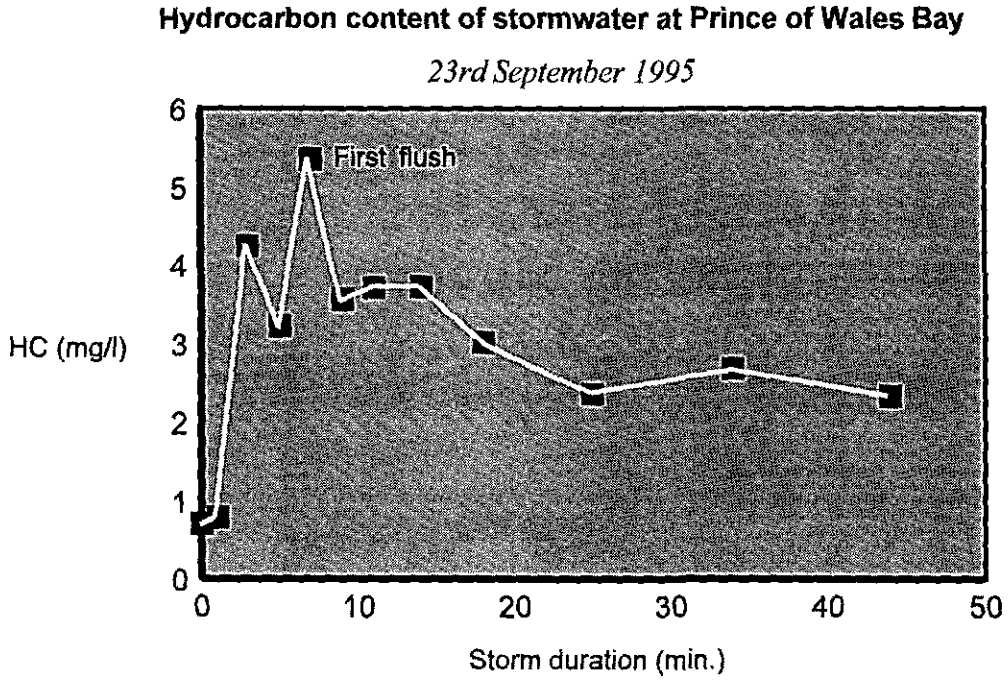


Fig. 16: Chart depicting the change in total aliphatic hydrocarbon content (measured using TLC-FID) of stormwater discharge at Prince of Wales Bay throughout the duration of Storm Event 7 (Table 5).

The mean concentration of hydrocarbons in stormwater collected from the Prince of Wales Bay outfall throughout the study period was 2.88 mg/l, which is within the range reported from stormwater studies elsewhere (Table 8). However, the maximum recorded hydrocarbon concentration in this study of 22.7 mg/l at the peak flow of a short summer storm, was comparatively high (Table 8) and equivalent to a discharge rate of 178 g/s (flow rate 7,720 l/s) of hydrocarbons into Prince of Wales Bay.

Table 8: Published concentrations of aliphatic hydrocarbons and PAHs in urban runoff

Location	PAH	Aliphatic hydrocarbons	Reference
	Total PAH		
Sydney, Australia*	0.2 - 41.3 ng/l*	0.6 - 21 µg/l*	Nichols *et al.*, (1993)
Hobart (Prince of Wales Bay)	**0.19 - 5.35 µg/l**	**0.26 - 22.7 mg/l**	**This study**
Madrid, Spain	15.1 - 35.5 µg/l	0.47 - 2.18 mg/l	Bomboi & Hernandez, (19
Philadelphia, USA	0.68 - 1.72 mg/l	1.50 - 3.58 mg/l	Hunter *et al.*, (1979)
Los Angeles, USA		11.5 mg/l	Eganhouse & Kaplan, (19
San Francisco, USA	0.40 - 3.53 mg/l	0.28 - 29.7 mg/l	Fam *et al.*, (1987)
Seattle, USA		0.2 - 7.50 mg/l	Wakeham, (1977)
Narragansett Bay, USA		0.98 - 2.15 mg/l	Hoffman *et al.*, (1982)
London, England		5.8 - 18.2 mg/l	Gavens *et al.*, (1982)

* Collected during dry weather (base level flow)

3.3.2 Aliphatic hydrocarbon composition of hydrocarbons in stormwater of the Derwent Park catchment.

Aliphatic GC-FID hydrocarbon profiles have shown the presence of biogenic, diesel fuel and lubricating oil hydrocarbons in stormwater samples. Hydrocarbon chromatograms with prominent UCM's, Pr/Ph ratios and biomarker profiles all suggested automotive sump oils as the major hydrocarbon source to Hobart stormwater. The way in which the relative composition of hydrocarbons in stormwater changes throughout a storm event is presented in Fig. 17. Lubricating oils, which were quantified by integration of the UCM, were the highest contributor of hydrocarbons to urban runoff throughout the storm event. Urban runoff at base flow was highly enriched in lubricating oils (96%). The relative contribution of lubricating oils fell during storm flow as other hydrocarbon inputs (predominantly diesel) became established (Fig. 17). Diesel fuel, quantified by integration of hydrocarbons between n-C_{14} and n-C_{24} in GC-FID chromatograms, was the second highest identifiable

contributor to hydrocarbon contamination in stormwater in this study and reached a level of 14% of total hydrocarbons during high flow shortly after the 'first flush' (Fig. 17). Biogenic hydrocarbons were the smallest detectable component of total hydrocarbons (0-2.6%) in urban runoff and were undetected at base flow (Fig. 17).

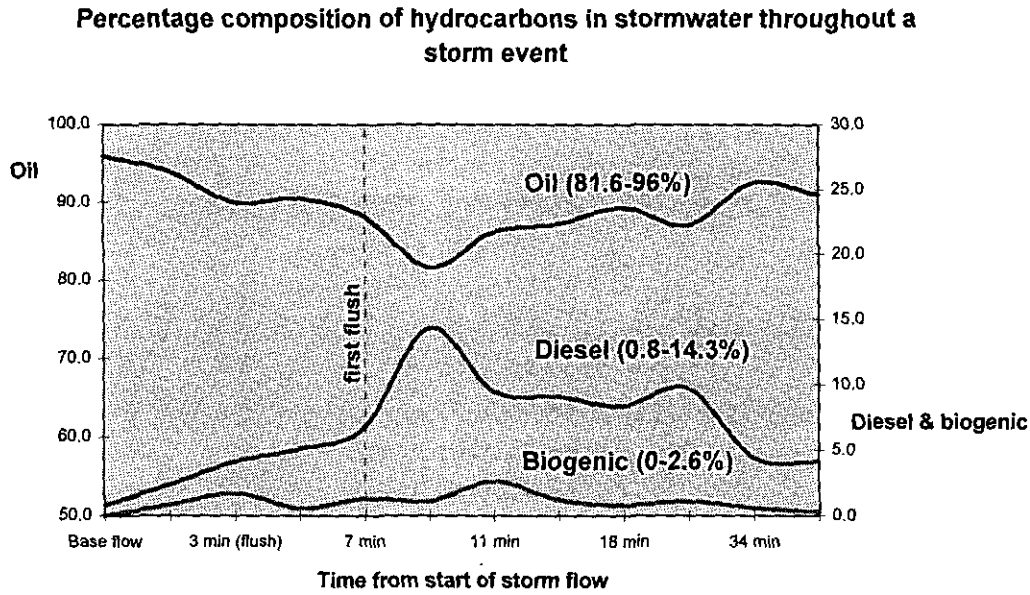


Fig. 17: Percentage composition of hydrocarbons in stormwater throughout a storm event. Samples collected at Prince of Wales Bay outfall, 23/9/95.

3.3.3 Aliphatic hydrocarbon composition of hydrocarbons in stormwater from other Hobart catchments

Lubricating oils were the highest contributor to the hydrocarbon load in stormwater samples collected at other Hobart catchments (Table 9). There did appear, however, to be a link between catchment land use type and the resultant hydrocarbon composition of stormwater samples. Catchments with a primarily residential land use had a higher proportion of biogenic compounds in stormwater hydrocarbon profiles whereas lubricating oils were more predominant in industrialised catchments (Table 9).

Table 9: Hydrocarbon composition of stormwater according to catchment characteristics

Catchment	Major land use	% biogenic	% diesel	% lub./sump oil
Waimea	residential	6.4	8.2	82.1
Hobart Rivulet	commercial / residential	3.7	13.3	81.2
NewTown Rivulet	mixed	1.1	13.8	81.9
Prince of Wales Bay	light industry	0 - 2.6	0.8 - 14.3	81.6 - 96

3.4 ASSESSMENT OF TOTAL HYDROCARBON INPUTS TO THE DERWENT ESTUARY

3.4.1 Lubricating oil budget for Hobart

Seven million litres of automotive lubricating oil is imported into Tasmania every year (AIP, 1993). A significant proportion of this oil is lost directly to the environment through engine leakage (approximately 18-27% - see below). It is believed that for every 1000 km of roadway at least 0.2 to 0.3 litres of lubricating oil per vehicle is lost (Vazquez-Duhalt, 1989). This compares to an Australian Institute of Petroleum figure of 1 litre oil use per 3,500 km travelled (Table 10). Based on these figures and an average annual mileage of 20,000 km per vehicle in Tasmania, 4-6 l of oil per vehicle per year or a total of 1.26 - 1.90 million litres of oil is lost from engines in Tasmania per year (based on 316,000 registered vehicles in Tasmania)(Table 10). This figure is likely to be higher if oil loss by incomplete combustion is also taken into account. In the Hobart urban area there are 114,000 vehicles, hence approximately 460,000-680,000 l litres of automotive lubricating oil is lost to road surfaces in the city per year. Hence the annual oil load detected in Hobart's stormwater of 149,000 l represents 22-32% of automotive lubricating oil lost to the environment in the urban area. This is higher than survey findings of Hoffman *et al.* (1980) who found that 13% of used oil is released directly to urban waterways by dumping and leaking of used motor oil directly into storm drains and sewers and onto road surfaces.

Table 10: Oil consumption and waste generation in Tasmania

Automotive lubricating oil imported / year*	7,000,000 l
Waste oil collected / year**	~5,200,000 l
Automotive oil lost to the environment / year	1.26 Ml - 1.90 Ml
Oil lost to the environment in Hobart / year	456,000 - 684,000 l
Number of vehicles in Hobart	114,000
Number of vehicles in Tasmania***	316,000
Number of vehicles in Australia***	11,200,000

*(AIP, 1993)
**Figure from Department of Environment and Land Management, Tas.
***From Australian Bureau of Statistics, Australian Year Book 1996.

It should be noted that disposal of waste oil at rubbish tips or in the backyard can also affect coastal or estuarine water quality by contaminating land runoff, urban runoff and groundwater. The survey of Hoffman *et al.* (1980) found that the amount of oil disposed in this way accounts for 71% of total oil disposal, however the impact of these practices on water quality is difficult to assess.

3.4.2 Stormwater

Total hydrocarbon discharge to receiving waters was estimated for all storm events studied. To estimate the total discharge rate of hydrocarbons in any given storm event it is necessary to collect samples that encompass the discharge from start to finish. Storm event mass loads are estimated by integration of the discharge rates in mass of hydrocarbons per unit of time over each storm period (Hoffman *et al.*, 1983).

The greatest hydrocarbon discharges at Prince of Wales Bay occurred when the prior rain-free days exceeded one week and ranged from 10.3 kg for a rainfall event of 3 mm to 341 kg for a rainfall event of 148 mm (Table 11). Hydrocarbon discharge at Prince of Wales Bay as a function of rainfall ranged from 2.3 - 37.3 kg/mm with a mean discharge of 12.4 kg/mm for all storm events studied at this catchment (Table 11). Based on these figures, and an average annual rainfall in Hobart of 626 mm, an

estimated 7,760 kg of hydrocarbons are discharged into Prince of Wales Bay every year from runoff during storms (Table 11). This equated to an estimated annual discharge 17.1 kg HC/ha from this catchment (Table 11). This figure applied to the total urban area of Hobart (7,700 ha) yields to an estimated total annual wet weather hydrocarbon discharge of 132,000 kg (120,000 litres)(Table 12) from the urban area of Hobart. This is an estimate based on the assumption that hydrocarbon generation rates in the Derwent Park catchment are representative of the greater Hobart area.

Table 11: Total (particulate) hydrocarbon discharge (wet weather) at Prince of Wales Bay outfall, Hobart. Storm event information is presented in Table 5.

Date 1995	Prior rain-free days	Rainfall (mm)	Duration of rainfall	Hydrocarbon discharge (kg)	HC (kg) / rainfall (mm)
Jan. 4-6	48	7.5	2 days	280 kg	37.3
Feb. 16	2	6	1 hour	84 kg	14.0
Aug. 17	8	41.5	1 day	205 kg	4.9
Sept. 23	1	3	45 min.	10.3 kg	3.4
Dec. 18-19	12	148	2 days	341 kg	2.3
					mean = 12.4 kg/mm
					mean annual = 7760 kg*
					mean annual/ha = 17.1 kg/ha**

*Based on a mean annual rainfall in Hobart of 626 mm
**Based on Derwent Park catchment size of 454 ha

Table 12: Total hydrocarbons (particulate) discharged per annum in stormwater from the *Hobart urban area**

	litres	kg
Wet weather discharge	120,000	132,000
Dry weather discharge	29,000	32,000
Total discharge / annum	**149,000**	**164,000**
HC discharge (kg) / hectare of urban area		21.2
HC (kg) / capita (170,000 people)		0.96
HC (g) / capita / day		*(2.6 g)*
Estimated HC discarge to the coast from stormwater in Australia / annum**		(14,900 tonnes)

* Estimate based on annual discharge of hydrocarbons at Prince of Wales Bay outfall
** Based on the Hobart figure of 0.96 kg per capita and applied to the coastal population of 15,480,000

Hydrocarbon discharge from urban areas is not only confined to wet weather and was detected in Hobart's stormwater at all times. For the Derwent Park catchment, the mean dry weather or base flow of 85 l/s contained an average hydrocarbon concentration of 0.70 mg/l at the Prince of Wales Bay outfall. With an annual dry weather water discharge at the Derwent Park catchment of 2.68×10^9 l (calculated from 85 l/s), then an estimated 1,880 kg or 4.14 kg/ha of hydrocarbons are discharged to Prince of Wales Bay annually during dry weather. Again, assuming that hydrocarbon generation rates within the Derwent Park catchment are representative of Hobart as a whole, then the estimated total dry weather hydrocarbon discharge from the Hobart urban area is 32,000 kg (29,000 litres)(Table 12).

The total combined wet and dry weather discharge of Hydrocarbons from the Hobart urban area to the Derwent estuary is an estimated 164,000 kg (149,000 litres)(Table 12), 80 % of which is from wet weather or storm flow. This is equivalent to a total hydrocarbon discharge of 21.2 kg per hectare of urban area per annum or 0.96 kg per capita per year based on a population of 170,000 people in Hobart (Table 12). This compares to estimates from the USA of 0.969 kg/capita/year at Narragansett Bay (Hoffman *et al.*, 1982) and 0.875 kg/capita/year for the Los Angeles River drainage basin (Eganhouse *et al.*, 1981).

The estimate of hydrocarbon discharge from the Hobart urban area to the Derwent estuary does not include sporadic hydrocarbon discharge due to accidental spillages or leakage of fuel from underground tanks. Inputs of this nature can be considerable. During the study period, leakage from an underground fuel storage tank in the Derwent Park catchment resulted in a discharge of approximately 10,000 litres of diesel directly to the stormwater drainage system at an average rate of 30 l/hour for two weeks.

A previous estimate of hydrocarbon discharge into Australian coastal waters from urban runoff alone was 5,000 tonnes per annum (Volkman *et al.*, 1994) based on an estimate of 1.0 g / capita / day in America (NRC, 1985). It would appear that the actual discharge figure in Australia is higher based on the findings of this study of 2.6

g HC / capita / day (Table 12), and a coastal population of 15,480,000 (86% of population in coastal towns and cities - Commonwealth of Australia, 1995). This accrues to a total annual discharge of 14,900 tonnes of hydrocarbons from urban runoff (Table 12) or the equivalent of one Exxon Valdez (45,150 tonnes spilt) every 3 years.

3.4.3 Sewage effluent

Twelve sewage plants with a combined discharge rate of 55,600 kL/day are located on the Derwent Estuary (Fig. 10). Almost without exception in 1972, sewage in Hobart received only primary treatment. By the end of 1994 the councils bordering the estuary had upgraded most of their sewage treatment plants to a minimum of secondary treatment (Tasmanian Government, 1996)(Table 4). The hydrocarbon content of sewage effluent is variable and dependent on the amount of industrial waste treated. Connell (1982) reported a mean hydrocarbon concentration of 3.8 mg/l in municipal wastewater in areas adjacent to New York, whereas Eganhouse and Kaplan (1982) reported a range of 6.1-16.3 mg/l in Southern Californian wastewater effluents. The hydrocarbon content of sewage effluent in Australia has previously been measured at 3.5 ± 0.65 mg/l (Nichols in Volkman *et al.*, 1994). Based on this figure an estimated 58,000 - 84,000 kg of hydrocarbons is discharged to the Derwent Estuary in sewage effluent every year (Table 18).

3.4.4 Industrial effluent

The two single largest effluent contributors to the Derwent Estuary are an electrolytic zinc (EZ) works and a paper mill (Fig. 10) which contribute 130,000 kL/day and 69,500 kL/day of effluent respectively. At the electrolytic zinc plant, effluent and some runoff from the site is treated through settling ponds and filtration. It is believed that processes implemented in recent years to meet guidelines for heavy metal and suspended solids regulations in effluent have also had a beneficial effect on reduction of hydrocarbon concentrations. The only potential for hydrocarbon discharge to the

estuary from the EZ works is through runoff from the site or accidental discharge from the wharf apron.

Hydrocarbon content of the factory effluents analysed (replicate x4) was between 0.24 and 0.41 µg/l giving rise to an estimated total annual discharge of between 18,000 and 31,000 kg of hydrocarbons to the Derwent estuary (Table 13). Effluent from light industry in Hobart is treated through wastewater treatment plants.

3.4.5 Atmospheric fallout

Biogenic *n*-alkanes, anthropogenic hydrocarbons, UCM and PAHs have all been previously detected in wet and dry atmospheric deposition samples (Webber, 1986). Previous work has demonstrated that hydrocarbon inputs to estuarine environments from atmospheric deposition could be of the same order of magnitude as contributed by municipal wastewaters (Webber, 1983). Measured deposition rates were 195 µg/m^2/day at an urban sampling station in Norfolk, Virginia, and a mean value of 69 µg/m^2/day at non-urban and coastal locations. These figures have been used as a guide to give an approximate figure for input of atmospheric particulate hydrocarbons to the Derwent Estuary. Given a surface area of 192 km^2 the estimated daily hydrocarbon input from atmospheric particles is 13.3 - 37.4 kg or 4,850 - 13,700 kg annually (Table 13).

Table 13: Estimation of the major aliphatic hydrocarbon inputs to the Derwent estuary.

	Volume (kL/day)	Concentration (mg/l)	Quantity (kg/day)	Quantity (tonnes/year)
Urban runoff				164
Sewage effluent	55,600	2.85 - 4.15	158 - 231	58 - 84
Industrial effluent	199,500	0.24 - 0.41	48 - 82	18 - 31
Atmospheric deposition			13 - 37	4.9 - 13.7

Estimation of the major aliphatic hydrocarbon inputs to the Derwent estuary on an annual basis has demonstrated that stormwater is potentially the largest contributor (164 tonnes), ahead of sewage effluent (58-84 tonnes), industrial effluent (18-31 tonnes) and atmospheric deposition (5-14 tonnes). Other hydrocarbon inputs to the Derwent estuary that were unable to be quantified include; runoff from factory sites with water frontage, spillage from tanker operations and fuel/oil holding facilities, and inadvertent releases from boating and shipping activities.

The hydrocarbon budget for the Derwent compares well with similar studies from elsewhere. Connell (1982) found that urban runoff and sewage effluent were the major contributors of hydrocarbons to the Hudson-Raritan Estuary, USA; Hoffman *et al.* (1983) suggested urban runoff as the major contributor (40% of the total input) of hydrocarbons to Narragansett Bay, Rhode Island; Wakeham (1977) found that the major hydrocarbon inputs to Lake Washington from Seattle are approximately equal contributions from river and stormwater discharges which together contribute 85% of the total input.

3.5 SOURCES OF ALIPHATIC HYDROCARBONS IN HOBART STORMWATER

Given the high hydrocarbon concentrations recorded in Hobart stormwater under some circumstances, and the potential this has for ecological, aesthetic and recreational disruption in receiving waters; source identification is essential for the subsequent management of the stormwater contamination. In this section, characteristic features of GC-FID chromatograms are discussed in relation to hydrocarbon source identification.

3.5.1 Biogenic hydrocarbons

Biogenic hydrocarbons were present in stormwater samples as a minor component of hydrocarbon profiles. They were identifiable as prominent 'odd-chain length' hydrocarbon peaks in the range n-C_{25} to n-C_{35} in GC-FID profiles from samples collected 1 minute after the onset of storm flow (Fig. 18). This indicates that plant material is rapidly mobilised from urban catchments into storm drains during rain events. The 'carbon preference index' (CPI), which is used as a measure of the predominance of molecules with an odd number of carbon atoms (Tissot & Welte, 1978), could not be calculated in most samples in this study due to the presence of an unresolved complex mixture (UCM) masking even chain hydrocarbons between n-C_{24} and n-C_{34}.

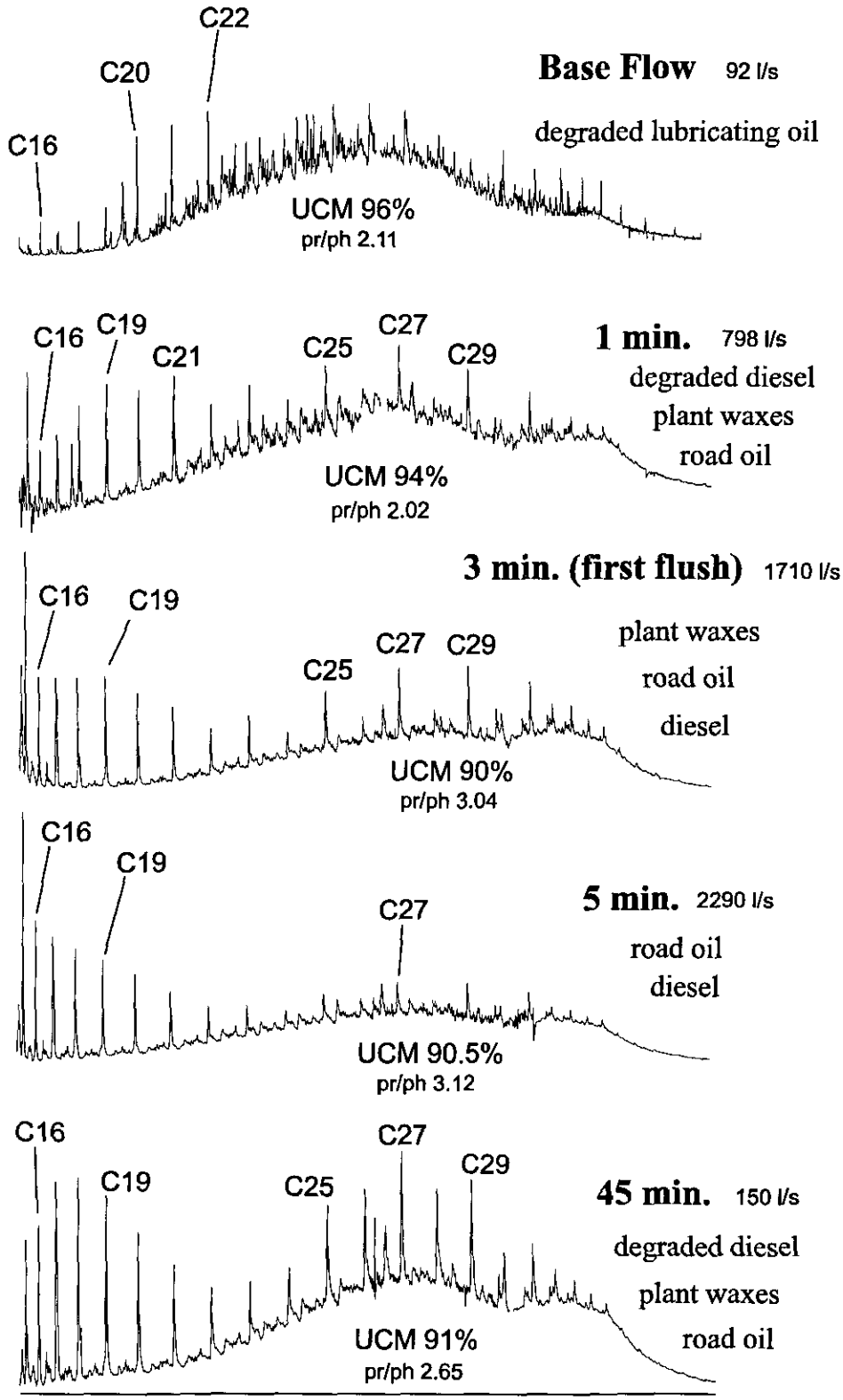


Fig. 18: Aliphatic hydrocarbon chromatogram series depicting the change in hydrocarbon inputs to stormwater samples throughout Storm Event 7 (Table 5). Samples were collected at the Derwent Park catchment, Prince of Wales Bay outfall. Hydrocarbon constituents were assessed by comparison to hydrocarbon source materials (**Fig. 15**).

3.5.2 *Diesel fuel*

Diesel fuel was readily detected in stormwater samples by the presence of an *n*-alkane series ranging from *n*-C_{14} - *n*-C_{24} in GC-FID chromatograms with a maximum at *n*-C_{16} and no odd/even predominance (CPI = 1)(see diesel fuel chromatogram in Fig. 15). In addition, the diesel fuels analysed exhibited a pristane/phytane (Pr/Ph) ratio of 9, significantly higher than in sump oil (pr/ph 2.09) and lubricating oil (pr/ph 0.65)(Table 14). This allowed diesel fuel contributions to stormwater to be clearly distinguished from other possible inputs. Partly degraded diesel fuel, most likely from road surfaces and seepage into drains during prior dry periods, was present in samples collected from the early stages of storm events. This was evidenced by an *n*-alkane profile maximising progressively from *n*-C_{22} to *n*-C_{16} and a gradual rise in the Pr/Ph ratio with increasing time (Fig. 18). Undegraded diesel fuel was detected in the *n*-alkane profile of stormwater samples at 5 minutes from the commencement of storm flow, and thereafter in all samples until flow receded to close to base level (Fig. 18).

Table 14: Biomarker ratios in source materials and in stormwater and sediment samples collected from Hobart stormwater catchments.

	Pr/Ph	Pr/nC17
Source materials		
diesel fuel	9.00	0.68
sump oil	2.09	0.56
lubricating oil	0.65	0.50
factory effluent	3.20	0.66
Stormwater		
Prince of Wales Bay	0.74 - 4.17 (2.54)	0.15 - 1.36 (0.65)
Newtown Rivulet	4.03	0.78
Hobart Rivulet	3.26	0.74
Waimea catchment	2.45	0.61
Sediments		
Prince of Wales Bay	2.0 - 3.7 (2.72)	0.62 - 3.55 (1.81)

There are a number of possible sources of diesel fuel in urban catchments including road and service station tarmac runoff, seepage from underground storage tanks, spillage, and industrial discharge. Industrial effluent does not appear to be a

significant contributor of diesel to stormwater in this study as effluents sampled from within the Derwent Park catchment contained slightly weathered diesel with an *n*-alkane maximum at *n*-C_{18} (Fig. 15).

3.5.3 Isoprenoid ratios

In stormwater at Prince of Wales Bay Pr/Ph ratios were variable and ranged from 0.74 - 4.17 (Mean 2.54)(Table 14). This can be explained in terms of the different relative inputs from diesel, sump oil, lubricating oil and other hydrocarbon inputs to stormwater throughout a rain event. The similarity between Pr/Ph ratios in stormwater samples and automobile sump oil provides evidence that sump oil is potentially the largest contributor to hydrocarbon contamination in urban runoff in Hobart (Table 14).

The isoprenoid/*n*-alkane ratio, pristane/*n*-C_{17}, was used in this study as a biodegradation index. Pr/*n*-C_{17} ratios in stormwater were only marginally higher than those of source materials (Table 14). This indicates that petroleum hydrocarbons had not undergone substantial degradation prior to incorporation into stormwater implying that fresh input of hydrocarbons, rather than re-suspension of degraded hydrocarbons from sediments, is the primary hydrocarbon source to stormwater. There were no observable trends in Pr/*n*-C_{17} ratios throughout storm events which suggest a relatively uniform hydrocarbon input, in terms of the degree of weathering, to storm drains during catchment flushing. This observation contrasted with findings of Eganhouse *et al.*, (1981) who, in a study of stormwater runoff in Los Angeles, observed that stormwater samples collected earlier in a storm contained hydrocarbons relatively unweathered and hence more recently deposited.

3.5.4 Unresolved complex mixture (UCM)

A large UCM (80%-97% of hydrocarbons) in GC-FID chromatograms was characteristic of all stormwater samples collected throughout the study period. Some examples are shown in Fig. 18. A similar feature was noted in stormwater samples

collected from the Los Angeles River in which resolved components accounted for, on average, less than 17% of the total integrated area (Eganhouse *et al.*, 1981). The presence of the UCM as a dominant component in stormwater samples indicates a substantial contribution from petroleum products, more specifically lubricating oils (Volkman *et al.*, 1994).

3.5.5 Biomarker profiles

Hydrocarbon biomarker profiles (hopane and sterane mass fragmentograms) for potential source inputs to stormwater such as sump oil, lubricating oil and two factory effluents exhibited a strong similarity due to the component oils being formulated from imported oils. The hopane distributions (*m/z* 191) all exhibited a $C_{29} > C_{30}$ distribution with 17α(H),21β(H)-hopanes as the predominant triterpenoid hydrocarbons (Fig. 19). This tends to be characteristic of oils derived from carbonate sources such as the Middle East (Peters & Moldowan, 1993). Oils from this region are the major source of oils imported for refining in Australia (AIP, 1993). Oils produced from within Australia (Gippsland crudes) exhibit *m/z* 191 profiles with $C_{30} > C_{29}$ and *m/z* 217 distributions dominated by C_{29} steranes and diasteranes (Philp *et al.*, 1982). Although sterane distributions of hydrocarbon source materials showed a high degree of similarity, potential differences in the C_{27} diasterane S:R ratio could be exploited for source identification (Fig. 20). The 20S/20R ratios were 1.4 in automotive lubricating oil and sump oil, 1.1-1.3 in all stormwater samples and 3.4 in the textile and glass factory effluents (Fig. 20). This provided further evidence of automotive oils, rather than the factory effluents, as the major contributor to hydrocarbons in urban runoff in this catchment.

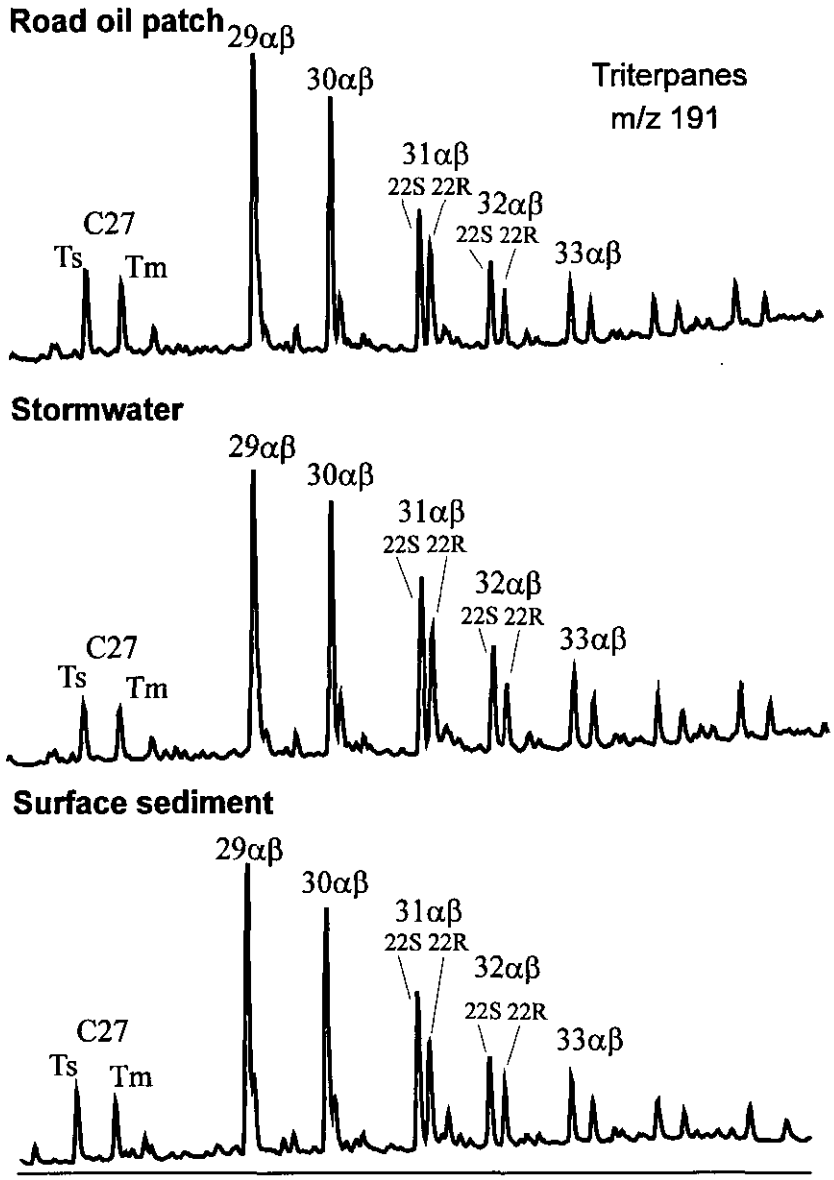


Fig. 19: Mass fragmentograms (*m/z* 191) for pentacyclic triterpanes (hopanes) from a road oil patch, stormwater and surface sediment sample at Prince of Wales Bay. Hopanes are given by *x*αβ where "*x*" is the number of carbon atoms and where αβ denote the orientation of hydrogen atoms at positions 17 and 21. Stereoisomerism at position 22 is given as 22S and 22R.

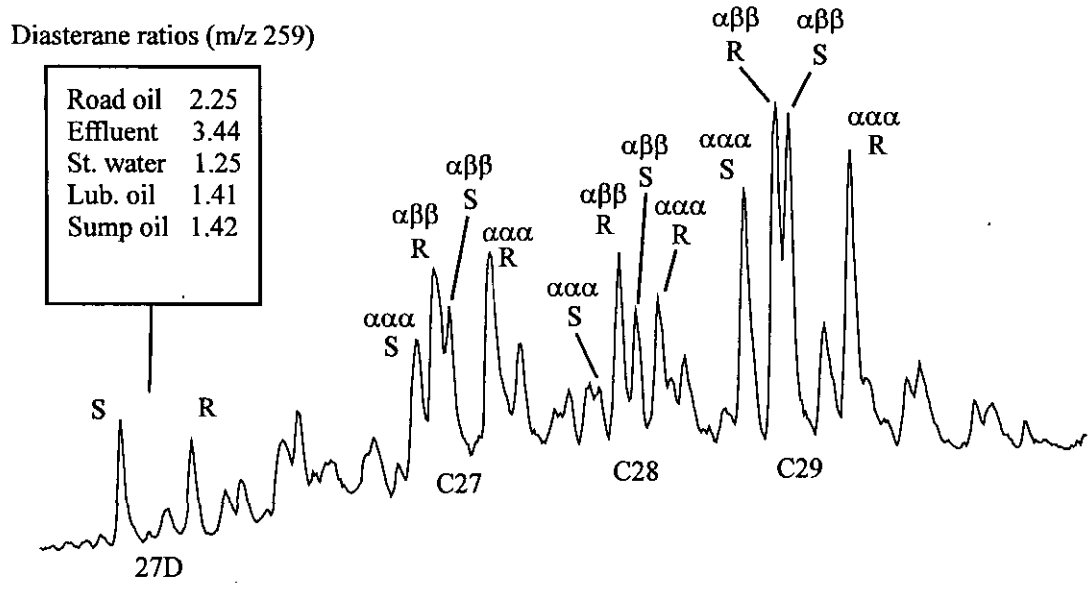


Fig. 20: Mass fragmentograms (*m/z* 217) showing the distribution of steranes in Hobart stormwater samples. Steranes are given by C*x* and diasteranes by *x*D where "*x*" is the number of carbon atoms. Stereochemistry at position 20 is denoted by S and R and by ααα etc. for hydrogen atoms at positions 5, 14 and 17. Diasterane 20S/20R ratios are also shown and were calculated from the *m/z* 259 mass fragmentogram.

3.6 ALIPHATIC HYDROCARBONS IN ESTUARINE SEDIMENTS

3.6.1 Content and composition of aliphatic hydrocarbons in estuarine sediments

Sediment samples were collected from the shoreline of Prince of Wales Bay in order to determine the degree of hydrocarbon contamination and the potential sources of the contamination. Sediment sampling locations at Prince of Wales Bay, together with hydrocarbon concentrations and GC-FID traces of aliphatic hydrocarbons for each site, are shown in Fig. 21.

Hydrocarbons have previously been measured in sediments of the upper Derwent Estuary (Volkman *et al.*, 1989) and the lower Derwent Estuary (Volkman *et al.*, 1988)(Table 15). At Prince of Wales Bay, hydrocarbon contamination in sediment samples was indicative of extreme pollution (400-10,100 μg/g)(Fig. 21; Table 15). Some of this contamination is potentially derived from a major industrial source on the upper Derwent estuary (Volkman *et al.*, 1989), and deposited in Prince of Wales Bay through estuarine water or tidal movements.

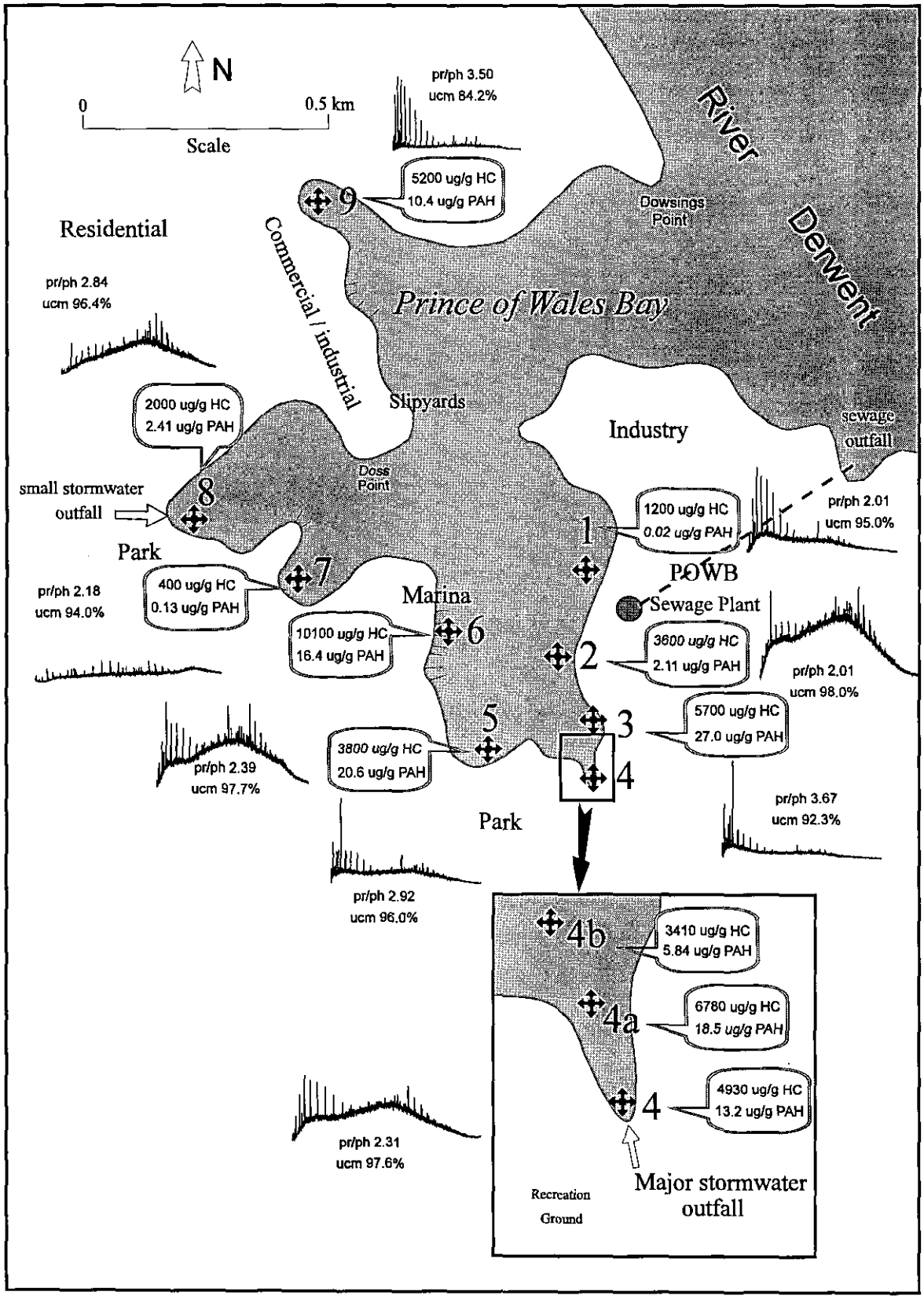


Figure 21: Map of Prince of Wales Bay on the Derwent estuary showing the stormwater outfall and sediment sampling sites.

GC-FID chromatograms of aliphatic hydrocarbons for each sediment is shown.

Many of the sediment samples collected within Prince of Wales Bay contained hydrocarbon concentrations higher than any yet reported for Australian marine surface sediments (Table 15) and compared to concentrations of 26-101 µg/g near Sydney's Malabar sewage outfall which were considered high for a coastal marine environment (Nichols & Espey, 1991). The concentrations are also well above those recorded in sediments from Victoria Harbour, Hong Kong (60-646 µg/g; Hong *et al.*, 1995) and Alexandria Harbour, Egypt (61-1,360 µg/g; Aboul-Kassim & Simoneit, 1995)(Table 15) which is considered to be heavily polluted with sewage-derived hydrocarbons.

Table 15: Concentrations of total aliphatic hydrocarbons in marine surface sediments of Australia and elsewhere

Location	Hydrocarbons (ug/g)	Reference
Westernport Bay, Victoria	2.3 - 5,271	Burns & Smith, (1977)
Port Phillip Bay, Victoria	6 - 1,520	Burns & Smith, (1982)
Great Barrier Reef, Queensland	0.2 - 0.8	Coates *et al.*, (1986)
Sydney coastal, NSW	3 - 42.3	Nichols & Leeming, (1991)
Malabar Sewage outfall, Sydney	26 - 101	Nichols & Espey, (1991)
Rowley Shelf, Western Australia	0.02 - 0.05	Pendoley, (1992)
Lower Derwent Estuary, Tasmania	0.8 - 6.4	Volkman *et al.*, (1988)
Upper Derwent Estuary, Tasmania	1,100 - 4,600	Volkman *et al.*, (1989)
Prince of Wales Bay	**400 - 10,100**	**This study**
Victoria Harbour, Hong Kong	60 - 646	Hong *et al.* (1995)
Alexandria Harbour, Egypt	61 - 1,360	Aboul-Kassim & Simoneit (1995)

3.6.2 Sources of aliphatic hydrocarbons in estuarine sediments

In sediments from Prince of Wales Bay an UCM was the dominant feature of GC-FID chromatograms, reaching 98% of hydrocarbons at sites 2,4 and 6 (Fig. 20). This feature, together with Pr/Ph ratios in the sediments ranging from 2.01 - 3.67 (Mean 2.72)(Table 14; Fig. 20), suggests a predominant contribution to the sediments from used lubricating oils. Biomarker compounds such as pristane and hopanes along with some degraded diesel *n*-alkanes (maximising between n-C_{17} and n-C_{20}) were the major individual hydrocarbon compounds identified in sediment samples.

The highest sediment hydrocarbon concentration was associated directly with stormwater discharge was 6,780 μg/g at site 4a (Fig. 20). Other stations exhibiting high concentrations of hydrocarbons were between sites 2 and 6 in close proximity to the major stormwater outfall at Prince of Wales Bay (Fig. 20). The highest concentration of hydrocarbons detected in Prince of Wales Bay sediment (10,100 μg/g - or 1% by weight of hydrocarbons) was at site 6 which was collected from beneath a marina complex (Fig. 20). The high percentage UCM (98%) and pristane / phytane ratio of 2.4 at this site suggested a used lubricating oil source for the hydrocarbon contamination which, however, may not be unequivocally assigned to a stormwater source at this stage as chronic industrial effluent discharges to the Derwent estuary also contain lubricating oil (Volkman *et al.,* 1989), and exhaust from boats is known to contain combusted lubricating oil (Collier *et al.*, 1995). Further interpretation regarding source elucidation of sediment hydrocarbons by using PAH analysis is discussed in Section 3.7.2

SECTION THREE
PAH CONTENT, COMPOSITION AND SOURCES IN HOBART STORMWATER AND ESTUARINE SEDIMENTS

3.7 PAH CONTENT AND SOURCES IN STORMWATER SAMPLES

3.7.1 PAH content of stormwater samples

PAH concentrations in stormwater in this study ranged from 0.19 to 5.35 µg/l (Table 7). This includes both dry and wet weather flow samples from all four catchments studied. Many of the PAH concentrations observed in this study were above the recommended Australian guidelines of 3 µg/l, which is believed to be the threshold for the protection of aquatic life in marine and fresh waters (ANZECC, 1992). PAH concentrations recorded in stormwater were also above the recommended level for primary contact in New South Wales (NSW) of 0.2 µg/l (Water quality criteria, NSW), but were generally less than 1% of total hydrocarbons and lower than most concentrations reported in the literature (Table 8).

Published PAH concentrations are characterised by a large amount of variability ranging from ng/l concentrations to mg/l concentrations (Table 8). This variation between studies may be due to the presence of strong localised sources of PAHs, including pavement attrition, tar-based pavement sealants, used crankcase oil, deposition from car exhausts, and industrial sources (Marsalek, 1990). Alternatively, there is great variation between laboratories of techniques, procedures and instrumentation used to extract, fractionate, identify and quantify PAHs. For example, methods currently employed to analyse and quantitate PAHs include HPLC, HPLC with UV/Vis absorption spectra, GC-FID, GC-MS and UV fluorescence.

3.7.2 PAH composition and sources in stormwater samples

Analysis of aliphatic hydrocarbons showed that stormwater samples and receiving sediments are impacted by hydrocarbons predominantly originating from automotive

lubricating oils. Only the Pr/Ph ratios (Table 8) provided any evidence that this oil contamination may be predominantly from sump oil rather than from unused lubricating oil. Other biomarker ratios, biomarker mass fragmentograms, and GC-FID chromatograms proved inadequate to distinguish between these two hydrocarbon sources as the major hydrocarbon contributor to stormwater. PAH profiles have the potential to provide this additional information and may offer a means of estimating the contribution of combustion derived PAHs to stormwater and sediment samples.

The composition of PAHs in stormwater particulates, sediments and source materials is summarised in Table 16. There was a noticeable difference in PAH composition of stormwater particulates between catchments. Stormwater entering Prince of Wales Bay was characterised predominantly by 2-3 ring compounds (39-88%, mean 71%), a high degree of alkylation (39-85%, mean 65%), phen./ant. ratios in the range 1.3-18 (mean 6.0), and dimethylphenanthrenes and methylphenanthrenes as the most abundant PAH compounds (Table 16). This information does not unambiguously distinguish between lubricating and sump oils as the major contributor of PAH to the oil content in stormwater samples, however the wide range of concentrations suggests inputs from both oil types.

Table 16: Composition of PAHs in oils, effluents, stormwater and sediment samples

	% 2-3 rings	% alkylated	phen./anth.	fluor./pyr (*m/z* 202)	b(a)ant./chry. (*m/z* 228)	b(a)p/b(e)p (*m/z* 252)	% phen.	% m-phens.	% dm-phens.
Source materials									
sump oil	46.8	50.4	4.9	0.65	0.72	0.92	10.9	19.0	12.0
road oil patch	53.2	60.1	10.3	0.78	0.94	0.64	10.2	24.7	17.1
lubricating oil	72.3	74.0	34.8	0.43	0.05	-	9.2	23.0	36.6
factory effluent	100.0	76.5	48.0	-	-	-	20.9	34.8	41.1
boat exhaust (2 stroke)	23.2	36.0	3.7	0.62	0.34	1.10	3.7	5.2	11.1
Stormwater									
Prince of Wales Bay	39-88 (71)	39-85 (65)	1.3-18 (6.0)	0.28 - 0.96	0.34 - 0.75	0.49 - 1.30	1.8-13.5 (7.0)	5-39 (23.5)	21-59 (36)
Newtown Rivulet	45	44	5.3	0.81	0.73	0.94	4.9	16.5	22.3
Hobart Rivulet	26	22	4.4	0.85	0.96	1.79	6.7	11.4	6.5
Waimea catchment	36	27	4.6	0.60	0.79	1.51	9.3	12.2	11.4
Sediments									
Prince of Wales Bay	15 - 94	21 - 95	0.4 - 10.6	0.63 - 6.0	0.03 - 0.66	0.09 - 1.81	0.01 - 7.5	3.4 - 31	0 - 64

Cluster analysis, a form of multivariate statistical analysis, has been used as an additional tool to process PAH data collected from both stormwater and sediment samples using the statistics package *Statistica for Windows V 5.0.* Data was standardised by;

$$\frac{(\text{value}-\text{mean})}{\text{Standard deviation}}$$

A distance matrix was then created using cluster analysis on the standardised data (using squared euclidean distances). The correlation matrix was then developed by multidimensional scaling. Multivariate analysis is a technique which allows a number of variables, measured for a number of samples from a data matrix, to be tested for inter-sample correlations with the aim of reducing data in both size and complexity (Revill, 1992). Multivariate analysis has been successfully used previously to distinguish between PAH sources (e.g., Yunker & McDonald, 1995 - Mackenzie River coastal region of the Canadian Arctic; Yunker *et al.*, 1996 - Beaufort and Barents Seas).

Multivariate analysis using multidimensional scaling, incorporating PAH data collected from four storm events and four different catchments, indicated that a majority of stormwater samples have a composition pattern more closely related to sump oil than to unused lubricating oil (Fig. 22). There was no noticeable trend in PAH composition throughout storm events that indicated a change in oil or combustion derived inputs with time, in fact, the data presented as a cluster diagram show that samples collected from individual storm events tend to be closely related (Fig. 22). This indicated that PAHs are derived from a single predominant hydrocarbon source at the time of each storm event. This may be related to catchment practices at the time of the storm event such as traffic density, oil spills and oil dumping, or to seasonal inputs such as atmospherically transported combustion PAHs. The cluster diagram also confirms that hydrocarbons in stormwater during storm flow are almost exclusively derived from automotive oils rather than factory discharges within the Derwent Park catchment (Fig. 22).

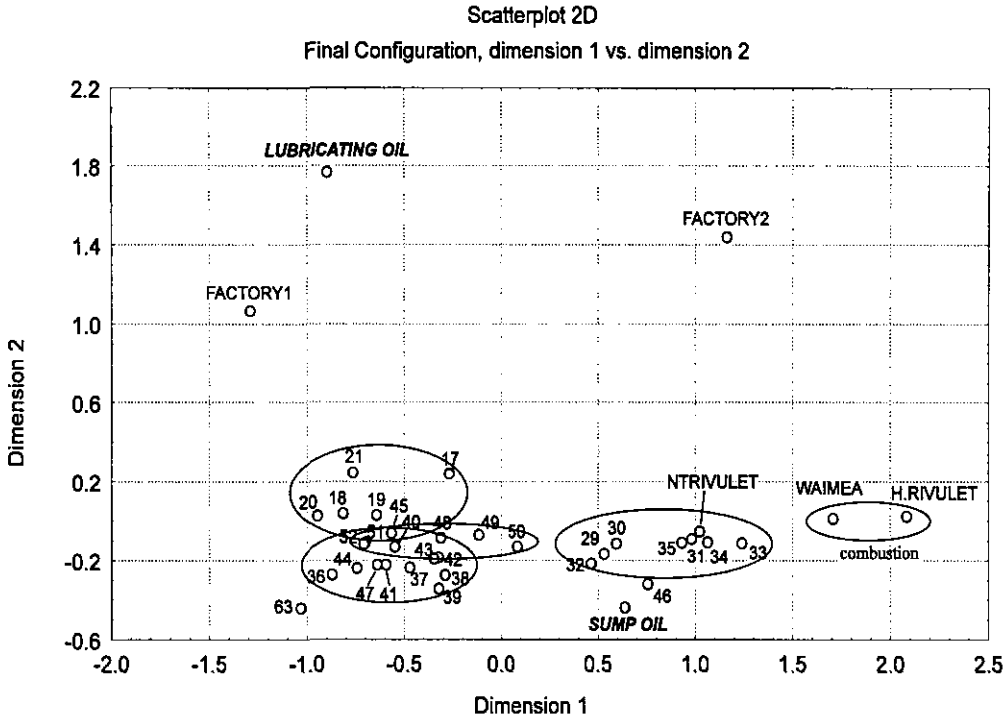


Fig. 22: Cluster analysis of hydrocarbon source materials and stormwater samples collected at Prince of Wales Bay outfall unless otherwise marked. Samples collected from particular storm events are grouped within circles. Cluster analysis, a form of multivariate statistical analysis, was conducted using PAH compositional data by the statistics package *Statistica 5.*

Stormwater samples in the Hobart Rivulet (HR) and Waimea catchments (WC) contained PAHs with a signature implying predominant input from combustion-derived products. PAHs formed by combustion are distinguished from those in oils by the dominance of 4 to 6 ring compounds (HR-74%; WC-64%)(Table 12) over the lower molecular weight di-aromatic and tri-aromatic compounds and by the dominance of the unsubstituted compounds (HR-78%; WC-73%)(Table 8) over their corresponding alkylated homologues (Bence *et al.*, 1996).

Ratios for PAH isomer pairs at *m/z* 202, *m/z* 228, and *m/z* 252 to determine the relative amounts of kinetic and thermodynamic isomers (see section 1.7) are given in Table 16 and show values indicative of enhanced combustion product input to both Hobart Rivulet and Waimea catchment samples. Also, the predominance of methyl phenanthrenes over phenanthrene and higher alkylated homologues indicated car exhaust as the major contributor to the combustion-derived PAH profiles at these

catchments (see Aboul-Kassim & Simoneit, 1995). At Prince of Wales Bay, in two of the nine storm events studied, an increasing trend in isomer pair ratios, and hence combustion product input, was noted as catchment flushing progressed. As mentioned above, trends such as this were not obvious in the cluster analysis. This stresses the importance of monitoring individual source specific parameters in addition to cluster analysis which proved useful for broad groupings according to PAH composition.

3.8 PAH CONTENT AND SOURCES IN SEDIMENTS OF PRINCE OF WALES BAY

3.8.1 PAH content of Prince of Wales Bay sediments

Total PAHs in sediments of Prince of Wales Bay varied greatly in concentration, ranging over four orders of magnitude. As was the case for aliphatic hydrocarbons, PAH contamination in Prince of Wales Bay was found to be localised occurring predominantly in sheltered embayments, adjacent to stormwater outfalls and beneath marinas (Fig. 20). Sediment from site 1 had a total PAH concentration of 0.02 μg/g (Fig. 21) which is relatively unpolluted and compares to a level of 0.007 μg/g recorded in Antarctica (Green *et al.*, 1992) and <0.0004 μg/g in a Great Barrier Reef sediment (Smith *et al.*, 1985)(Table 17). Sediment from site 3, near a major stormwater outfall, had a PAH concentration of 27.0 μg/g (Fig. 21) which is the highest concentration for marine sediments recorded in Australia (Table 17). This concentration is comparable to grossly polluted sites overseas, such as 29.6 μg/g PAHs in San Francisco Harbour (Periera *et al.*, 1996). The value is also above those recorded in many other areas of North America and Europe and indicates significant pollution. It is however well below values recorded for extremely polluted sites; e.g., 718 μg/g PAHs in a surface sediment from Boston Harbour (Shiaris & Jambard-Sweet, 1986)

Table 17: Concentrations of total PAHs in surface, marine and lacustrine sediments of Australia. PAH concentrations are presented in **µg/kg** (dry weight).

Location	Total PAH	Reference
Yarra Estuary, Melbourne	120 - 10,900	Bagg *et al.*, (1981)
Corio Bay, Geelong	490 - 3,000	Bagg *et al.*, (1981)
Mallacoota Inlet, Victoria	80 - 1,100	Bagg *et al.*, (1981)
Greenwich Bay, Melbourne	16,406	Clementson, (1981)
John Brewer Reef, Qld	< 0.4	Smith *et al.*, (1985)
Gladstone Harbour, Qld	1,497	Smith *et al.*, (1985)
Townsville Harbour,Qld	42 - 13,400	Smith *et al.*, (1985)
Brisbane River, Qld	3,940 - 16,110	Kayal & Connell, (1989)
Malabar sewage outfall, Sydney	180	Nichols & Espey, (1991)
George's River, Sydney	56 - 21,400	Brown & Maher, (1992)
Lake Burley Griffin, Canberra	80 - 538	Leeming & Maher, (1992)
Derwent Estuary, Hobart	**21 - 27,040**	**This study**

3.8.2 Sources of PAHs in Prince of Wales Bay sediments

The sediments of Prince of Wales Bay contained PAHs from several different sources. Adjacent to the major stormwater outfall (sites 3, 4, 4a, 4b, 5) and in poorly flushed back waters (site 9)(Fig. 21), automobile sump oil appeared the major contributor to hydrocarbon contamination in the sediments based on comparison to the PAH composition of source materials (Table 7). This contribution from automobile sump oil was evidenced by a high relative proportion of 2 and 3 ring compounds (76 to 94%) and a large proportion of alkylated versus parent PAHs (68 to 95%) at these sites (Table 18). The relationship between PAH composition of these sediments is illustrated by a cluster diagram (Fig. 23). The diagram also confirms a greater contribution by sump oil to the sediments of the Bay than by factory discharges and lubricating oil. Hence, despite the relatively low content of PAHs in stormwater in Hobart, evidence suggests that there is a cumulative effect leading to localised very high levels of contamination by automotive sump oil in sediments of receiving waters.

Table 18: Percentage composition of aromatic hydrocarbons and PAHs in Prince of Wales Bay sediments. Station numbers correspond to sampling sites in Fig. 21.

		1	2	3	4	4a	4b	5	6	7	8	9
PAH	m/z											
(2 rings)												
naphthalene	128	3.77	0.11	0.01	0.11	0.04	0.03	0.01	0.01	0.07	0.06	0.03
methyl naphthalenes		13.10	0.13	0.08	0.19	3.53	3.59	0.04	0.01	0.04	0.05	0.05
(3 rings)												
phenanthrene	178	2.69	0.69	0.07	7.52	6.11	4.01	1.39	0.01	0.10	0.04	0.04
anthracene	178	6.57	0.16	0.01	0.75	1.15	0.70	0.13	0.03		0.02	
methyl phenanthrenes		6.28	20.50	30.64	34.96	26.81	27.65	24.03	3.43	3.26	7.25	24.63
dimethyl phenanthrenes	206		28.48	63.36	37.48	32.58	40.23	50.70	12.84	11.33	32.72	63.85
% 2-3 ring compounds		**32.41**	**50.06**	**94.17**	**84.00**	**76.00**	**81.00**	**76.31**	**16.33**	**14.81**	**40.15**	**88.59**
(4 rings)												
fluoranthene	202	4.88	22.12	1.26	2.29	3.35	3.39	3.42	7.40	12.12	11.50	3.25
pyrene	202	1.76		0.72	3.61	4.97	4.25	3.74	8.23	8.49	4.31	0.54
b-fluorenes/m-pyrenes	216	15.60	3.55	0.56	2.36	2.77	2.40	1.63	6.00	5.92	3.57	1.30
benz(a)anthracene	228	6.74		0.06	0.68	1.26	0.85	1.41	4.62	4.33	0.79	0.06
chrysene	228	24.89	12.47	0.91	1.24	1.91	1.48	2.31	8.69	14.10	10.67	2.13
(5 rings)												
benzo fluoranthenes	252	4.46	8.57	1.26	1.89	3.30	2.25	4.44	18.82	17.23	16.08	2.78
benzo(e)pyrene	252	2.58		0.46	0.73	1.26	0.91	1.96	8.24	6.33	6.00	0.78
benzo(a)pyrene	252	2.46			1.33	2.23	1.49			0.56		
perylene	252	0.50			0.29	0.38	0.30	0.03	1.52	0.97		
dibenz (ah)	278		0.30	0.05	0.16	0.17	0.13	0.36	2.82	0.97	0.81	0.08
(6 rings)												
indeno (123)	276		2.45	0.41	0.77	0.89	0.69	2.26	9.04	7.52	4.58	0.39
benzo (ghi)	276	3.73	0.47	0.14	1.09	1.56	1.05	2.14	8.30	6.65	1.56	0.09
% alkylated compounds		**34.98**	**52.65**	**94.64**	**75.00**	**68.00**	**77.00**	**76.41**	**22.28**	**20.56**	**43.60**	**89.83**
Total PAH (ug/g)		**0.02**	**2.11**	**27.04**	**13.22**	**18.46**	**17.42**	**20.60**	**16.42**	**0.13**	**2.41**	**10.35**
Total HC (ug/g)		1200	3600	5700	4930	6780	3410	3800	10100	400	2000	5200
phenanthrene/anthracene		**0.41**	**4.17**	**5.21**	**10.01**	**5.30**	**5.72**	**10.61**	**0.24**		**1.95**	
fluoranthene/pyrene		2.77		1.74	0.634	0.674	0.80	0.91	0.90	1.43	2.67	5.97
b(a)ant/chry		0.27	0.00	0.06	0.55	0.66	0.58	0.61	0.53	0.31	0.07	0.03
b(e)p/b(e)p		0.95		0.00	1.81	1.77	1.65	0.00	0.00	0.09	0.00	0.00

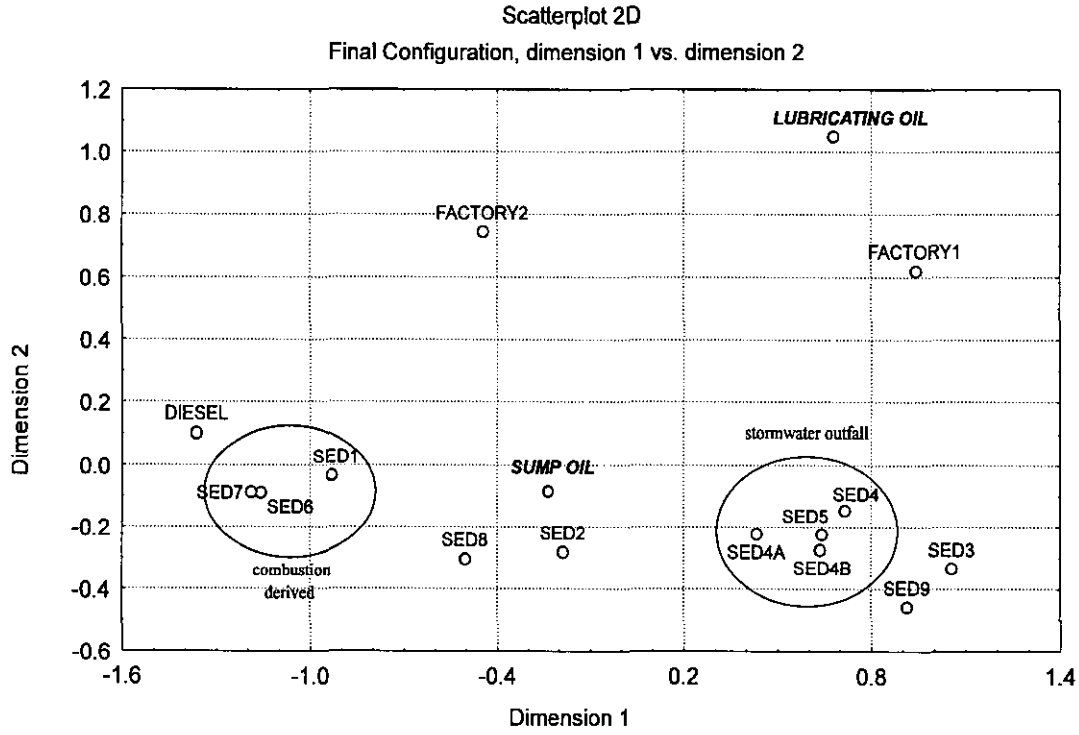


Fig. 23: Cluster analysis of hydrocarbon source materials and sediment samples collected from Prince of Wales Bay. For sediment sample locations see Fig. 21. Cluster analysis, a form of multivariate statistical analysis, has been conducted using PAH compositional data by the statistics package *Statistica 5.*

PAH compounds derived from boating activities and combustion were identifiable at sites away from stormwater discharge points. Combustion derived PAHs were predominant at sites 1 and 7 which was evidenced by a low proportion of 2 and 3 ring compounds (15 to 32 %) and a low proportion of alkylated *versus* parent PAHs (21 to 35%)(Table 14). Sediment collected from site 6, beneath a marina complex (Fig. 20), was heavily impacted by PAHs (16,400 µg/kg), primarily from a combustion derived source (22.3% alkylated compounds)(Table 14). It is believed that these compounds were introduced directly to the sediments from discharge of diesel exhaust from boats using the marina. Combusted lubricating oil and/or fuel are one of the main sources of PAHs in diesel exhaust (Collier *et al.*, 1995). In section 3.4 it was speculated as to whether the high concentration of aliphatic hydrocarbons detected in sediment collected from beneath the marina complex was from stormwater discharge, factory effluent or from boating activity. It would appear that significant contribution from the latter is most likely based on the PAH composition of the sediment.

The ratio of phenanthrene to anthracene (phen/ant) proved to be a useful indicator of PAH source in this study. Sediments heavily impacted by used oil had phen/ant ratios in the range 5.2 to 10.6 (Table 18), sediments containing predominantly combustion derived PAHs had phen/ant ratios in the range 0.4 to 1.6 whilst those containing PAHs from mixed sources had phen. ant ratios of 2.0 to 4.9 (Table 18). This is in accordance with findings of Bomboi & Hernandez (1991) who suggested a phen./ant. ratio of 1 to 8 as an indicator of used oil in urban runoff and Kayal and Connell (1989) who suggested a phen/ant ratio of 3 to 5 as an indicator of an urban origin of PAHs containing some petroleum products. Analysis of source materials in this study showed a phen/ant ratio of 3.7 in outboard motor exhaust, 4.9 in automotive sump oil, 10.3 in road oil, 34.8 in unused automotive lubricating oil and 48 in factory effluent (Table 18).

3.9 SUMMARY

In this study chemical marker techniques were utilised to identify specific sources of hydrocarbons in selected stormwater catchments of Hobart. The mean concentration of hydrocarbons in stormwater during this study was found to be 2.88 mg/l with a total annual discharge to the Derwent Estuary on the order of 164,000 kg/year. Assessment of the major hydrocarbon inputs to the Derwent estuary on an annual basis demonstrated stormwater as the largest contributor. Estimates of other hydrocarbon inputs to the Derwent estuary were; sewage effluent (58,000-84,000 kg/annum), industrial effluent (18,000-31,000 kg/annum) and atmospheric deposition (5,000-14,000 kg/annum).

Source elucidation of hydrocarbons demonstrated inputs to stormwater from automotive oils, diesel fuel, and plant waxes. Analysis of polycyclic aromatic hydrocarbon (PAH) profiles by gas chromatography-mass spectrometry and multivariate analysis suggests, in most cases, that automobile sump oil, rather than unused lubricating oils are the major component of oil in stormwater. This is not surprising, as up to 680,000 l of automotive lubricating oils are believed to be lost from engines annually in Hobart. Vehicle exhaust fumes were believed to be a significant contributor of hydrocarbon contamination to stormwater in some catchments.

In sheltered embayments of the Derwent Estuary a clear link was demonstrated between urban stormwater and the build-up of hydrocarbon contaminants in sediments. Localised extreme hydrocarbon concentrations were found to be associated with stormwater discharge and boat mooring areas. Concentrations of aliphatic hydrocarbons (10,100 μg/g) and PAHs (27 μg/g) in sediments at Prince of Wales Bay were the highest yet reported for estuarine sediments in Australia.

CHAPTER FOUR

CHARACTERISATION OF FAECAL MATERIAL IN STORMWATER

Life is like a sewer. What you get out of it depends on what you put in.

Tom Lehrer, 1953

4. CHARACTERISATION OF FAECAL MATERIAL IN STORMWATER

4.1 INTRODUCTION

A study of source identification of faecal pollution from the urban catchments of Hobart is presented in this chapter. The study builds on the body of research that uses both traditional faecal indicators and faecal sterol techniques for the elucidation of the source of faecal material in catchments. Aside from providing local information essential to the management of urban runoff and receiving waters, the study compliments recent research projects on the distribution of organic matter in the Derwent Estuary. Included in the study is an assessment of the faecal pollution load of stormwater in relation to sewage inputs to the Derwent estuary.

4.2 SOURCE IDENTIFICATION OF FAECAL MATERIAL IN HOBART STORMWATER DRAINS

4.2.1 Derwent Park catchment - stormwater sterols

The sterols of stormwater samples collected from the Derwent Park catchment at the Prince of Wales Bay outfall were dominated largely by cholesterol and β-sitosterol (from here on referred to as sitosterol)(Fig. 24). The sterol content and composition of all stormwater samples collected at the Prince of Wales Bay outfall are shown in Appendix 3. The average sterol content and composition from all stormwater samples collected is presented in Table 20. On some occasions stigmasterol (24-ethylcholesta-5,22E-dien-3β-ol) was the dominant sterol, for example storm event 3 (Table 21). The sterol profile of many stormwater samples was generally similar to that of dog faeces (Fig. 25). This feature provided the first line of evidence that domestic pets are potentially significant contributors to faecal contamination in urban runoff from Hobart, although it is recognised that cholesterol and sitosterol are ubiquitous sterols in environmental samples (R. Leeming, pers. comm.).

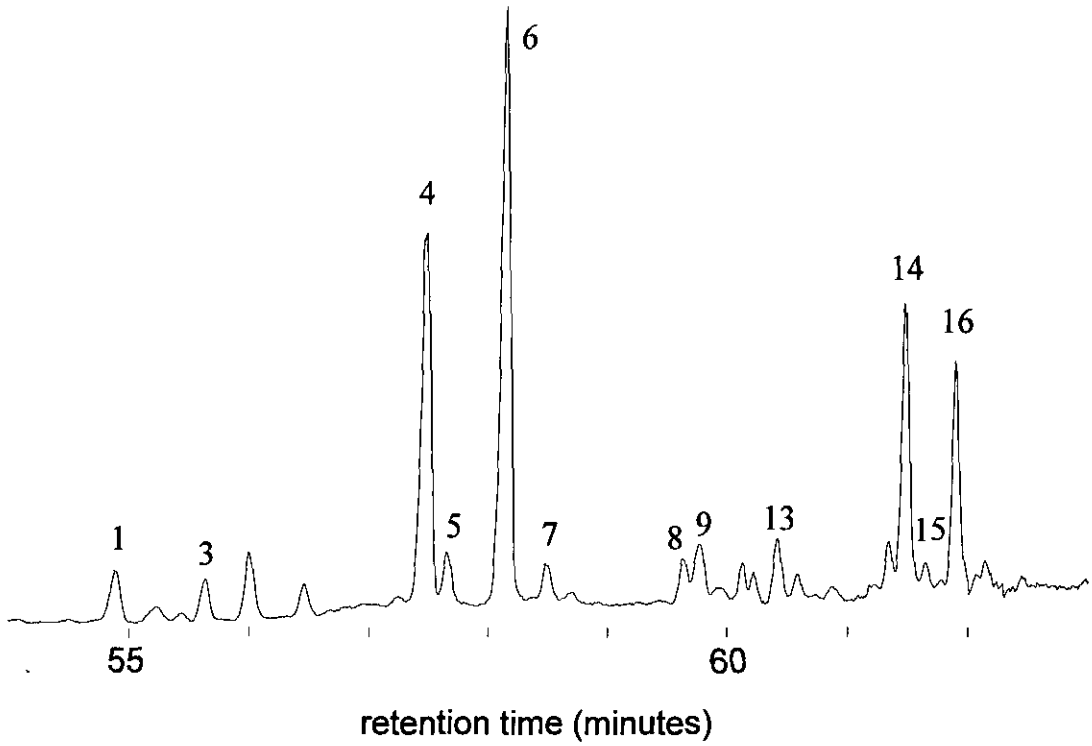


Fig. 24: Partial gas chromatogram of sterols in Prince of Wales Bay stormwater (Storm event 7, 23/9/95, 9 min. into storm - Table 5). For peak number identification refer to Table 19.

Table 19: Peak identification numbers for sterols and alcohols.

Peak number	Sterol / alcohol	Common name(s)
1	5β-cholestan-3β-ol	coprostanol
2	5β-cholestan-3α-ol	epicoprostanol
3	27:0 alcohol	
4	cholest-5-en-3β-ol	cholesterol
5	5α-cholestan-3β-ol	cholestanol
6	28:0 alcohol	
7	24-methylcholesta-5,22E-dien-3β-ol	brassicasterol
8	24-methylcholesta-5,24(28)E-dien-3β-ol	24-methylenecholesterol
9	24-methylcholest-5-en-3β-ol	campesterol / 24-methylcholesterol
10	24-ethyl-5β-cholestan-3β-ol	24-ethylcoprostanol
11	24-methyl-5α-cholestan-3β-ol	campestanol / 24-methylcholestanol
13	24-ethylcholesta-5,22E-dien-3β-ol	stigmasterol
14	24-ethylcholest-5-en-3β-ol	β-sitosterol / 24-ethylcholesterol
15	24-ethyl-5α-cholestan-3β-ol	sitostanol / 24-ethylcholestanol
16	30:0 alcohol	

Table 20: Content and composition of sterols and alcohols in Prince of Wales Bay stormwater. Mean content and composition of all samples (n=61).

Sterol	mean µg/l	mean %
coprostanol	0.28	5.0
cholesterol	1.74	37.4
cholestanol	0.13	2.6
brassicasterol	0.20	4.2
24-methylenecholesterol	0.20	3.6
campesterol	0.27	5.8
stigmasterol	0.77	16.6
sitosterol	1.06	22.8
sitostanol	0.10	2.0
Mean sterol content (µg/l)	**4.65**	

Table 17: Content and composition of sterols and alcohols in Prince of Wales Bay stormwater. Storm event 3 - 20th January 1995 (Table 5). First flush is denoted by ff.

	powb 10		powb 11		powb 12		powb 13	
	2hr after ff		1 hr later		2 hr later		11 hr later	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.07	**0.8**	0.10	**1.2**	0.07	**0.8**	0.13	**3.3**
cholesterol	1.03	**13.0**	1.47	**18.3**	0.91	**10.6**	0.99	**25.2**
cholestanol	0.03	**0.4**	0.06	**0.8**	0.04	**0.4**	0.05	**1.2**
brassicasterol	0.22	**2.8**	0.35	**4.4**	0.21	**2.4**	0.16	**4.0**
24-methylenecholesterol	0.07	**0.8**	0.19	**2.4**	0.10	**1.2**	0.28	**7.2**
campesterol	0.28	**3.6**	0.24	**3.1**	0.25	**2.9**	0.13	**3.2**
stigmasterol	4.74	**60.3**	3.66	**45.8**	5.24	**61.3**	1.03	**26.1**
sitosterol	1.26	**16.0**	1.75	**21.9**	1.66	**19.4**	1.08	**27.5**
sitostanol	0.17	**2.2**	0.18	**2.2**	0.08	**0.9**	0.09	**2.4**
Total sterols (ug/l)	**7.86**		**8.00**		**8.56**		**3.93**	

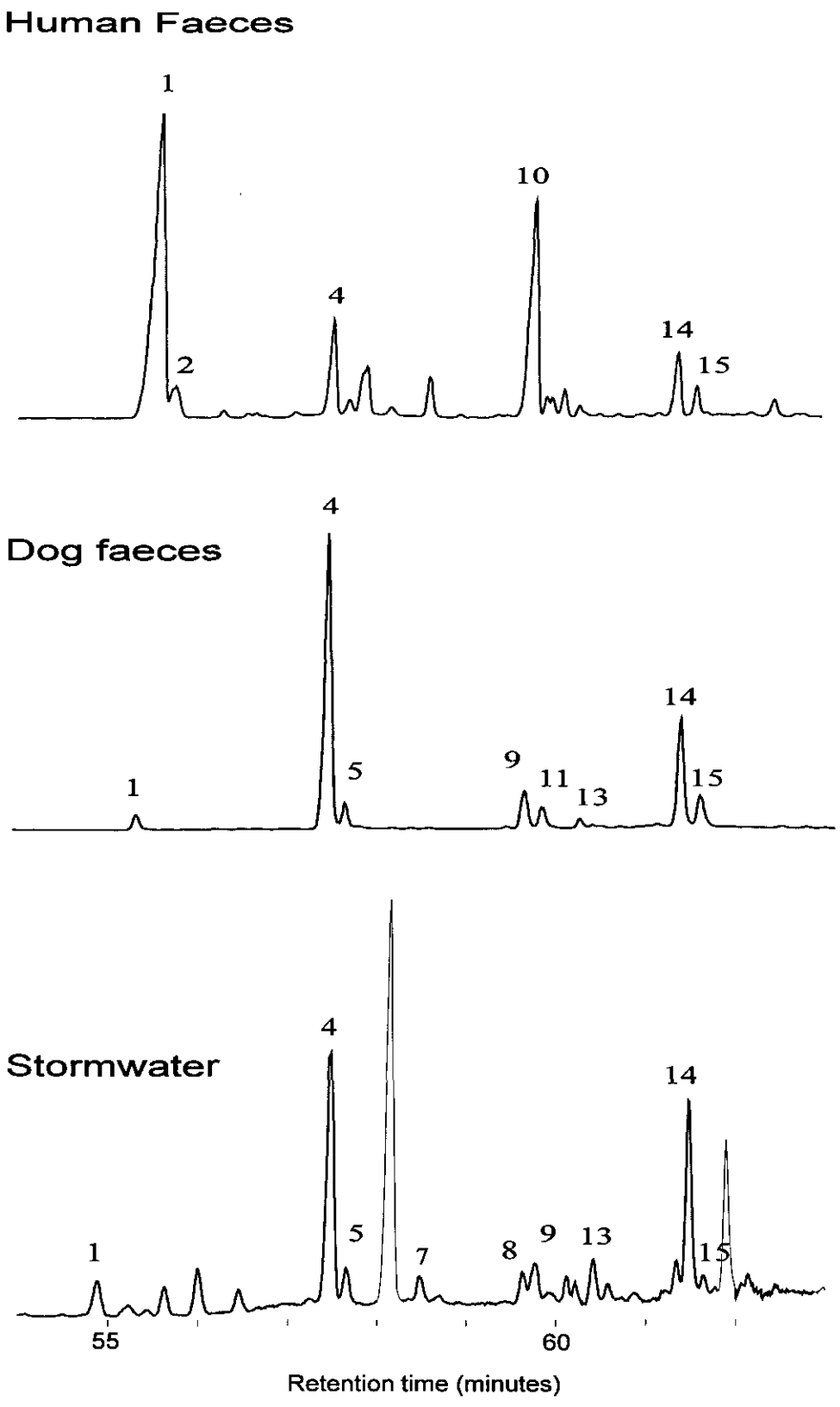


Fig. 25: Comparison of sterol profiles from faeces samples and stormwater collected from the Derwent Park catchment in Hobart. Chromatogram for human faeces from Leeming *et al.*, 1994). For peak identification refer to Table 19. Alcohol peaks derived from higher plant input have been shaded.

The predominant sterol used to identify human faecal material, coprostanol, was present in stormwater samples at concentrations between 0 - 1.68 μg/l, with a mean of 0.28 μg/l for the whole study period (Table 20). In other Australian stormwater studies coprostanol concentrations reported were 0.01 - 0.98 μg/l in Sydney (Nichols *et al.*, 1996) and 0.01 - 0.11 μg/l in Wyong, NSW (Leeming *et al.*, 1997). The coprostanol concentrations reported for stormwater in this study fall within the range identified from studies elsewhere as "highly polluted waters" (Laureillard & Saliot, 1993). However, compared to wastewater effluent, which in Hobart has a mean coprostanol concentration of 230 μg/l for secondary treated effluent (Leeming & Nichols, 1996a), the mean coprostanol concentration of 0.28 μg/l reported in this study for stormwater samples is very low and equivalent to an effluent dilution factor of 1:820.

The mean proportion of coprostanol in stormwater samples from all events studied was 5% of total sterols (Table 20). It was, however, detected at levels approaching 50% of total sterols during prolonged wet periods (0.19-0.21 μg/l in storm event 9)(Table 22). During this storm event the high level of coprostanol was accompanied by a corresponding large increase in bacterial load in the stormwater samples (Table 22). This provided evidence that cross contamination from the sewerage system possibly occurred at this time. This phenomenon, common in Melbourne (Melbourne Water, 1993) and Sydney (Ngo *et al.*, 1992; O'Loughlin, 1990; Scott, 1996), typically occurs during periods of prolonged heavy rain.

Table 22: Bacterial indicators of faecal pollution in Prince of Wales Bay stormwater

Sample No.	Date collected	Faecal coliforms cfu/100 ml	*E. coli* cfu/100 ml	*C. perfringens* /100 ml	Flow rate l/s	Coprostanol ug/l	Coprostanol %
22	7/4/95	15 000	15 000		9280	nd	0.0
Storm event 5							
23	27/6/95	25 000	25 000		1830	0.30	6.2
24		50 000	50 000		2080	0.38	28.0
25		12 000	12 000		2160	0.49	18.5
26		18 000	18 000		2510	0.13	6.0
28	7/8/95	21 000	13 000		290	0.07	5.3
Storm event 6							
29	17/8/95	4 600	3 500	2 200	1560	0.06	6.2
30		3 500	2 600	4 100	3700	0.09	2.8
31		4 900	4 900	3 400	3430	0.10	2.5
32		4 000	2 000	6 000	4970	0.04	0.8
33		10 000	10 000	1 700	3430	0.02	0.8
34		2 200	1 600	1 600	2150	0.02	1.0
35		22 000	11 000	2 300	350	0.02	0.7
Storm event 8							
48	12/10/95	18 000	1 000		85	0.03	6.1
49		29 000	10 000		507	0.06	7.5
50		5 100	2 900		1570	0.15	10.0
51		12 000	12 000		1600	0.49	16.4
52		24 000	24 000		1550	0.14	6.1
Storm event 9							
53	18/12/95	3 000	3 000		1400	0.01	6.0
54		9 000	9 000		800	tr	
55		3 900	3 200		1570	tr	
56		3 700	3 400		2220	nd	0.0
57		5 400	5 400		6030	0.01	2.3
58		4 000	4 000		2760	tr	
59		4 000	4 000		3480	0.01	5.6
60		13 000	13 000		10500	0.02	11.0
61		12 000	10 000		6150	0.19	48.4
62		40 000	29 000		3560	0.21	46.7

Despite the measured high proportions of coprostanol in the sterol profiles during flood conditions it is also significant to note its presence in stormwater samples collected at base flow, for example; sample 36 - 0.20 µg/l 9.4%)(Table 23), and sample 48 - 0.03 µg/l (6.1%)(Table 24). This demonstrated that human faecal infiltration, or *in situ* formation of coprostanol from cholesterol under anoxic conditions, occurs in the stormwater system as an everyday occurrence, not just during times when contamination from the sewerage system under high flow occurred.

Table 23: Content and composition of sterols in Derwent Park stormwater, sampled at Prince of Wales Bay. *Storm event 7 - 23rd September 1995* (Table 5).

	powb 36		powb 37		powb 38		powb 39		powb 40		powb 41	
	base flow		flush start		flush							
	11:10 am 23/9/95		1:36 PM		1:39 PM		1:41 PM		1:43 PM		1:45 PM	
Sterol	µg/l	%	µg/l	%	µg/l	%	µg/l	%	µg/l	%	µg/l	%
coprostanol	0.202	9.4	0.55	13.1	0.18	2.5	0.15	2.8	0.27	2.6	0.40	6.3
cholesterol	1.229	57.2	2.08	49.6	2.51	36.0	2.35	44.4	4.40	42.2	2.73	43.0
cholestanol	0.069	3.2	0.22	5.3	0.28	4.1	0.18	3.3	0.26	2.5	0.27	4.2
brassicasterol	0.039	1.8	0.08	1.9	0.25	3.6	0.23	4.3	0.23	2.2	0.23	3.7
24-methylenecholesterol	0.029	1.4	0.14	3.2	0.35	5.1	0.19	3.5	0.54	5.2	0.24	3.8
campesterol	0.073	3.4	0.19	4.6	0.85	12.1	0.32	6.1	0.58	5.6	0.38	5.9
stigmasterol	0.055	2.5	0.18	4.2	0.53	7.6	0.30	5.7	0.61	5.9	0.37	5.9
sitosterol	0.411	19.1	0.65	15.4	1.91	27.3	1.47	27.7	3.38	32.4	1.63	25.6
sitostanol	0.043	2.0	0.11	2.6	0.12	1.7	0.12	2.3	0.15	1.4	0.10	1.5
Total sterols (µg/l)	2.15		4.20		6.98		5.30		10.43		6.35	

Table 24: Content and composition of sterols in Derwent Park stormwater, sampled at Prince of Wales Bay. *Storm event 8 - 12th October 1995* (Table 5).

	powb 48		powb 49		powb 50		powb 51		powb 52	
	base flow		flush start		light rain		v. light rain		easing off	
	8:10 pm 12/10/95		8:35 pm		8:40 pm		8:50 pm		9:05 pm	
Sterol	µg/l	%	µg/l	%	µg/l	%	µg/l	%	µg/l	%
coprostanol	0.03	6.1	0.06	7.5	0.15	10.0	0.49	16.4	0.14	6.1
cholesterol	0.23	55.4	0.36	48.4	0.62	42.4	1.24	41.6	1.03	44.4
cholestanol	tr		0.01	1.4	0.03	2.3	0.06	2.1	0.03	1.4
brassicasterol	nd		0.01	1.7	0.06	4.2	0.09	2.9	0.07	3.2
24 methylenecholesterol	nd		0.01	1.8	0.06	3.9	0.07	2.3	0.06	
campesterol	0.04	10.1	0.07	9.8	0.15	10.0	0.25	8.3	0.16	6.8
stigmasterol	0.02	4.8	0.03	4.5	0.06	3.8	0.10	3.3	0.07	3.0
sitosterol	0.10	23.6	0.18	24.1	0.31	21.3	0.63	21.2	0.74	31.6
sitostanol	nd		0.01	0.9	0.03	2.1	0.05	1.8	0.02	1.0
Total sterols (µg/l)	0.42		0.75		1.46		2.97		2.33	

The faecal sterol, 24-ethylcoprostanol, which is predominantly derived from the faeces of herbivorous animals (Leeming *et al.*, 1994), was largely absent in stormwater samples, however its coelution with other sterols made it difficult to quantify accurately (see section 2.4.5). The absence, or extremely low abundance, of 24-ethylcoprostanol in stormwater samples basically precluded herbivores as significant contributors to the faecal matter entering stormwater drains of this catchment under most conditions. The faeces of dogs and birds do not contain 24-ethylcoprostanol or coprostanol, or for that matter any significant faecal biomarkers (Leeming *et al.*, 1996). However, the fact that their faeces contains high levels of faecal bacteria, and the similarity of Prince of Wales Bay sterol profiles to dog faeces, implicates these animals as the predominant source of faecal material in stormwater samples which were also demonstrated to commonly contain high levels of faecal bacteria (see section 4.2.3).

4.2.2 Sterol composition of stormwater from other catchments

Other Hobart catchments from which stormwater samples were collected are shown in Fig. 11. The sterol profiles from stormwater samples collected from these catchments during storm flow were almost identical (Fig. 21) and showed a great deal of similarity to Prince of Wales Bay stormwater sterols. This observation suggests a degree of uniformity in sterol composition of stormwater across Hobart catchments, with cholesterol, sitosterol and stigmasterol dominant and only low levels of human derived faecal sterols detected.

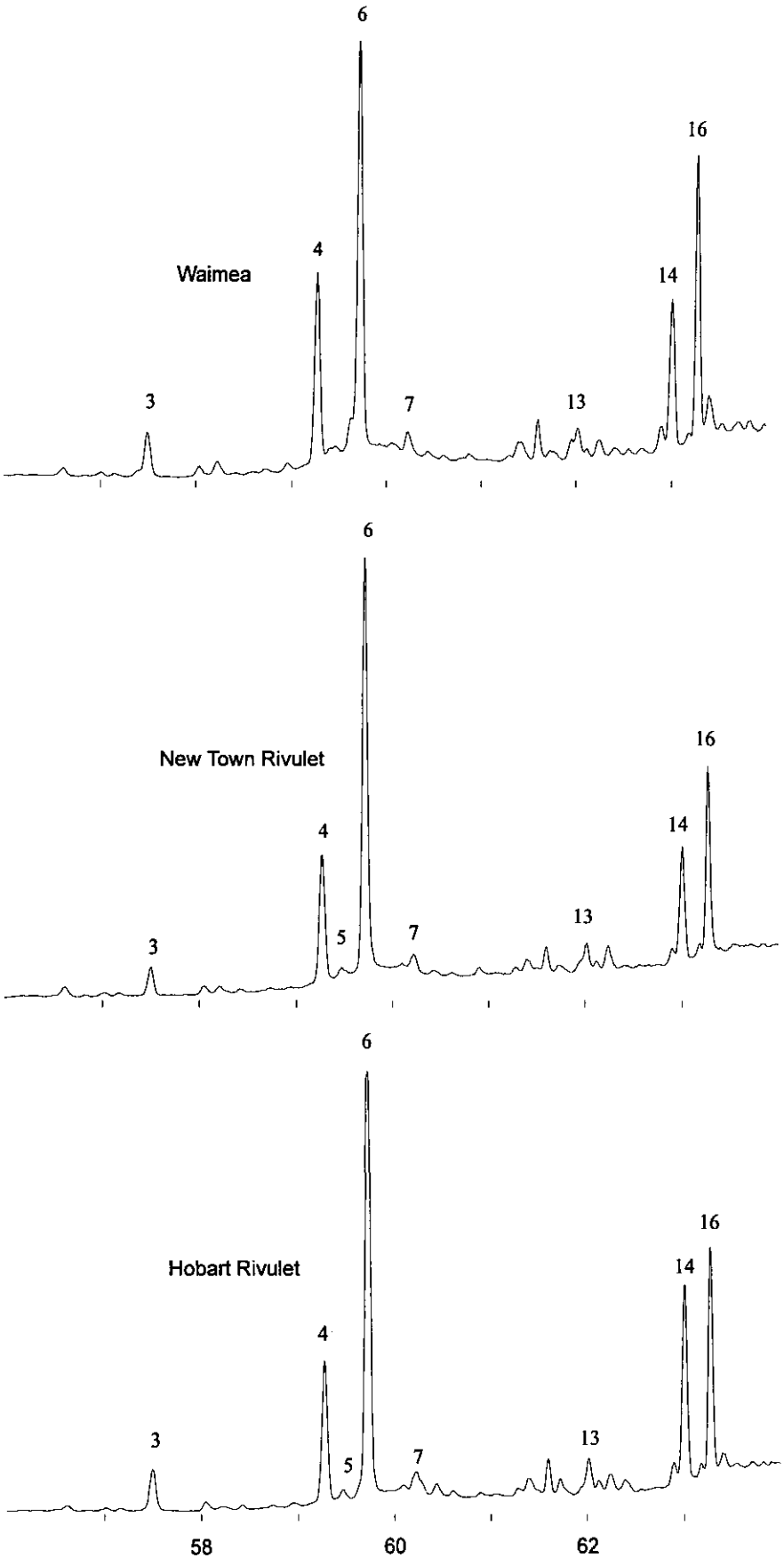


Fig. 26: Sterol profiles from Hobart stormwater samples. For peak identification refer to Table 19, and for sample locations refer to Fig. 11.

4.2.3 Derwent Park catchment - bacterial contamination

Bacterial contamination in Hobart stormwater was not determined for all samples collected throughout the study period because access to microbiological laboratories was not always possible. The bacterial indicators measured were total faecal coliform, *E. coli* and in one storm event *Clostridium perfringens* spores (Table 22). The mean concentration of faecal coliforms in stormwater during the course of the study was 13,500 cfu/100 ml (range = 3,000 - 50,000) and for *E. coli* 10,800 cfu/100 ml (range = 1,000 - 50,000). All bacterial concentrations reported assume minimal die-off due to UV light exposure between collection and analysis of samples due to rapid delivery to the laboratory. All recorded bacterial concentrations were well above primary contact (representing full immersion in the water) and secondary contact (representative of minor contact e.g. splashing) levels for faecal coliforms in Australia of 150 colony forming units (cfu)/100 ml and 1,000 cfu/100 ml, respectively, and demonstrate that stormwater is a major contributor to faecal pollution in the Derwent estuary.

Bacterial levels in Hobart stormwater samples were generally lower than those recorded in Sydney and Melbourne studies. In Sydney, levels of up to 1,000,000 faecal coliforms per 100 ml were detected at stormwater outfall pipes during storm flow (Table 25) with up to 9,000,000/100 ml during dry weather flow (Rowlands *et al.*, 1992). In Melbourne, *E. coli* levels in the Yarra River ranged from 200-17,000/100 ml with much higher levels (30,000-1,800,000/100 ml) in samples collected from street runoff (Melbourne Water, 1992)(Table 25).

Table 25: Bacterial indicator numbers in recent Australian studies of urban and rural runoff

Location	Faecal Coliforms	*E. coli*	*C. perfringens*	Reference
	cfu/100 ml	cfu/100 ml	spores/100 ml	
Hobart - This study	**3,000 - 50,000**	**1,000 - 50,000**	**1,600 - 6,000**	
Wyong	6,400 - 49,000		680 - 11,000	Leeming *et al.*, 1996
Sydney	800 - 1,000,000			Rowlands *et al.*, 1992
Melbourne				Melbourne Water, 1992
Yarra River	200 - 17,000			
Street runoff	30,000 - 1,800,000			

The stormwater bacterial counts measured in this study were above those recorded at many of Hobart's sewage treatment plant outfalls (Table 26). New regulations in Hobart specify discharge levels of faecal coliforms to be below 750 cfu/100 ml (Pitman, pers. comm.). At secondary treatment plants, due to chlorination as part of the treatment process, contribution of faecal coliforms to receiving waters is generally much lower than the regulation level, often below 60 cfu/100 ml (Table 26).

Table 26: Faecal coliform inputs to the Derwent Estuary from sewage treatment plants*

Treatment plant	Flow kL/day	Faecal coliforms /100 ml**	Faecal coliforms /day	Suspended solids mg/l	Suspended solids kg/day
New Norfolk	2,000	53	1.06E+09	17	34
Bridgewater	2,200	54	1.19E+09	9	20
Brighton	300	100,300	3.01E+11	66	20
Cameron Bay	4,800	7,000	3.36E+11	18	86
East Risdon	2,400	28	6.72E+08	21	50
Prince of Wales Bay	9,600	638	6.12E+10	18	173
Selfs Point	4,500	55	2.48E+09	26	117
Macquarie Point	14,200	3	4.26E+08	18	256
Rosny	7,400	336	2.49E+10	71	525
Sandy Bay	4,200	6,830,000	2.87E+14	214	899
Taroona	800	100,400	8.03E+11	94	75
Blackmans Bay	3,200	5,500	1.75E+11	79	253
Total	55,600		2.89E+14		2,508

*Information based on data supplied by the Tasmanian Department of Environment and Land Management
**Geometric mean

High counts of faecal coliforms were also detected in stormwater samples at base flow, for example, sample 48 - 18,000 cfu/100 ml (Table 22). In the previous section the presence of the human faecal marker coprostanol at base flow was acknowledged and some of this bacterial contamination in stormwater may be attributed to a human faecal source. This human contribution is, however, believed to be small based on the assumption that 0.06 µg/l of coprostanol corresponds to 150 cfu/100 ml of faecal coliforms (Leeming & Nichols, 1996). Only 0.03 µg/l of coprostanol was detected in sample 48. A similar situation was observed in Sydney stormwater studies where dry weather coprostanol levels in stormwater were low (0.006 - 1.0 µg/l)(Nichols *et al.*, 1996) and faecal coliform levels were mostly in excess of the Clean Waters Act limit of 1000 cfu/100 (Rowlands *et al.*, 1992).

The large excess of bacteria relative to coprostanol in sample 48 must be attributed to a faecal source(s) other than from humans or be attributed to the potential degradation of coprostanol in the storm drains. Coprostanol can be microbially degraded under aerobic conditions. Although half-lives are variable and depend on experimental conditions, they are generally less than 10 days at 20°C (Takada & Eganhouse, 1997). On the other hand, under aerobic conditions microbial degradation of coprostanol is much slower (Takada & Eganhouse, 1997).

It is unlikely that, in a fully piped catchment, that the bacterial contamination at base flow would be from water birds, herbivores or domestic animals, unless defecation by these animals directly into the storm drain occurred. The sterol profile of base flow water samples at the Derwent Park catchment was, however, similar to both the sterol profiles of stormwater samples collected during rainfall and to that of dog faeces. It is, however, possible that some of the bacterial contamination was derived from the faeces of rats which are known to live in the storm drains of Hobart.

Rat faeces collected from the Hobart Rivulet storm drainage system was analysed for faecal sterols and the results are presented in Table 27. The principal sterols present in rat faeces were coprostanol, cholesterol and epicoprostanol (Fig. 27). The fact that the rat faeces analysed contained epicoprostanol, 24-ethylcoprostanol and 24-ethylepicoprostanol, all of which were largely undetected in stormwater samples collected throughout the study period, suggest that rats aren't a significant contributor to faecal contamination in Hobart's stormwater. Although rat faeces contained coprostanol as a major sterol it was present at much lower concentration (634 μg/g - Table 27) than typically found in the faeces of humans (1,400-5,800 μg/g)(Leeming *et al.*, 1994).

Table 27: Mean sterol content and composition of stormwater*, rat faeces**, and storm drain sediment*** samples.

	stormwater*		rat faeces		storm drain sediment	
Sterol	**mean ug/l**	**%**	**ug/g (dry wt.)**	**%**	**ug/g (dry wt.)**	**%**
coprostanol	0.28	5.0	634	26.3	0.21	6.5
epicoprostanol			400	16.6		
cholesterol	1.74	37.4	634	26.3	0.54	17.0
cholestanol	0.13	2.6	68	2.8	0.04	1.2
brassicasterol	0.20	4.2	73	3.0	0.15	4.8
24-methylenecholesterol	0.20	3.6				
campesterol	0.27	5.8			0.19	5.9
24-ethylcoprostanol			172	7.1		
24-ethylepicoprostanol			105	4.3		
stigmasterol	0.77	16.6	58	2.4	0.15	4.7
sitosterol	1.06	22.8	196	8.2	1.75	54.6
sitostanol	0.10	2.0	67	2.8	0.17	5.5
Total	**4.65 ug/l**		**2410 ug/g**		**3.20 ug/g**	

*Mean value from all stormwater samples collected at Prince of Wales Bay outfall
**Collected from Hobart Rivulat storm drainage system
***Collected from Derwent Park storm drainage system

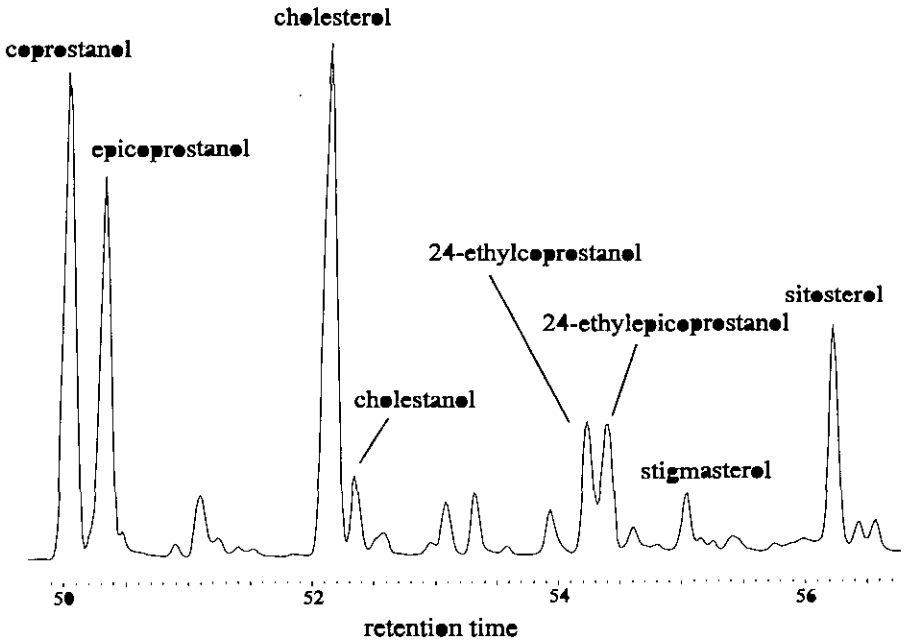


Fig. 27: Partial gas chromatogram of sterols in rat faeces.

Septic tank leachate as a potential source of the bacterial contamination to stormwater during dry flow may be ruled out due to the extremely low incidence of septic tank usage in the Hobart urban area. Additionally, a decrease in faecal coliform numbers with passage of leachate from septic tanks through soil has been well documented (Gilbert *et al.*, 1976). Infiltration from contaminated soils is another potential source

of the bacterial load in storm drains during dry weather. Glanton *et al.* (1992) found that most of Houston's polluted stormwater was derived from broken wastewater collection systems. A more likely source of the human component of the dry weather bacterial contamination in the stormwater drains is through direct sewage connection from households. Council tracer surveys have demonstrated the occurrence of this practice in the Derwent Park catchment.

Despite the potential human sources of bacterial contamination mentioned above, the potential for faecal bacteria to survive in sediments must also be considered. The occurrence of faecal coliform bacteria is generally accepted to be a sign of recent human sewage or animal faecal pollution, however, the survival of faecal coliform bacteria in specific environments has been documented. Marino & Gannon (1991) believe that sediments in storm drains act as reservoirs of high concentrations of faecal bacteria which exhibit the ability to multiply in drain sediment under favourable conditions. It is possible that animal or human faeces are not completely flushed from the drainage system during rain events to become lodged in an environment away from direct sunlight. Such conditions are potentially ideal for the multiplication of bacterial populations. From here the occurrence of bacterial inoculation of the overlying water, giving rise to high levels of bacterial contamination during base flow, would be possible.

To investigate the possibility of bacterial multiplication in Hobart storm-drain sediments, samples of sediment were collected from the interior of a drain in the Derwent Park catchment. The sediment samples contained very low faecal coliform counts (10-300 cfu/g) in comparison to typical bacterial levels encountered in storm drain sediments of 10^5 to 10^6 cfu/g (House *et al.*,1993). Despite this finding, the potential for storm drain sediments to inoculate overlying water with faecal bacteria cannot be discounted, particularly as localised occurrences of bacterially contaminated sediments may occur. A connection, in terms of sterol composition, was, however, demonstrated between the sterol profile of stormwater samples and storm drain sediments from the Derwent Park catchment (Table 27). Storm drain sediments contained sitosterol and cholesterol as the major sterols, some human faecal content

(coprostanol 6.5%), and the absence of the herbivore faecal biomarker 24-ethylcoprostanol (Table 27).

Bacterial levels were variable throughout the storm events studied, and there was poor correlation between faecal coliform counts and coprostanol concentration in stormwater samples collected at the Prince of Wales Bay stormwater outfall (correlation coefficient $r^2 = 0.442$)(Fig. 28). Re-analysis of the data disregarding the outlying points at coprostanol 0.49 μg/l (Fig. 28), resulted in a correlation coefficient of $r^2 = 0.71$. This re-assessment suggested that there may be some relationship between the human faecal sterol marker and the bacterial counts in Derwent Park stormwater, however, more data would be useful to assign greater significance to this relationship. Coprostanol concentration and faecal coliform counts have, on occasion, been shown to correlate well, for example in seawater from the Clyde estuary (Goodfellow *et al.*, 1977).

There was also poor correlation between *C. perfringens* spores and coprostanol concentration in stormwater samples from the Derwent Park catchment ($r^2 = 0.393$)(Fig. 29), however the relationship was only based on seven data points. The fact that low correlation was observed between *C. perfringens* and coprostanol or between coprostanol and faecal coliforms in this study provided additional evidence of faecal input to stormwater other than from humans.

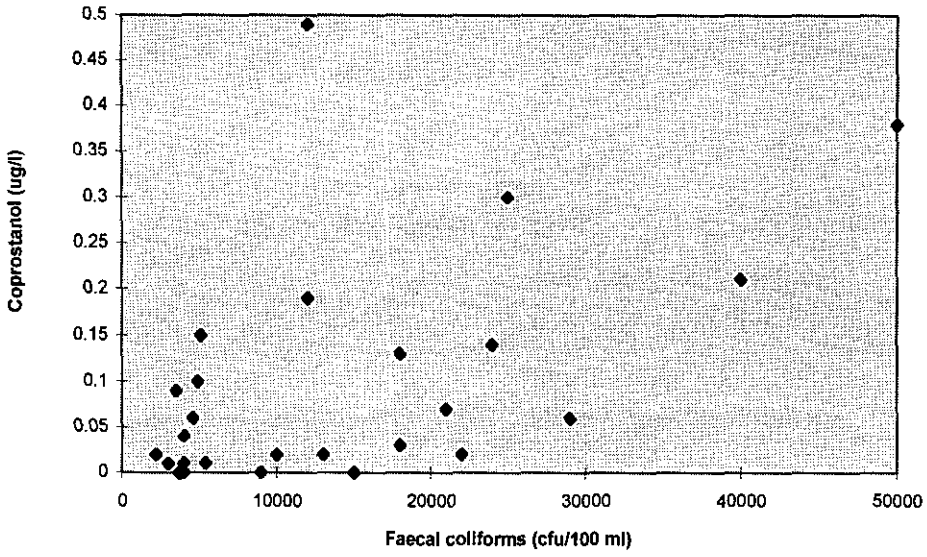


Fig. 28: Scatter plot demonstrating the relationship between coprostanol and faecal coliforms ($r^2 = 0.442$).

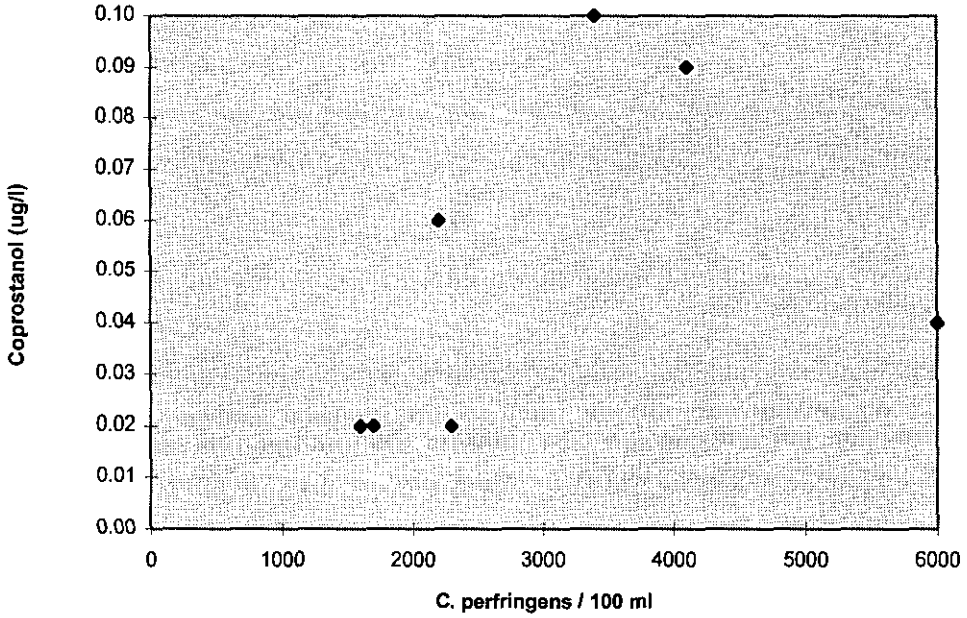


Fig. 29: Scatter plot demonstrating the relationship between coprostanol *and C. perfringens* ($r^2 = 0.393$).

4.2.4 Bacterial contamination in other Hobart stormwater catchments

In the open storm-drainage systems of Hobart, a more complex pattern of faecal input is likely. For example, urban rivulets may support large bird populations which are known to contribute significantly to aquatic bacterial contamination (Leeming *et al.*, 1997; Valiela, 1991). With catchments extending to the forested slopes of Mt. Wellington, faecal inputs to the streams from the local wildlife also probably occur. However, water samples collected outside Hobart's urban area have been shown to contain very low bacterial counts (Blacklow, 1995).

In a recent microbiological study of Hobart's major urban stream, the Hobart Rivulet (Blacklow, 1995), a major assumption was that human sewage-contaminated soils were the primary contributor to faecal contamination of the rivulet during dry weather. As over 400 pipes enter the lower reaches of the rivulet, direct sewer connections were also suggested as a possible contamination source. During wet weather, faecal coliform levels were significantly higher and attributed to surface runoff of dispersed animal faeces. Interestingly the study showed the highest levels of bacterial contamination in the lower reaches of the rivulet, away from sunlight, where much of the rivulet flows beneath the Hobart city centre.

4.2.5 Domestic pets

Dogs have previously been implicated as major contributors of *E. coli*, pathogens and nutrients to Australian waterways (Melbourne Water, 1993). It is believed that Melbourne's 300,000 dogs deposit an estimated 90 tonnes of faeces every day (Melbourne Water, 1993). In Hobart there are approximately 25,000 dogs each of which are believed to deposit 100-400 g of faeces per day. This equates to somewhere between 2.5 and 10 tonnes of dog faeces per day deposited in Hobart. With faecal coliform levels in the order of 2.0×10^8/g in dog faeces (Leeming *et al,* 1997), this represents an enormous potential bacterial contribution directly to the environment every day. It must be considered, however, that possibly only a small percentage of the faeces deposited by domestic pets would end up in urban waterways. Only those

faecal droppings deposited directly onto impervious surfaces or in the vicinity of urban waterways would available for direct flushing into the drainage system.

Evidence of domestic pet inputs of faecal material is provided by the fact that faecal coliform and *C. perfringens* counts in stormwater samples are of the same order of magnitude (Table 18, storm event 6). A hypothesis proposed by Leeming *et al.*, (1997) is based on the fact that the faeces of native birds contains 10^6 - 10^8 cfu/g of faecal coliforms but generally less than 10^2 cfu/g of *C. perfringens* spores, whereas dog and cat faeces contain approximately equal and comparatively high numbers (10^6 - 10^8 cfu/g) of both faecal coliforms and *C. perfringens*. Hence, if birds, humans and herbivores are assumed to have no *C. perfringens* compared to domestic pets (the proportion is < 0.01%)(Leeming *et al.*, 1997), we have an additional means by which to elucidate the source of faecal material where mixed inputs are likely.

4.2.6 Comparisons to other studies

As stated in the introduction, very few studies have elucidated sources of faecal material in stormwater. Although limited data exists, it was noted that there was a degree of similarity in the results of this study, in terms of bacterial counts (Table 21) and sterol composition, to those of the inland waters of Wyong in NSW, Australia (Leeming *et al.*,1997). The sterol profiles of water samples from both studies were dominated by cholesterol and sitosterol with low levels of coprostanol. In the Wyong study, human faecal matter was found to be only a minor component of the total faecal pollution which, in most cases, was found to be dominated by faeces from birds and pets.

4.2.7 Summary

The major findings of this section all implicated dog faeces as being the most obvious and major contributor to faecal contamination in Hobart's stormwater. These were;

- A similarity in the sterol profiles of dog faeces and stormwater samples.
- Low levels or absence of sterol markers for other sources of faeces.
- High bacterial levels bearing only low correlation to coprostanol levels.
- Up to 10 tonnes of dog faeces is deposited in Hobart every day.
- Similarity between faecal coliform and *C. perfringens* counts in stormwater samples.

Human faecal material, identified by tracing coprostanol, was a minor component of total faecal content in stormwater except during very wet periods. This finding supported findings from elsewhere in Australia where sewage contamination in stormwater is a common occurrence during heavy rain. Low levels of human faecal pollution also were detected in storm drains during dry weather implicating the occurrence of direct sewer connections or the fact that coprostanol may be degraded in storm drains under aerobic conditions. Bacterial contamination of stormwater during dry weather was attributed, to a small extent, to human faecal material. There remains uncertainty as to the major source of bacterial contamination in urban runoff during dry weather conditions due to the low levels of bacteria measured in drain sediments which were suggested as a source of bacterial inoculation to the overlying water. A more extensive survey of faecal sterols and bacterial counts in storm drain sediments is required as localised areas of bacterial contamination, or discharge of contaminated waste, may occur.

4.3 STEROL CONTENT, COMPOSITION & SOURCES IN DERWENT ESTUARY SEDIMENTS

4.3.1 Composition and sources of sterols in sediments of Prince of Wales Bay

Sterol profiles were determined for selected estuarine sediments in order to investigate whether stormwater is a significant contributor to faecal pollution in the Derwent Estuary. Sediment sampling sites at Prince of Wales Bay are shown in Fig. 30. Sediment sterols at the stormwater outfall of Prince of Wales Bay (stations 4, 4a, 4b) were dominated by sitosterol, cholesterol, brassicasterol and stigmasterol (Table 28). Coprostanol was present at 1.6 - 8.3% of total sterols in the stormwater outfall sediments, which although demonstrating the presence of human faecal contamination, was lower than that detected at all other sites in Prince of Wales Bay (Tables 28 & 29; Fig. 30). The marker for herbivore faecal contamination, 24-ethylcoprostanol, was undetected in the vicinity of the stormwater outfall (stations 4, 4a, 4b). This observation for sediments at the stormwater outfall reflected the sterol content of the stormwater discharge which contained predominantly low to undetectable levels of human and herbivore faecal material.

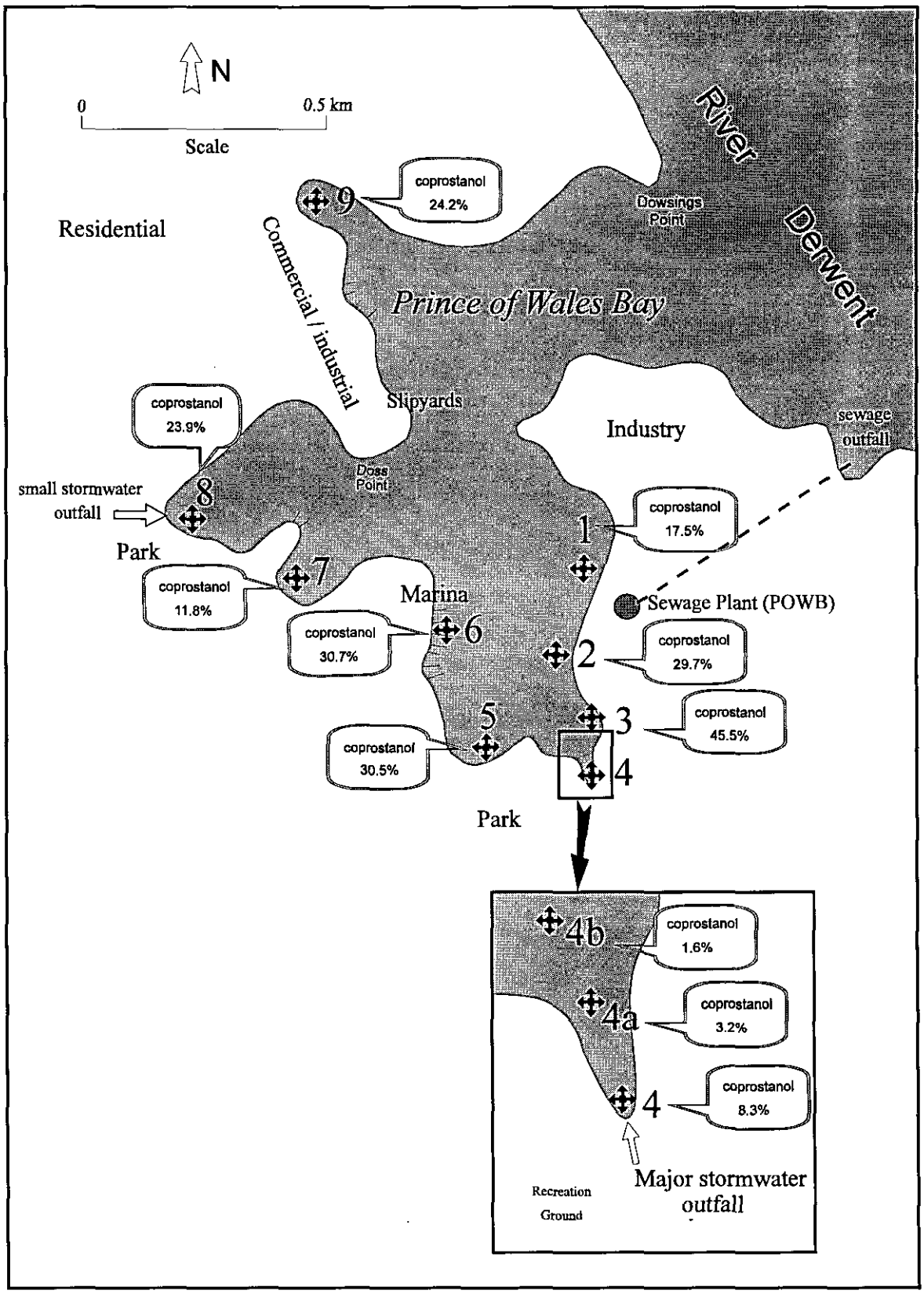


Figure 30: Map of Prince of Wales Bay showing the stormwater outfall and sediment sampling sites. Coprostanol is presented as percentage of total sterols in sediment samples.

Table 28: Sterol content and composition of Prince of Wales Bay sediments at stormwater outfall (Refer to Fig. 30 for location of sediment samples).

	sediment 4		sediment 4a		sediment 4b	
Sterol	ug/g	%	ug/g	%	ug/g	%
coprostanol	0.58	**8.3**	0.74	**3.2**	0.87	**1.6**
epicoprostanol						
trans-22-dehydrocholesterol	0.11	**1.6**	0.34	**1.4**	**2.73**	**5.1**
cholesterol	1.44	**20.7**	3.32	**14.3**	9.59	**18.1**
cholestanol	0.28	**4.1**	0.63	**2.7**	1.14	**2.2**
brassicasterol	1.01	**14.5**	3.26	**14.0**	10.22	**19.3**
24-methylenecholesterol						
24-ethylcoprostanol						
campesterol	0.50	**7.2**	1.77	**7.6**	4.06	**7.7**
24-ethyl-epicoprostanol						
stigmasterol	0.84	**12.1**	3.64	**15.6**	8.09	**15.3**
sitosterol	2.18	**31.5**	9.58	**41.2**	16.32	**30.8**
sitostanol						
Total stanols	0.86	**12.4**	1.37	**5.9**	2.01	**3.8**
Total sterols (ug/g dry wt.)	**6.94**		**23.28**		**53.01**	

Table 29: Sterol content and composition of Prince of Wales Bay sediments

	sediment 1		sediment 2		sediment 3		sediment 5		sediment 6		sediment 7		sediment 8		sediment 9	
Sterol	ug/g	%	ug/g	%	ug/g	%	ug/g	%	ug/g	%	ug/g	%	ug/g	%	ug/g	%
coprostanol	0.14	**17.5**	0.88	**29.7**	6.73	**45.5**	1.85	**30.5**	3.37	**30.7**	0.12	**11.8**	4.32	**23.9**	16.50	**24.2**
epicoprostanol	0.05	**6.5**	0.17	**5.7**	1.36	**9.2**	tr		0.30	**2.7**	0.05	**4.9**	0.65	**3.6**	2.67	**3.9**
trans-22-dehydrocholesterol										**0.0**		**0.0**				
cholesterol	0.10	**11.9**	0.16	**5.6**	0.34	**2.3**	0.26	**4.3**	0.41	**3.7**	0.11	**10.8**	0.69	**3.8**	1.83	**2.7**
cholestanol	0.12	**15.3**	0.39	**13.4**	1.35	**9.1**	0.27	**4.5**	1.07	**9.8**	0.26	**25.5**	3.24	**18.0**	13.20	**19.4**
brassicasterol																
24-methylenecholesterol																
24-ethylcoprostanol	0.03	**3.2**	0.39	**13.3**	2.37	**16.0**	1.00	**16.5**	1.72	**15.7**	0.10	**9.8**	1.22	**6.8**	10.20	**15.0**
campesterol																
24-ethyl-epicoprostanol	0.08	**10.4**	0.21	**7.3**	0.97	**6.6**	0.67	**11.0**	0.89	**8.1**	0.10	**9.8**	0.87	**4.8**	5.14	**7.6**
stigmasterol																
sitosterol	*0.04*	***4.8***	*0.16*	***5.5***	*0.33*	***2.2***	*0.78*	***12.9***	*0.61*	***5.6***	*0.07*	***6.9***	*2.06*	***11.4***	*3.05*	***4.5***
sitostanol	0.25	**30.4**	0.57	**19.5**	1.34	**9.0**	1.23	**20.3**	2.60	**23.7**	0.21	**20.6**	4.99	**27.7**	15.47	**22.7**
Total stanols	0.67	**83.3**	2.62	**88.9**	14.11	**95.5**	5.01	**82.8**	9.95	**1230.5**	0.84	**28.5**	15.29	**103.5**	63.18	**1042.8**
Total sterols (ug/g dry wt.)	**0.81**		**2.94**		**14.77**		**6.06**		**10.97**		**1.02**		**18.04**		**68.06**	

Sediments from stations 1-3 and 5-9 collected away from the Prince of Wales Bay stormwater outfall (Fig. 30) exhibited an entirely different sterol profile to those collected at the stormwater outfall. Sediments of Prince of Wales Bay contained sterol profiles dominated by the stanols, coprostanol, sitostanol, 24-ethylcoprostanol and cholestanol (Table 29; Fig. 31). Stanols comprised 82 - 96% of total sterols in these sediments as compared to 4 - 12% stanols in sediments at the stormwater outfall. The high proportion of stanols in Prince of Wales Bay sediments is very unusual in comparison to other sediment studies in the Derwent estuary, for example Leeming & Nichols (1996a), and indicates that sediments of Prince of Wales Bay are highly reduced (anoxic).

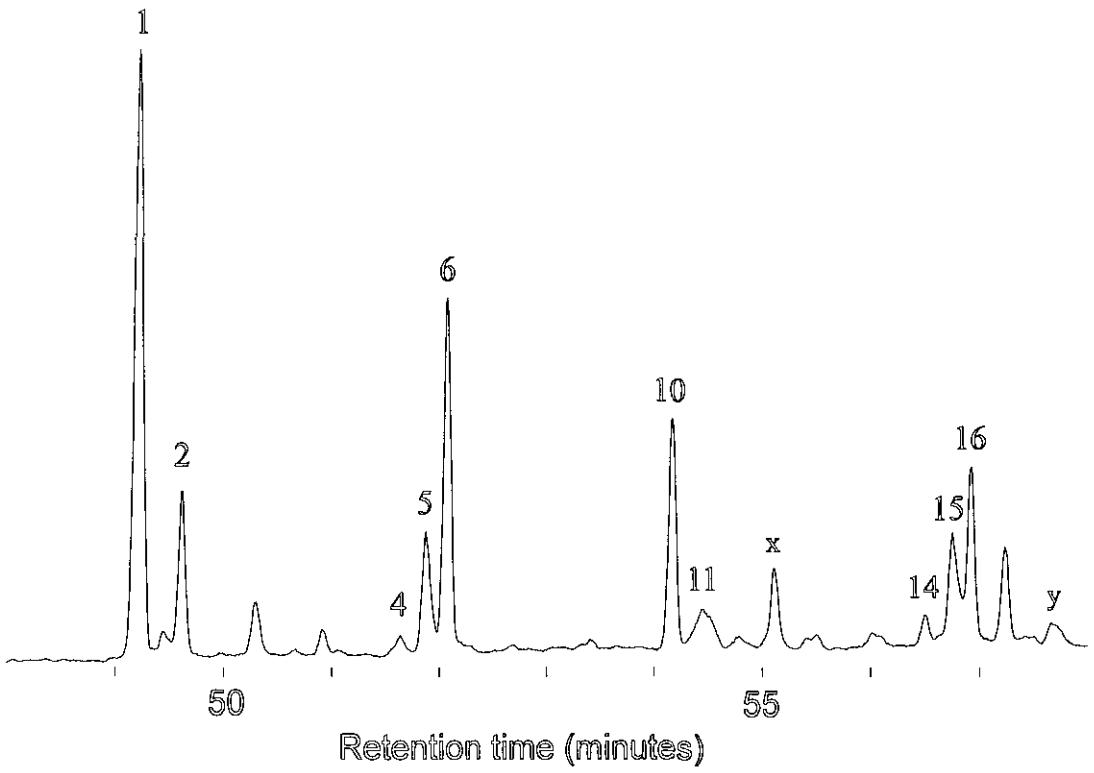


Fig. 31: Partial gas chromatogram of sterols in Prince of Wales Bay sediment. For peak number identification refer to Table 19. Refer to text for explanation of peaks x and y.

Coprostanol content of Prince of Wales Bay sediments was very high (0.12 - 16.5 μg/g or 12 - 46% of sterols)(Table 29; Fig. 30) and together with high concentrations of 24-ethylcoprostanol indicated the potential presence of significant levels of human faecal contamination. In fact, the percentage of coprostanol detected in Prince of

Wales Bay sediments was not much lower than that recorded in human faeces of 40-60% of total sterols (Leeming *et al.*, 1994). The coprostanol concentrations reported for Prince of Wales Bay sediments were up to an order of magnitude higher than those recorded in previous studies of organic matter in the Derwent Estuary by Volkman *et al.*, (1989) and Leeming & Nichols (1996a), and are compared to some other findings in Australia and overseas in Table 30. The coprostanol concentrations in Prince of Wales Bay sediments were comparable to those recorded in Mersey River sediment (9 µg/g) which was considered as grossly contaminated with sewage (Readman *et al.*, 1986)(Table 30). The concentration of coprostanol in sewage sludge is typically 1 mg/g (Readman *et al.*, 1986) but has been reported as high as 4.05 mg/g (Table 30). Hence, the highest concentration of coprostanol detected in Prince of Wales Bay sediments of 16.5 µg/g represents a dilution in the order of 355x from sewage sludge.

Table 30: Coprostanol concentrations in sewage sludge and surface sediments in Australia and from elsewhere. * signifies maximum recorded concentration.

Location	***Coprostanol*** ***µg/g (dry wt.)***	Reference
Australia		
Port Phillip Bay, Vic.	0.01 - 0.55	O'Leary *et al.*, 1994
Sydney coastal, NSW	2.9*	Nichols *et al.*, 1993
Sydney coastal, NSW	0.01 - 1.1	Nichols *et al.*, 1996
Derwent Estuary, Tas.	0.6 - 1.9	Volkman *et al.*, 1989
Derwent Estuary, Tas.	0.01 - 1.95	Leeming & Nichols, 1996a
Derwent Estuary, Tas.	0.12 - 16.5	**This study**
elsewhere		
Davis, Antarctica	0.01 - 0.88	Green & Nichols, 1995
Mersey Estuary, UK	9*	Readman *et al.*, 1986
New York Bight, USA	26.7*	Boehm, 1983
Narragansett Bay, USA	0.13 - 33.3	LeBlanc *et al.*, 1992
Venice canals, Italy	1.0 - 41.0	Sherwin *et al.*, 1993
Kaohsiung Harbour, Taiwan	0.58 - 128	Jeng & Han, 1994
Sewage sludge	***Cop. (mg/g)***	
UK (primary sludge)	0.91 - 3.1	Takada & Eganhouse, 1997
UK	1.28	Takada & Eganhouse, 1997
Spain (Primary sludge)	1.4 (ave.)	Takada & Eganhouse, 1997
USA (digested sludge)	0.23 - 2.94	Takada & Eganhouse, 1997
France (digested sludge)	2.28 - 4.05	Takada & Eganhouse, 1997

In addition to high coprostanol concentrations, total sterol content of Prince of Wales Bay sediments was also very high (0.81 - 68.1 μg/g - Tables 28 & 29) but fell within or below ranges previously reported for Derwent estuary sediments; (0.6 - 112 μg/g; Leeming & Nichols, 1996a) and (100 - 1,000 μg/g; Volkman *et al.,* 1989). Elsewhere in Australia, the total sterol content of Port Phillip Bay sediments was mostly lower than 20 μg/g (O'Leary *et al.,* 1994), and in Sydney - 5.2 μg/g of sterols in sediments adjacent to the Malabar sewage outfall (Nichols & Espey, 1991). High sterol concentrations in Derwent estuary sediments have previously been attributed to unnaturally high loads of organic matter derived from pulp fibre sludge (Volkman *et al.*, 1989; Leeming & Nichols, 1996a) from a paper mill situated on the upper Derwent estuary (Fig. 10). It is possible that this source of organic material also contributes to the high sterol concentrations in Prince of Wales Bay sediments.

The most abundant sterol constituent of pulp fibre sludge is sitosterol (24-ethylcholest-5-en-3β-ol)(Volkman *et al.,* 1989). This compound comprises greater than 60% of the sterols in paper mill effluent and has been proposed as a marker for pulp effluent (Leeming & Nichols, 1996a). Although concentrations of sitosterol in sediments of Prince of Wales Bay were comparatively low; 0.04 - 3.1 μg/g (compared to 2 - 5 μg/g previously reported for the middle Derwent estuary by Leeming & Nichols, 1996a), the reduced forms of the compound, sitostanol (24-ethyl-5α-cholestan-3β-ol) and 24-ethylcoprostanol (24-ethyl-5β-cholestan-3β-ol) were very abundant 0.28 - 25.7 μg/g (sum of the two compounds (Table 29). These compounds were possibly formed in pulp mill fibre sludges, from biohydrogenation of sitosterol during the anaerobic decomposition of organic matter (Volkman *et al.,* 1989), and subsequently transported to Prince of Wales Bay. Further analysis of Prince of Wales Bay sediments for compounds unique to pulp fibre sludges, such as resin acids, needs to been undertaken in order to ascertain the source of the reduced forms of sitosterol.

While pulp fibre was identified as a probable source of sitosterol and its reduced forms in Prince of Wales Bay, the source of the high levels of human sewage contamination in sediments of the bay remains to be determined. It is possible to distinguish between the faeces of humans and herbivores in environmental samples

using a technique developed by Leeming *et al.*, (1997). The technique is based on a relationship between the faecal sterols coprostanol and 24-ethylcoprostanol. In human faeces the percentage of coprostanol relative to the sum of coprostanol and 24-ethylcoprostanol is 73% ± 4%. In faecal matter of herbivorous origin (five species of herbivores tested) the ratio is 38% ± 4%. Hence with a coprostanol ratio greater than 73% faecal origin may be assumed to be wholly from humans and below 38% solely from herbivorous sources (Leeming *et al.*, 1997). Relative proportions of human and herbivore faecal contribution to an environmental sample may be calculated as demonstrated in Leeming *et al.* (1997) and is based on the addition of a factor of 2.86 for every one percent below 73%.

These calculations were made for Prince of Wales Bay sediments and are presented in Table 31. The results, however, need to be treated with caution due to the possible source of 24-ethylcoprostanol derived from pulp fibre sludges mentioned above. Regardless of this fact, the coprostanol concentrations in Prince of Wales Bay sediments are so high relative to those of 24-ethylcoprostanol, that it may be assumed that the sewage contamination in Prince of Wales Bay is largely of human origin. The potential presence of 24-ethylcoprostanol derived from pulp sludge in the sediments of Prince of Wales Bay would have the effect of underestimating the human derived faecal input and overestimating the herbivore faecal input.

Table 31: $\frac{\text{Coprostanol (ug/g) x 100}}{\text{Coprostanol + 24-ethylcoprostanol (ug/g)}}$ ratios for Prince of Wales Bay sediments showing the potential major source of faecal material.

Sediment	Ratio (%)	Estimated human and herbivore faecal inputs
1	84.8%	100% human
2	69.0%	89% human / 11% other
3	73.9%	100% human
4	-	not of human or herbivore origin
4a	-	not of human or herbivore origin
4b	-	not of human or herbivore origin
5	64.9%	77% human / 23% other
6	66.2%	81% human / 19% other
7	54.6%	47% human / 53% other
8	77.8%	100% human
9	61.7%	68% human / 32% other

There are several scenarios which may explain the high levels of human sewage contamination in the sediments of Prince of Wales Bay:

- Transportation and deposition of contaminated water and solids from the Derwent estuary to Prince of Wales Bay by the estuarine salt wedge (saline bottom water) or through tidal movement.

- A history of past sewage contamination in Prince of Wales Bay prior to commissioning of the new treatment plant. Dumping of dried sewage sludge directly into the bay has been documented in the past.

- Overflows from the existing plant during periods of prolonged wet weather. Spillages from the sewage plant have also been previously documented.

- Malfunction of a pump station near the Prince of Wales Bay stormwater outfall, which in 1996 caused 1.2 megalitres of sewage (0.3% of the total annual sewage discharge from this plant) to be delivered to Prince of Wales Bay.

An unidentified homologous series of compounds was identified in all of the highly reduced sediments of Prince of Wales Bay. The first two compounds in this series are denoted by *x* and *y* in Fig. 31 and a mass spectrum of compound *x* in Fig. 31. The unknown compound *x* with a molecular ion at *m/z* 474 and an *m/z* peak at 215 is indicative of a C_{28} stanol. The mass spectrum of *x* does not however match those of 24-methyl-5α-cholestan-3β-ol (campestanol) or 4-methyl-5α-cholestan-3β-ol (4-methylcholestanol), two C_{28} stanols that characteristically elute in this region of a sterol chromatogram. The unidentified compounds were possibly products of microbial reduction in the heavily reducing environment of the Prince of Wales Bay sediments.

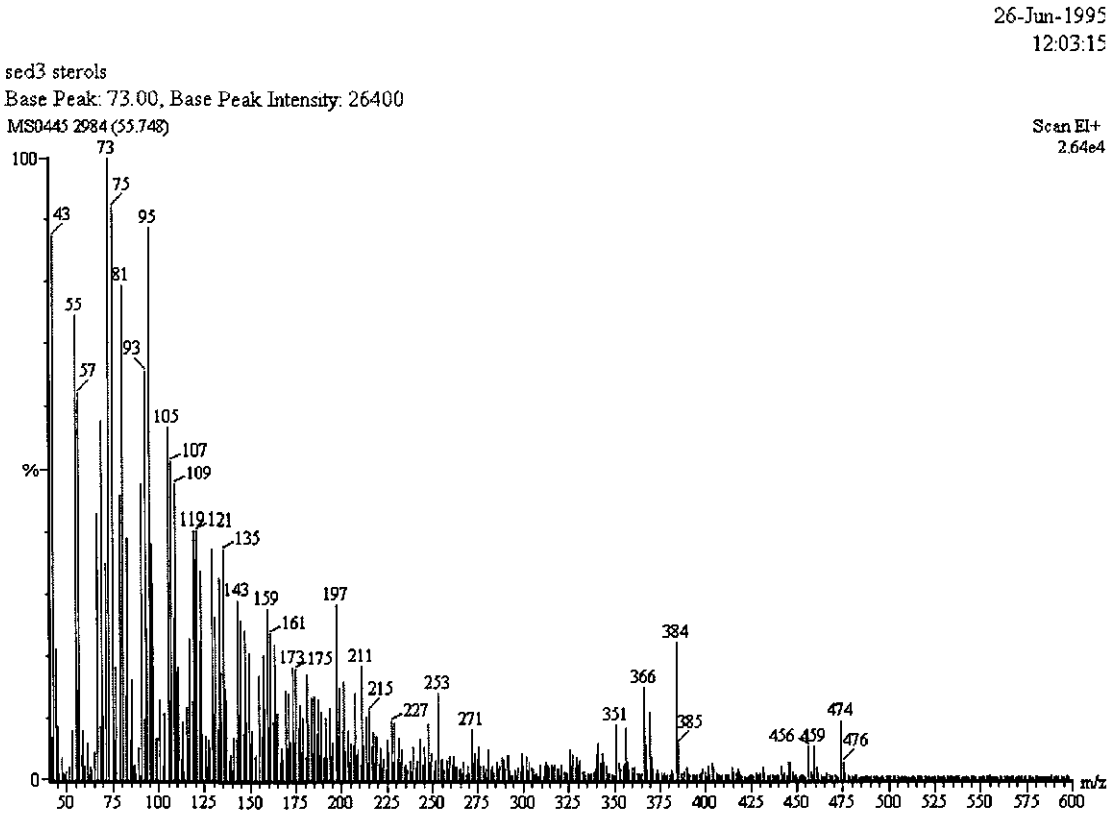


Fig. 32: Mass spectrum of unknown compound x, believed to be a C_{28} stanol. Refer to Fig. 31.

4.3.2 Sterols in sediments at other stormwater outfalls on the Derwent Estuary

Sediment sampling sites adjacent to other stormwater outfalls in the Derwent estuary are shown in Fig. 11. Sterol profiles from these sediments were largely similar to those found at the Prince of Wales Bay outfall, in that they were dominated by cholesterol and sitosterol and only low relative levels of stanols and faecal sterols (0.5 - 6.5% coprostanol)(Table 32). Despite this observation, absolute levels of coprostanol were high in some Derwent estuary sediments. At the outfalls of New Town Rivulet (6.5 μg/g) and Hobart Rivulet (4.9 μg/g)(Table 32) coprostanol concentrations recorded were higher than reported in a comprehensive study of sterols in Derwent estuary sediments which were identified as severely contaminated by sewage (Leeming & Nichols, 1996a)(Table 30).

The high coprostanol concentrations detected in some of the Derwent estuary sediments are possibly of faecal origin. However, the low concentration of coprostanol detected at Waimea stormwater outfall (Table 32) coupled with a coprostanol / cholestanol ratio of 0.2 at this site (Table 32) suggests that background levels of coprostanol may exist under localised anaerobic conditions where *in situ* formation is possible (see Nishimura, 1982). The ratio of coprostanol to cholestanol may be used as a measure of sewage contamination in organic-rich, partly anoxic sediments. At pristine sites the ratio is generally well below 0.3 and greater than 1 at sites severely contaminated with sewage (Leeming & Nichols, 1996a).

Table 32: Sterol composition of Derwent estuary sediment samples at stormwater discharge points. Refer to Fig. 11 for sample locations.

	New Town Rivulet	Cornelian Bay	Hobart Rivulet	Sandy Bay Rivulet	Lambert Ave.	Waimea Ave.	Lipscombe Ave.
Sterol	%	%	%	%	%	%	%
coprostanol	6.5	0.8	4.9	0.9	1.7	0.9	0.5
epicoprostanol							
trans-22-dehydrocholesterol	3.0	1.0		2.0	6.6	30.2	1.2
cholesterol	19.9	9.6	40.8	15.7	22.8	17.7	6.3
cholestanol	10.7	1.5	2.4	1.0	2.6	4.6	0.8
brassicasterol	7.3	4.6	5.5	3.8	5.2	24.4	2.3
24-methylenecholesterol	1.3	1.8		9.2	3.8	1.1	0.9
24-ethylcoprostanol							
campesterol	9.8	5.2	5.7	5.1	6.7	6.0	4.8
campestanol	7.4	1.7	5.0	1.6	2.2	1.7	1.4
stigmasterol	4.4	4.7	3.8	3.4	6.5	2.7	5.8
sitosterol	15.4	53.8	23.8	44.1	29.8	6.3	67.7
sitostanol	12.4	10.2	8.1	8.5	10.0	4.4	5.0
coprostanol / cholestanol	*0.61*	*0.53*	*2.04*	*0.90*	*0.65*	*0.20*	*0.63*
Sterol content (ug/g dry wt.)	**0.98**	**5.90**	**0.14**	**38.0**	**1.50**	**0.53**	**41.0**
Coprostanol content (ug/g dry wt.)	**0.06**	**0.05**	**0.01**	**0.34**	**0.03**	**0.01**	**0.20**

It was established earlier that stormwater was not a significant contributor of human faecal matter to the Derwent estuary under most conditions. The high levels of coprostanol recorded in sediments at rivulet outfalls mentioned above are likely to be a result of sewage effluent input. Sewage treatment plants are located near the mouths of both Hobart and Newtown Rivulets (Fig. 10) and it has been previously established that the bulk of particulate matter from sewage effluents settle in the vicinity of the outfalls (Leeming & Nichols, 1996a). Despite this, sewage movement may occur both upstream and downstream in the Derwent estuary depending upon the depth of sewage discharge. Bottom waters follow a salt-wedge movement upstream, whereas surface waters move predominantly downstream with river flow (Leeming & Nichols, 1996a).

4.3.3 Summary

In Prince of Wales Bay the sterol composition of sediments in the direct path of stormwater discharge was vastly different from those elsewhere in the bay. The stormwater outfall sediments contained low levels of human faecal indicators whilst elsewhere in the bay sediments were highly reduced with variable, though generally high, concentrations of coprostanol. High concentrations of particular sterols in Prince of Wales Bay were believed to be from pulp sludge, which is ubiquitously distributed in the upper and middle estuary. It was concluded that human faecal material in Prince of Wales Bay was largely not from stormwater discharge but either due to spillage from a nearby treatment plant, through transportation from elsewhere in the Derwent estuary or remnant from earlier discharge in the region with subsequent preservation in anoxic sediments.

4.4 AN ASSESSMENT OF FAECAL INPUTS TO THE DERWENT ESTUARY FROM STORMWATER & SEWAGE

Given the high bacterial levels carried by stormwater relative to sewage (section 4.2), stormwater would be expected to contribute a high proportion of the total annual bacterial load to the Derwent estuary. Moreover, stormwater discharge would constitute an intense bacterial load during periods of rainfall and catchment flushing.

It has been established from previous work (ie: Leeming & Nichols, 1996a) and this study that human faecal pollution in the Derwent estuary is, or has been extensive. From the results of this study it appears that stormwater contributes only a minor amount of human faecal pollution to the estuary, except during heavy rains or flood conditions. At these times it would appear that the stormwater conveyance system provides a means by which raw faecal effluent may bypass the sewage plants and enter the estuary untreated. In this section an estimate will be made of the total flux of faecal pollution, in terms of bacterial numbers, to the Derwent estuary from both stormwater and sewage treatment plants.

4.4.1 Sewage

Sewage effluent discharge to the Derwent estuary is in the order of 55.6 million litres daily, delivered *via* 12 treatment plants (Table 26; Fig. 10). The degree of sewage treatment ranges from macerated raw sewage to secondary treatment with chlorination (Leeming & Nichols 1996a)(Table 4). The Selfs Point treatment plant (Fig. 10) is currently being upgraded to tertiary treatment with a focus on nutrient removal and the commissioning of UV treatment of effluent to replace chlorination. It is believed that this plant will become the benchmark for future sewage treatment plant upgrades in Hobart (T. Pitman, pers. comm.). A summary of effluent discharge by treatment plant is given in Table 26. The geometric mean content of faecal coliforms and total suspended solids in effluent from each plant (1994 data) is also given.

In March 1997, effluent from the Sandy Bay sewage treatment plant (Fig. 10), which contributed over 99% of the total sewage-derived bacterial load to the Derwent

estuary at $6.8x10^6$ cfu/100 ml, $2.9x10^{14}$ faecal coliforms per day and $1.1x10^{17}$ faecal coliforms per annum (Table 26), was directed to the Selfs Point plant for treatment. The Selfs Point sewage treatment plant currently has a faecal coliform output in the vicinity of 55 cfu/100 ml (Table 26). This diversion of effluent and subsequent upgrade in sewage treatment has resulted in a massive reduction in faecal coliform discharge to the Derwent estuary from $1.1x10^{17}$ to $6.2x10^{14}$ cfu/annum.

4.4.2 Stormwater

i) Derwent Park catchment (piped). Based on results from this study, stormwater in the Derwent Park catchment carries a mean faecal coliform load of $1.3x10^4$ cfu/100 ml (based on all seasons and all flow rates) which is in excess of bacterial levels discharged by many of the sewage treatment plants. With an average annual rainfall of 626 mm in this part of Hobart, and a catchment surface which is largely impervious, then an annual runoff in the order of $3x10^9$ litres may be estimated for the Derwent Park catchment. This represents a faecal coliform discharge in the vicinity of $3.840x10^{14}$ cfu per annum or *$8.5x10^{11}$ cfu/hectare annually.*

ii) Hobart Rivulet catchment. Stormwater in the Hobart Rivulet carries on the order of 5,350 cfu/100 ml of faecal coliforms near its outfall into the Derwent estuary (based on four years of data collection from 1990-93 by Hobart City Council - n=43). With an annual runoff from the urbanised area of this catchment in the order of $3x10^9$ litres, then the total annual faecal coliform discharge from the Hobart Rivulet is calculated to be approximately $1.6x10^{14}$ cfu or *$3.4x10^{11}$ cfu/hectare annually.* These figures were based on the fact that most of the faecal contamination is generated within the built-up-area of the catchment (481 hectares or 22% of the catchment), and also assumes that runoff approaches 100% in this area and a mean annual rainfall of 626 mm.

4.4.3 Hobart total

If the Derwent Park catchment and the Hobart Rivulet are assumed to be typical of piped and open stormwater carriageways respectively in Hobart, and given that the

metropolitan area of Hobart draining to the Derwent estuary covers an area of approximately 7710 hectares, then an estimated 2.6×10^{15} - 6.5×10^{15} faecal coliforms are delivered to the Derwent estuary annually from the urban drainage system. Prior to the diversion and treatment upgrade of Sandy Bay effluent in March 1997, this represented only 2.3 - 5.8% of the estimated combined annual load of faecal coliforms (1.1×10^{17} cfu) entering the estuary. However, after the sewage system upgrade and a reduction in annual faecal coliform load to the estuary from this system to 6.2×10^{14} cfu, the contribution by stormwater to annual bacterial load to the Derwent estuary is now in the vicinity of 80-91%.

As sewage treatment in Hobart is constantly being upgraded, there will be an expected continued decrease in bacterial loads to the Derwent estuary from sewage treatment plants. Hence the relative proportion of faecal pollution that stormwater conveys to the Derwent estuary will continue to increase in the future, unless stormwater management practices are implemented.

CHAPTER FIVE

CASE STUDY: HYDROCARBONS AND STEROLS IN MARINE SEDIMENTS AND SOILS AT DAVIS STATION, ANTARCTICA

Only one who has sledged or travelled in mid-winter in the Antarctic can appreciate the luxury and sheer beauty of a modern lavatory.

Robert Dovers, 1957

This study has been published in the journal *Antarctic Science* as;

Green G.J. and Nichols, P.D. (1995). Hydrocarbons and sterols in marine sediments and soils at Davis Station, Antarctica: A survey for human derived contaminants. *Antarctic Science* 7, pp. 137-144.

5.1 ABSTRACT

A survey of hydrocarbons and sterols in marine and shoreline sediments was undertaken adjacent to Davis Station in Princess Elizabeth Land, Prydz Bay, Eastern Antarctica to determine the impact of a human settlement, including a sewage outfall, on the local marine environment. Soil samples from selected locations onshore were also analysed to ascertain the extent of hydrocarbon contamination emanating from fuel storage facilities and other potential sources. The faecal sterol coprostanol was detected at 13.2 μg/g (60% of total sterols) in sediments at the Davis sewage outfall and up to 5.0 μg/g on the shoreline at Davis Beach. These concentrations indicate significant faecal contamination. The absence of coprostanol in faeces from the local wildlife confirmed a human origin for this sewage biomarker. Hydrocarbons on the shoreline near Davis were present at up to 5.5 μg/g (dry weight of sediment). Biomarker profiles indicated an anthropogenic origin for these hydrocarbons. Onshore, degraded hydrocarbons derived from Special Antarctic distillate were found at relatively high levels in soils at the fuel storage depot (up to 220 μg/g). The source of these hydrocarbons appeared to be spillage from fuel storage tanks with possible contributions from fuel pipeline leakage and vehicle usage. Concentrations of polycyclic aromatic hydrocarbons in the soils were very low, generally below 1 ng/g (dry weight of sediments) for individual compounds.

5.2 INTRODUCTION

Davis Station (established 1957 by the Australian National Antarctic Research Expedition) is located on the shoreline of the Vestfold Hills in Princess Elizabeth Land on Prydz Bay, eastern Antarctica (68°35'S, 77°58'E). The Vestfold Hills is an ice free area of approximately 400 km^2 bounded by the Antarctic ice plateau and the Sorsdal Glacier. The proximity of Davis to unique biological communities including penguin colonies, geological, glacial and periglacial features, fjords, islands, and a unique diversity of lacustrine environments make it a key location for Australian Antarctic research. Davis Station is normally occupied over winter by 20 - 30 personnel. During summer (November - March) the population may reach 100 which includes seasonal scientific parties and summer construction personnel (Antarctic Division 1993). There are a number of locations at Davis which constitute a 'point source' contamination risk. These include the sewage treatment plant, the fuel storage depot, refueling stations and the power generation plant.

All human waste and wastewater from the new station complex at Davis receives primary and secondary treatment before discharge of the effluent through an outfall pipe into the sea close to the shoreline. The sewage treatment plant was completed in the summer of 1990/91. Prior to 1990, solid waste was disposed of onshore by combustion. The optimum population size served by the installation is approximately 60 persons (Antarctic Division 1993). There is potential for sewage contamination in Davis Bay due to mechanical breakdowns and the propensity of the system to be overtaxed in summer.

Electrical power at Davis Station is provided by four diesel motors coupled to 125 kW generators. These are fuelled by Special Antarctic Blend (SAB) distillate which, due to high consumption, constitutes the greatest potential source of hydrocarbon contamination at Davis. The storage capacity of SAB is 10^6 litres, and fuel consumption in the past four years has been between 6.6 and

$7.5x10^5$ litres per year. Other fuels used at Davis include: unleaded petrol (quikes, utilities, 2 stroke); aviation turbine kerosine (helicopters); and a range of oils and greases are also used. The use of fuels, oils and greases constitutes a risk with regard to potential direct release of hydrocarbons into the environment as well indirectly through incineration and exhaust emissions which are a source of polycyclic aromatic hydrocarbon (PAH).

Cripps (1992a) has reviewed the major marine hydrocarbon pollution incidents in the Antarctic. Although some of these incidents have caused severe localised short-term effects on biota (Kennicutt & Sweet 1992; Eppley 1992), recovery, particularly on high energy shorelines, has been shown to be rapid. Aside from the examination of major pollution events, it is only in recent years that studies have focussed on hydrocarbon and sewage pollution resulting from routine operations at Antarctic stations (Cripps 1992b; Kennicutt *et al.* 1992; Venkatesan & Mirsadeghi 1992; McFetters *et al.* 1993). The aim of this study is to identify, by use of particular hydrocarbon and sterol marker compounds, the extent of existing contamination at the 'point source' risk sites at Davis Station and to provide a basis on which future monitoring of pollutants or assessments of the state of the environment can be based. The study also includes analysis of elephant seal (*Mirounga leonina*) and Adelie penguin (*Pygoscelis adeliae*) faeces for sterols which was undertaken to more precisely elucidate the source of the faecal sterol coprostanol in the marine environment at Davis Station.

5.3 SAMPLING

Sediment samples were collected in the austral summer of 1992 - 93 to assess the levels of faecal sterols and hydrocarbons at point sources around Davis Station. Marine sediments were collected at the sewage outfall into Davis Bay, in a transect to 200 m from the outfall, and at various other locations in the bay and along the shoreline (Fig. 33). The shallow water depth of the nearshore environment at Davis enabled marine sediments to be collected manually with a scoop. A sample of sewage effluent was taken directly from the outlet pipe of the treatment plant. Surface soil samples were collected at the fuel storage depot and at 50 m intervals downslope towards the coast (Fig. 33). The collection area was downwind from the generator plant in order to detect the presence of any combustion derived hydrocarbons in the environment. All samples were stored at -20°C and returned to Australia for analysis.

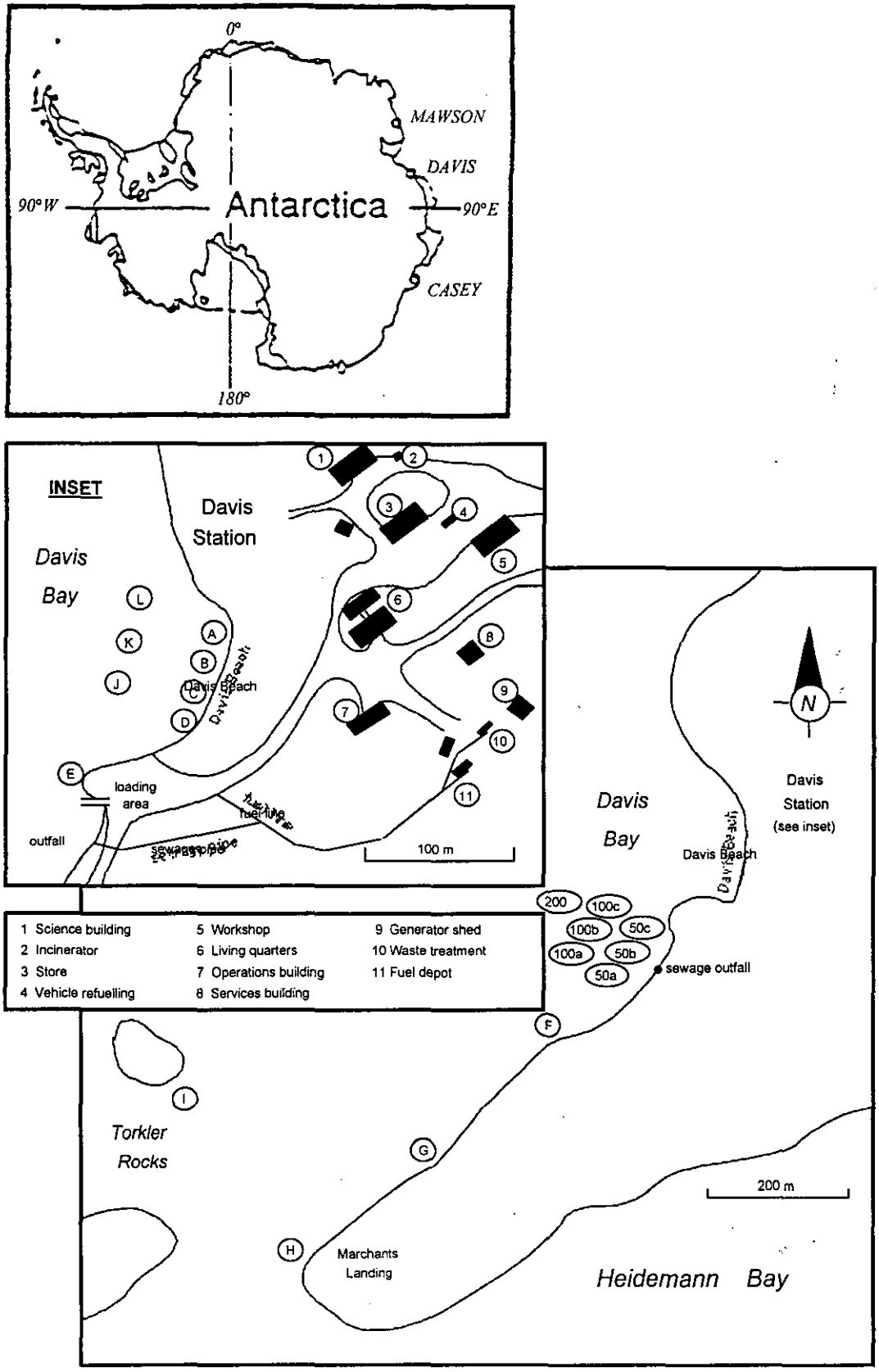


Fig. 33: Map of Davis Station and local area showing sampling locations

5.4 STEROLS

A selection of samples and sediments from near Davis Station have been analysed for sterols primarily to determine the extent of sewage pollution emanating from the outfall into the bay. Sterols such as coprostanol, epi-coprostanol and 5β-ethylcoprostanol can be used as indicators of sewage pollution. The presence of these sterols at different relative amounts can be diagnostic in differentiating various sources of mammalian faecal waste (Venkatesan & Santiago 1989). There are three documented sources of coprostanol and epicoprostanol in the Antarctic marine environment: i) human faeces (e.g. this study and Venkatesan & Mersadeghi 1992); ii) marine mammalian faeces, particularly whales, (Venkatesan & Santiago 1989); iii) *in situ* formation in reducing environments such as anoxic bottom waters of fjords (Green *et al.* 1992; Nishimura 1982). In addition to coprostanol and epi-coprostanol, other sterols can be used to fingerprint the presence of detrital material from zooplankton and various classes of phytoplankton in sediments.

The highest concentrations of total sterols in sediments were at Davis Beach (up to 119 μg/g; Table 33). The lowest detected concentration of sterols (0.46 μg/g; Table 34) was at site I, the most distant sample location, 1 km southwest of Davis Station. The sterol content was much higher than concentrations found previously in Antarctica, for example; Bransfield Strait, 0.15 - 0.42 μg/g (Venkatesan *et al.* 1986); Mc Murdo Sound, 0.40 - 8.0 μg/g (Venkatesan 1988a); Vestfold Hills, 1.0 - 16.2 μg/g (Skerratt 1992).

There were two distinct types of sterol profiles. The first was dominated by coprostanol and/or cholesterol (Table 33; Fig. 34a) and were found in sewage outfall sediments, shoreline sediments and faeces from wildlife. The second was found in sediments from Davis Bay and was dominated by algal sterols (Table 34; Fig. 34b).

Table 33: Sterol content and composition of Davis sewer effluent, Davis shoreline sediments and local wildlife.

Sterol	Common name	Peak umber	Sewage tank	Sewage outfall	*Sample sites* A	B	C	D	E	Elephant seal faeces	delie penguin faeces
Composition (% of total sterols)											
5b-cholestan-3b-ol	*coprostanol*	2	60.5	60.3	6.3	3.9	4.2	0.5	4.0		
5b-cholestan-3a-ol	*epicoprostanol*	3	3.7	1.8							
cholest-5-en-3b-ol	*cholesterol*	5	18.1	13.6	92.3	94.1	95.1	97.0	92.5	> 99	> 99
5a-cholestan-3b-ol	*cholestanol*	6	2.7	4.1	1.4	1.9	0.7	2.5	3.6	tr	
5b-ethylcholestan-3b-ol	*5b-ethylcoprostanol*	9	12.4	14.6							
24-ethylcholest-5-en-3b-ol	*24-ethylcholesterol*	10	2.6	2.6							
Others				3.0							
Total sterol content (ug/g)**			*1 926 (ug/l)*	*21.8*	*32.8*	*52.2*	*119*	*43.6*	*3.80*	*3 900*	
Coprostanol content (ug/g)**			*1 166 (ug/l)*	*13.2*	*2.06*	*2.04*	*4.98*	*0.22*	*0.15*		

** Peak numbers refer to Figure 3.*

*** Dry weight of sediment*

Table 34: Sterol content and composition of Davis Bay sediment samples

Sterol	Common name	Peak number*	Sample location													
			50a	50b	50c	100a	100b	100c	200	F	G	H	I	J	K	L
Composition (% of total sterols)																
24-norcholesta-5, 22E-dien3b-ol	*24-norcholesterol*	1								1.2	2.1	5.4	*tr*	*tr*	1.2	1.2
5b-cholestan-3b-ol	*coprostanol*	2	2.7	1.8	3.3	3.1	1.4	4.6	3.6	*tr*	*tr*	0.3		2.9	4.7	1.5
5b-cholestan-3a-ol	*epicoprostanol*	3	*tr*			*tr*	*tr*	*tr*	*tr*							
cholesta-5, 22E-dien-3b-ol	*trans-22-dehydrocholesterol*	4	13.9	14.8	14.5	33.6	27.6	27.9	21.2	24.3	13.3	22.8	10.5	13.8	13.1	15.3
5a-cholest-22E-en-3b-ol	*trans-22-dehydrocholestanol*		5.3	2.7	4.1	6.7	5.2	7.3	4.7	1.5	2.2	1.0	4.4	7.9	8.6	6.1
cholest-5-en-3b-ol	*cholesterol*	5	31.2	30.8	23.1	8.2	8.0	7.1	10.9	24.8	37.9	36.0	24.0	11.1	13.2	10.8
5a-cholestan-3b-ol	*cholestanol*	6	9.6	6.2	9.1	5.1	4.4	4.7	5.4	9.1	8.3	2.6	11.1	9.7	10.3	10.6
24-methylcholesta-5, 22E-dien-3b-ol	*brassicasterol*	7	*tr*	*tr*	2.4	6.4	4.1	3.1	2.6	4.2	5.1	8.3	10.6	6.1	6.2	6.9
24-methyl-5a-cholest-22E-en-3b-ol	*brassicastanol*		7.6	9.4	4.7	6.6	5.0	5.8	8.0							
24-methylcholesta-5, 24,28-dien-3b-ol	*24-methylenecholesterol*	8								6.2	7.0	12.0	9.1	7.1	5.4	5.2
23,24-dimethylcholesta-5,22E-dien-3b-ol			4.7	6.7	9.8	22.6	20.7	13.7	8.6	4.6	2.1	1.1		11.3	10.6	13.7
24-ethylcholest-5-en-3b-ol	*24-ethylcholesterol*	10	10.3	11.5	12.2	2.4	2.1	3.8	6.7	3.5	5.8	4.0	16.6	12.9	9.7	9.7
4, 23, 24-trimethylcholest-22E-en-3b-ol	*dinosterol*	11	3.6	2.3	3.1	1.9	1.6	1.6	2.0	0.8	1.6	0.8	*tr*	2.9	2.2	2.6
Others			11.1	13.9	13.8	3.2	19.9	20.5	26.4	19.8	14.7	5.8	13.8	14.3	14.8	16.5
Total sterol content (ug/g)**			*2.08*	*2.68*	*1.94*	*28.6*	*28.4*	*6.48*	*2.42*	*12.3*	*6.10*	*14.6*	*0.46*	*3.06*	*9.32*	*4.16*
Coprostanol content (ug/g)**			*0.06*	*0.04*	*0.06*	*0.88*	*0.04*	*0.30*	*0.09*	*tr*	*tr*	*0.04*		*0.08*	*0.44*	*0.06*

** Peak numbers refer to Figure 3.*

*** Dry weight of sediment*

tr - trace amount (near detection limits)

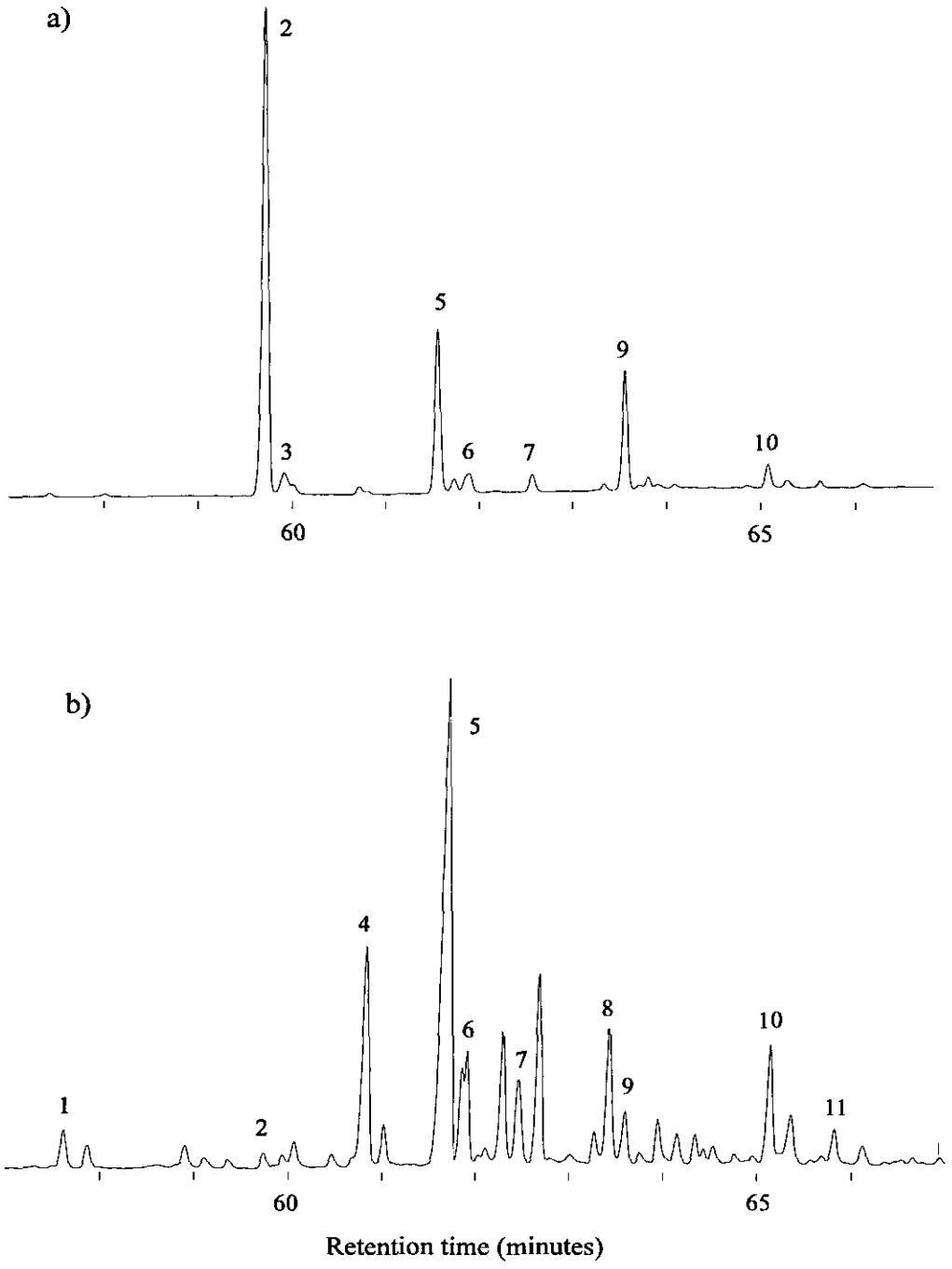


Fig. 34: Partial gas chromatograms (TMSi derivatised sterols) showing: a) sterol profile from Davis shoreline sewer outfall sediment sample and; b) sterol profile from Davis Bay sediment sample G. Key to sterols: **1**. 24-norcholesterol; **2**. coprostanol; **3**. epicoprostanol; **4**. trans-22-dehydrocholesterol; **5**. cholesterol; **6**. cholestanol; **7**. brassicasterol; **8**. 24-methylenecholesterol; **9**. 24-ethylcoprostanol; **10**. 24-ethylcholesterol; **11**. dinosterol.

5.4.1 Davis Shoreline Sites

The sterol composition of sediment at the Davis sewer outfall was similar to that of the sewage digester tank (Table 33). The faecal sterol coprostanol was dominant [13.2 μg/g (dry weight) and 60.3% of the total sterols]. The co-occurrence of epicoprostanol (1.8%) at the Davis sewer outfall was typical of the amount found in human faecal waste. The sterol 24-ethylcoprostanol (14.6%; Table 33) was also present in human sewage. The concentration of coprostanol in sewage sludge is typically 1000 μg/g, and in grossly contaminated sediment 9 μg/g (Nichols & Leeming 1991). The concentration of coprostanol found in sediments in this study was much lower than that found at the McMurdo sewage outfall (up to 3 000 μg/g, Venkatesan & Mersadeghi 1992) which represents the impact of raw sewage effluent from about 1 000 people. Compared to McMurdo Station, the disposal of sewage at Davis Station has only occurred since 1991 prior to which it was burnt.

Coprostanol was present at high concentrations at the shoreline locations at Davis, in particular at site C (5.0 μg/g, Table 33). As coprostanol was not be detected in elephant seal (*Mirounga leonina*) faeces or Adelie penguin (*Pygoscelis adeliae*) faeces, the presence of this stanol on the Davis Station shoreline, and at sites up to 1 km from the station, is due solely to human contamination.

At sites A to E from Davis Beach, cholesterol was the dominant sterol detected (92 - 97% of total sterols, Table 33). The cholesterol in the shoreline sediments probably originated from the faeces of elephant seals and Adelie penguins. This is supported by analysis of faeces from these animals, both of which have a sterol composition consisting almost entirely of cholesterol (> 99%)(Table 33).

5.4.2 Davis Bay Sediments

The sterol profiles in Davis Bay sediments were dominated by cholesterol (7 - 38% of total sterols), trans-dehydrocholesterol (10 - 34%), 23,24-dimethylcholesta-5,22E-dien-3β-ol (0 - 23%), 24-ethylcholesterol (2 - 17%) and cholestanol (3 - 11%; Table 34; Figure 34b). This sterol profile indicated input primarily from algae. The sterols 24-norcholesterol, dehydrocholesterol, cholesterol, brassicasterol, 24-methylenecholesterol, 24-ethylcholesterol and dinosterol have all been reported as components in Antarctic sea-ice diatoms (Nichols *et al.* 1989). The small amount of dinosterol in all samples probably originated from dinoflagellates. Coprostanol was detected in 13 of 14 sediments collected from Davis Bay indicating that transport and dilution of sewage effluent has occurred away from the source. The highest concentrations of coprostanol in the bay (0.30 - 0.88 μg/g) were found at sites that were 100 - 200 m distant from the sewage outfall (Table 34). The co-occurrence of coprostanol with trace amounts of epi-coprostanol suggests that anthropogenic sewage contamination is extending at least to 200 m offshore into Davis Bay.

5.5 ALIPHATIC HYDROCARBONS

The aliphatic hydrocarbon content of sediments from Davis Bay were in the range 0.25-9.0 μg/g (Table 36). These concentrations were similar to those previously reported for *n*-alkanes in Antarctic marine surface sediments (0.06-9.3 μg/g; Cripps 1992a) and were lower than a very localised and high level of contamination found adjacent to the wharf at McMurdo Sound (4500 μg/g; Lenihan *et al.* 1990). In a study of hydrocarbon contamination at Signy Station, South Orkney Islands, Cripps (1992b) found levels of *n*-alkanes at 0.04 - 1.7 μg/g in marine surface sediments.

There were also differences noted in the aliphatic hydrocarbon profiles. As for the sterols, the shoreline sediments contained compounds attributable to human activity and sediments from Davis Bay were dominated by biogenic compounds.

5.5.1 Hydrocarbons From Shoreline Sediment

The *n*-alkane series from the Davis shoreline sediments showed a maximum at *n*-C_{17} (Fig. 35a). The *n*-alkane series and presence of the isoprenoid hydrocarbons pristane and phytane in all shoreline sediments was characteristic of a degraded light fuel.

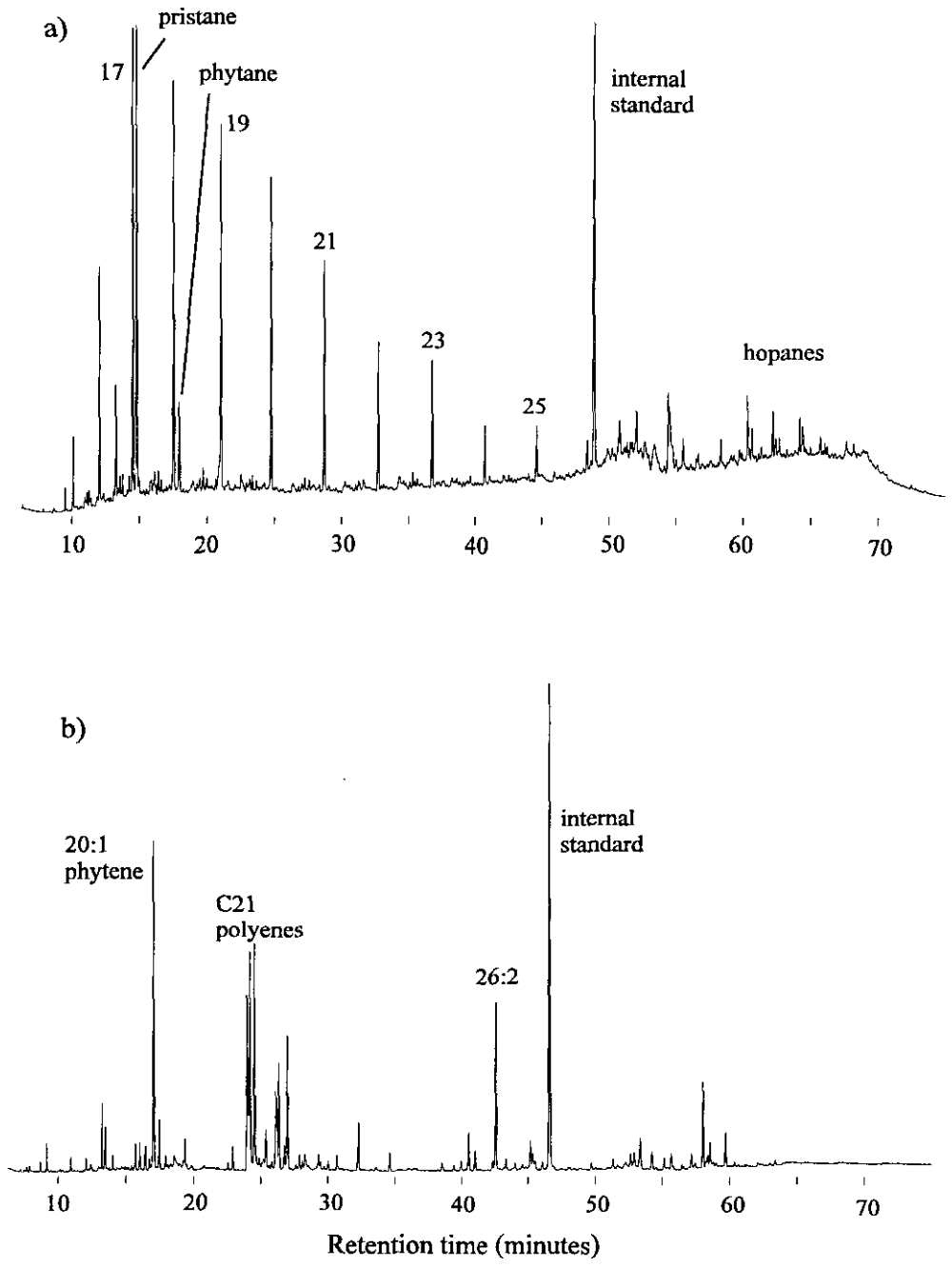


Fig. 35: Partial gas chromatograms showing: **a**. representative aliphatic hydrocarbon profile from Davis Beach sediment sample E and; **b.** representative aliphatic hydrocarbon profile from Davis Bay sediment sample L. Numbers adjacent to peaks indicate alkane carbon chain length.

The hydrocarbons from shoreline sediment, though at low levels, were probably derived from SAB (Special Antarctic Blend) fuel. SAB is composed almost entirely of low molecular weight hydrocarbons, nC$_8$-nC$_{16}$ alkanes. Loss of the *n*-alkanes nC$_8$-nC$_{15}$ (a major proportion of SAB fuel) from sediment adjacent to the fuel storage depot demonstrated, in the short term, this fraction of the fuel is almost completely evaporated, even under conditions of extreme cold. A previous study of the degradation of SAB fuel in Antarctica (Green 1992) showed a similar result and demonstrated loss of short-chain hydrocarbons by volatilisation within 30 days.

The pristane to phytane ratio in SAB for fuel residues found in soils and sediments at Davis Station was between 3.5 and 6.7 compared to 15 for SAB fuel. This change may be due to preferential degradation of pristane by physical/abiotic processes as previously found in marine sediments (see Barrick *et al.* 1980).

The presence of trace amounts of hopanes in the shoreline sediments was further evidence that petroleum contamination has occurred at these locations. Hopanes are ubiquitous constituents of crude oil and ancient rock and from these sources occur in the thermodynamically stable forms (Barrick & Hedges 1981). Hopanes were identified by the base peak ion (*m/z* 191) in the mass spectra of these cyclic hydrocarbons (*e.g.* Jones *et al.* 1986). Hopanes and steranes are used as crude oil markers as they are resistant to weathering and bacterial oxidation. The hopane distribution was similar to that of a widely used lubricating oil (Volkman *et al.* 1992).

The source of the hopane series in shoreline sediments at Davis was probably SAB fuel which contains a similar hopane series. In both sediments and SAB fuel, the hopane trace (*m/z* 191) is weak, and steranes are not detected. This indicates that the source of the hopanes was a refined product containing very few residual hopanes. The absence of a significant unresolved complex mixture

(UCM) of hydrocarbons in chromatograms from shoreline sediments suggested that a light fuel, rather than an oil, was the source of the hopanes.

The hydrocarbons detected in shoreline sediments may be derived from land runoff or from small spills during ship to shore fuel transfer. Depending upon sea ice conditions, fuel is transferred ashore either by pipeline or by barge. In recent years the pipeline and its couplings deteriorated to the point where it leaked fuel on most occasions when it was used. Other potential contamination sources were vehicles used for cargo loading and unloading operations, periodic discharge of effluent containing petroleum from the wastewater system and leaching from fuel spills onshore.

The aliphatic hydrocarbon profiles indicated that the discharge from the sewerage system was not the predominant source of the hydrocarbons on the shoreline at Davis. The aliphatic hydrocarbon distribution for sewage effluent showed the residue of a light fuel. Also present were nC$_{22}$-nC$_{32}$ alkanes associated with a small UCM which was indicative of the presence of fuel oil in the effluent. Squalene, which can be a major component in sewage effluent and is believed to derive from skin lipids, was the most dominant hydrocarbon in the Davis Station sewage effluent (31.5%; Table 35). The maximum level of squalene was 37.7% in sediment at the sewage outfall, and was present at two locations on Davis Beach at 23.7% (Table 35). Squalene was also found in nearshore sediments (trace - 2.2%; Table 36).

Table 35: Hydrocarbon content and composition of Davis sewer effluent and Davis shoreline sediments.

	sample						
	sewage tank	sewage outfall	A	B	C	D	E
Hydrocarbon content (ug/g	*370 ug/l*	*5.46 ug/g**	*3.24*	*1.10*	*2.69*	*1.00*	*1.49*
Hydrocarbon composition (%)							
n-alkanes	31.8	30.2	69.0	32.6	30.0	78.9	65.9
linear alkyl benzenes (LABs)	11.3	9.4					
pristane	0.5	3.9	9.2	5.7	8.2	7.9	12.2
phytane	*tr*	*tr*	1.8	2.1	2.2	3.4	2.9
21:2 alkene			0.9	1.3	6.9	*tr*	
squalene	31.5	37.7		23.7	23.7		
norhopanes	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*
others	24.9	18.8	19.1	34.6	29.0	9.8	19.0

** Dry weight of sediment*
tr - trace amount

Table 36: Hydrocarbon content and composition of Davis Bay sediments

	sample													
	50a	50b	50c	100a	100b	100c	200	F	G	H	I	J	K	L
*Hydrocarbon content (ug/g)**	*0.26*	*0.39*	*0.38*	*9.04*	*10.1*	*5.28*	*1.64*	*2.02*	*0.46*	*0.61*	*0.25*	*4.03*	*6.44*	*2.51*
Hydrocarbon composition (%)														
n-alkanes	24.1	23.3	16.5	*tr*	*tr*	*tr*	*tr*	8.6	16.2	14.7	9.5	7.8	5.4	8.4
20:1 phytene	1.2	0.7	2.7	3.4	5.5	7.5	4.7	0.9	2.5	0.9	2.4	19.7	34.9	12.5
polyenes (C21)	47.1	40.9	32.2	67.8	74.8	69.5	62.8	78.1	57.4	63.1	55.2	27.2	17.8	42.3
26:2 alkene	5.1	3.7	14.6	1.4	1.8	2.2	5.9	1.9	3.1	5.8	3.2	5.7	4.9	7.9
squalene				*tr*	*tr*	1.6	1.7					1.8	1.5	2.2
mono aromatic steroid (C29)				1.4	1.2	2.8	2.9					2.8	1.6	4.0
others	22.5	31.4	34.0	26.0	16.7	16.4	22.0	10.5	20.8	15.5	29.7	34.8	33.9	22.7

** Dry weight of sediment*
tr - trace amount

Linear alkyl benzenes (LABs with C_{11} to C_{14} side chains) derived from sewage effluent at Davis, were detected as a component of the aliphatic hydrocarbon fraction in sediment at the sewer outfall site (Σ LABs 9.4%; Table 35). LABs have been previously reported in sewage effluent at Davis Station (31% of hydrocarbons; Green *et al.* 1992). LABs are manufactured during the production of the linear alkylbenzenesulphonate surfactants used in commercial detergents

(Eganhouse *et al.* 1983). Their appearance in wastes results from incomplete sulphonation of the LABs and subsequent carry over in detergents. The ability of LABs to be preserved in the marine environment for up to 20 years (Eganhouse *et al.* 1983) means they are potentially good tracers for domestic and industrial waste in the marine environment. In Davis Bay, LABs are below detection at all sites away from the sewage outfall.

5.5.2 Hydrocarbons From Davis Fuel Storage Depot

Compared to sediments from the marine environment, relatively high concentrations of *n*-alkanes were detected at the Davis fuel depot (87 - 220 μg/g; Table 37). These levels were however lower than concentrations found at Signy Station, South Orkney Islands in soils heavily loaded with diesel (1220 μg/g; Cripps 1992b). A study by Kennicutt *et al.* (1992) also reported high concentrations of *n*-alkanes at 4.2 - 2300 μg/g in contaminated soils at Palmer Station on the Antarctic Peninsula. Along a transect (350 m to the south-west) from the Davis fuel depot (Fig. 33), hydrocarbon concentrations in soils were comparatively low (0.24 - 1.20 μg/g; Table 37) and similar in composition to those detected on the Davis shoreline (Table 35). The source of the contamination was probably from spillage or leakage from the fuel depot, use of SAB fuel at the station or from leaks in the fuel pipeline. The spread of these hydrocarbons throughout the station soils is probably by meltwater flow during the summer months.

Table 37: Hydrocarbons in Davis soil samples

Sample	Hydrocarbons (ug/g)	Most abundant n-alkane
Fuel depot 1	219	C 17
Fuel depot 2	87	C 16
100 m	0.74	C 18
150 m	0.80	C 18
200 m	0.92	C 18
250 m	1.20	C 20
300 m	1.22	C 20
350 m	0.24	C 18

Alkane chromatogram profiles from soils at Davis showed a trend to a greater degree of hydrocarbon weathering (loss of low molecular weight compounds) with increasing distance from the fuel depot. The dominant *n*-alkanes in soils at the depot were nC$_{16}$-nC$_{17}$, whilst towards the coast nC$_{18}$-nC$_{20}$ were predominant (Table 37).

5.5.3 Hydrocarbons in Davis Bay Sediments

The dominance of nC$_{21}$ polyenes in sediments from Davis Bay (Table 36; Fig. 35b) was indicative of micro-algal input. Odd-carbon numbered alkenes, particularly nC$_{21:6}$ (heneicosahexaene), which is derived from the corresponding docohexaenoic acid, predominate in marine phytoplankton species (Blumer *et al.* 1971). This alkene has previously been found as a major component in Antarctic sea-ice diatoms (Nichols *et al.* 1989).

Other alkenes detected in Davis Bay sediments included a phytene, a C$_{26}$ diene and trace concentrations of C$_{25}$ isoprenoid alkenes. Phyt-1-ene was detected in most sediments from Davis Bay including at up to 35% of total hydrocarbons at site K (Table 32). Phytenes are produced from bacterial and chemical degradation of naturally occurring lipids (Volkman *et al.* 1992). The C$_{26}$ diene was detected in all sediments collected from Davis Bay at concentrations between 1.4-14.6% of total alkanes (Table 36). This finding was unusual as, with the exception of nC$_{21:6}$, alkene hydrocarbons reported from algal lipids, are generally monounsaturated and fall within the chain-length range C$_{15}$-C$_{21}$ (Volkman *et al.* 1980). At the present time, the precise source of the C$_{26}$ diene is

not known. However the hydrocarbon and sterol profiles observed suggest marine algae.

5.6 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) are generally considered indicators of contamination from anthropogenic sources. Aside from minor contributions from bacteria and microalgae, PAHs are not known to be synthesised by organisms (Saliot 1981). The presence of PAHs may be attributed to both petrogenic and pyrogenic sources. Domination of unsubstituted compounds over their alkyl-substituted derivatives suggests a pyrogenic origin for PAHs. A petroleum source would be favoured when substituted derivatives dominate (Kayal & Connell 1989). As many PAHs are potential carcinogens and mutagens, there is concern about their occurrence in the environment (Smith 1990).

PAHs were present at Davis both in soils and marine sediments. The PAHs consisted primarily of naphthalenes, fluorenes and phenanthrenes which are present in trace amounts in SAB fuel. Kennicutt *et al.* (1992) found the same PAHs derived from diesel fuel as major contaminants at Palmer Station on the Antarctic Peninsula. The concentrations of PAHs in the environment at Davis were very low and close to the limits of detection. Only at the fuel depot and sewage outfall did the concentration of individual PAHs exceed 1 ng/g. This was in contrast to the findings of Kennicutt *et al.* (1992) who detected up to 51.3 μg/g of naphthalenes in soils at Palmer Station.

The major combustion derived PAHs detected in the environment at Davis were benzofluoranthene, fluoranthene, pyrene, benzoanthracene and indenopyrene. These PAHs were also present in sub ng/g amounts. The findings indicated that there was no serious contamination from these compounds in soils and sediments sampled at Davis Station. PAHs produced by fuel combustion at Davis appear to be well dispersed by aeolian and hydrological processes.

5.7 CONCLUSIONS

Analysis of coprostanol in sediments, beach sands, and seal and penguin faeces in Davis Bay indicate that human sewage contamination has accumulated to relatively high levels near the sewage outfall. The concentrations of coprostanol were, however, significantly lower than in sediments in an environment receiving untreated sewage from McMurdo Station. This comparison demonstrated the effectiveness of secondary treatment of effluent in Antarctica in reducing marine contamination. Coprostanol was detected at shoreline sites up to 1 km from the sewage outfall. The results of this study establish a baseline from which to gauge any future accumulation of sewage derived material in the marine environment at Davis Station.

Anthropogenically-derived hydrocarbons were present in soils and the marine environment at Davis, however, levels were lower, particularly for PAH, than in reports of contaminated soils in studies from elsewhere in Antarctica. This finding suggested that the Special Antarctic Blend refined fuel used at Davis contains significantly lower levels of PAHs than fuels used in some other areas of Antarctica, and hence is more suitable for use than other fuels in the sensitive and nearly pristine environment of Antarctica.

Measures are currently in place to address the contamination risk from the fuel storage depot at Davis. Containment structures which will hold fuel in the event of a spill were scheduled for installation in 1995. Improved valve functions as well as spill and leak detection facilities are also proposed (Antarctic Division 1993). The pipeline used for ship to shore fuel transfer has recently been repaired, and its condition is now monitored (Antarctic Division 1993). The implementation of management strategies and particularly the installation of containment bunding for fuel storage facilities can only help to minimise possible future contamination at Davis Station.

5.7.1 *Significance of the Davis Station environmental study in terms of the broader PhD study.*

Despite the remote location and low human population at Davis Station, there were some parallel findings in hydrocarbon and sewage impacts to those encountered at Hobart and the Derwent estuary. Despite no stormwater infrastructure at Davis, land run-off of hydrocarbons was demonstrated to have a localised impact along the adjacent shoreline with little evident wider impact on the marine environment. Although the Derwent estuary is known to be impacted widely by hydrocarbon contamination in sediments (Volkman *et al.*, 1988; Volkman *et al.*, 1989), the major hydrocarbon pollution impacts are also close to urban runoff outfall points. Additionally, urban runoff is thought to be the major contributor of hydrocarbons to the Derwent estuary.

The impact of human-derived sewage material at Davis was readily identifiable. This was facilitated by the absence of coprostanol, and distinctive sterol compositions, in the faeces of local wildlife and the low diversity of potential faecal inputs to the near-shore environment. Human faecal material at Davis was present in most samples collected, even up to 1 km from the Station. These findings demonstrated that in a system with low population and low output of sewage effluent that there is a measurable impact on the wider marine environment, not just near effluent outfall points. This finding demonstrates the potential magnitude of sewage dispersal and impact from a large populated centre such as Hobart, where sewage effluent discharges are enormously higher than at Davis.

CHAPTER SIX

CONCLUSIONS

Of making many books there is no end; and much study is weariness of the flesh. Let us hear the conclusion of the whole matter: for God shall bring every work into judgement, with every secret thing, whether it be good, or whether it be evil.

Ecclesiastes 12:12-14

6.1 CONCLUSIONS

In this study organic chemical markers (sterols, selected aliphatic hydrocarbons and PAHs), and bacterial markers, were used in conjunction to successfully trace sources of contamination in Hobart stormwater catchments. Hydrocarbon markers demonstrated that locally severe contamination of receiving sediments in the Derwent estuary has occurred as a result of a long history of unregulated and untreated stormwater discharge. Faecal sterol and bacterial markers demonstrated that the effects of stormwater discharge are more than just 'local', and have a widespread effect on the Derwent estuary.

In order to determine hydrocarbon load and sources in urban runoff to the Derwent Estuary, the content and composition of hydrocarbons in stormwater samples from several catchments in the Hobart urban area has been investigated. The mean concentration of hydrocarbons in Hobart stormwater, measured at the Prince of Wales Bay outfall, was 2.88 mg/l with an estimated total annual discharge to the Derwent Estuary of 164,000 kg/year. Based on these findings for Hobart, and assuming comparable hydrocarbon inputs to other coastal and estuarine environments in Australia, it is estimated that 14,900 tonnes of hydrocarbons are discharged annually into Australian coastal waters from urban runoff. This is higher than a previous estimate of 5,000 tonnes/annum (Volkman *et al.*, 1994). This study provides the first assessment of hydrocarbon discharge from stormwater to Australian coastal water based on locally collected data.

Assessment of the hydrocarbon input budget for the Derwent estuary showed an estimated annual input of between 245 and 293 tonnes. This assessment did not take into account potential inputs from tanker operations, runoff from factory sites or from boating and shipping activities, all of which were difficult to quantify.
Stormwater discharge was estimated as the largest contributor of hydrocarbons to the Derwent estuary annually (164 tonnes), followed by sewage effluent (58-84 tonnes), industrial effluent (18-31 tonnes) and atmospheric deposition (5-14 tonnes). Figures for sewage effluent and atmospheric deposition were not based on locally collected data.

Prior to this study there was a great deal of contention as to the extent to which stormwater drains contributed to bacterial contamination in receiving waterways. Based on numbers of faecal bacterial indicators, Hobart's stormwater was shown to carry very high levels of bacterial contamination. Bacterial numbers were often greater than observed for effluent discharged from sewage treatment plants. Continual upgrade of sewage effluent treatment in Hobart has lead to a rise in the proportion of bacterial contamination contributed to the Derwent estuary from the stormwater system. The current contribution of stormwater to estuarine bacterial contamination on an annual basis was estimated to be in the vicinity of 80-91%. During wet weather, in particular during flood conditions, the bacterial load in stormwaters result in considerable health risk to humans in estuarine receiving waters.

Source elucidation of hydrocarbons by use of GC-FID chromatograms and analysis of chemical marker compounds and ratios demonstrated inputs to stormwater from automotive oils, diesel fuel, and plant waxes. Automotive oils were the highest single contributor of hydrocarbons to stormwater (81-96%), as determined by GC-FID chromatograms, in all catchments studied. Analysis of PAH profiles by GC-MS and multivariate analysis suggested that automobile sump oil, rather than unused lubricating oils, were the major component of the oil in stormwater (often greater than 90%). Additionally, analysis of PAH profiles suggests that combustion products, including vehicle exhaust, are a major contributor of hydrocarbons to stormwater in two of the four Hobart catchments studied.

From previous studies of hydrocarbons in stormwater, a diverse range of sources have been determined (Eganhouse *et al.*,1981; Hoffman *et al.*, 1984; Webber, 1983 & 1986; Marsalek, 1990; Bomboi & Hernandez, 1991), however, sump oil is the most commonly detected predominant source (Hoffman *et al.*, 1982 & 1983; Fam *et al.*, 1987; Latimer *et al.*, 1990). This study has confirmed the variability in stormwater hydrocarbon input between sites and stresses the importance of further studies to raise awareness of local pollution characteristics. Additionally, it was demonstrated that samples collected from individual storm events tended to group together in multivariate cluster analysis based on PAH composition. This suggested that local catchment practices and seasonal

influences potentially also play a role in the type of hydrocarbons discharged from stormwater systems.

There have been relatively few studies aimed at source discrimination, using sterol profiles, of faecal material in urban stormwater catchments. Previous evidence suggested a significant input of faecal contamination to urban drainage systems from animals (Geldreich *et al.* 1968; Gannon & Busse, 1989; Melbourne Water, 1993). This study adds further evidence to this claim by demonstrating, through comparison of sterol profiles and by analysis of sterol and bacterial composition, that dog faeces are potentially a major contributor to faecal pollution in urban runoff in Hobart. There was no evidence of significant faecal input by cats or rodents to stormwater in Hobart.

The findings of this study that implicate dog faeces as a major contributor to faecal contamination in Hobart's stormwater are: a similarity in the sterol profiles of dog faeces and stormwater samples; low levels or absence of sterol markers for other sources of faeces; high bacterial levels bearing no correlation to coprostanol levels; the estimated deposition of up to 10 tonnes of dog faeces in Hobart every day; and the observed similarity between faecal coliform and *C. perfringens* counts in stormwater samples.

Human faecal material was detected in urban stormwater in this study; its presence, particularly during heavy rain, was assumed to be due to cross contamination from the sewerage system. This conclusion was drawn from the observation that bacterial loads increased in accordance with coprostanol levels during flood conditions, a time when cross contamination between the two conveyance systems is believed to be common in Australian cities. It is believed that ineffective septic tanks are not a significant contributor to bacterial pollution in urban waterways in Hobart. This observation was based on the fact that the use of septic tanks is very uncommon in the Hobart urban area.

At dry weather flow, low levels of coprostanol present in stormwater samples demonstrated the presence of sewage connections to the stormwater system. The presence of human faecal material, however, did not account for the very high bacterial levels in stormwater during low flow. It appeared likely that *in situ* bacterial production

from faeces deposited in drains during wet weather could be a contributor to the high bacterial loads during dry weather even though storm drain sediments contained only low faecal coliform counts.

In sheltered embayments of the Derwent Estuary a clear link was demonstrated between urban stormwater contamination and the build-up of hydrocarbon contaminants in sediments. Localised extreme hydrocarbon concentrations were found associated with stormwater discharge and boat mooring areas. Aliphatic hydrocarbons (10,100 μg/g) and PAHs (27 μg/g) in sediments at Prince of Wales Bay were the highest yet recorded levels for estuarine sediments in Australia. If contaminants continue to be generated at current rates in urban catchments, then the rate of sediment contamination in sheltered parts of the Derwent Estuary can only increase. This may lead to a potential increase in the extent of the affected areas in adjacent estuarine and coastal areas.

It was established from Derwent estuary sediments collected at stormwater outfalls that stormwater was not a significant contributor of *human faecal matter* to the Derwent estuary under most conditions. This was particularly noticeable at Prince of Wales Bay where a large difference was observed in the sterol composition of sediments in the direct path of stormwater discharge to those elsewhere in the bay. The outfall sediments contained comparatively low levels of human faecal indicators, whilst elsewhere in Prince of Wales Bay sediment sterol profiles were indicative of a highly reduced environment with variable, though generally high, levels of coprostanol. It was concluded that human faecal material in Prince of Wales Bay was largely not from stormwater discharge but either due to past dumping of sewage sludge, spillage from a nearby treatment plant or through transportation of contaminated sediments and effluents from elsewhere in the Derwent estuary.

This study has provided information that will assist in a more holistic understanding of the link between catchment activities and the type, extent and source of faecal pollution in the Derwent estuary. Although it was beyond the scope of the study to determine faecal inputs from the rural areas of the upper Derwent catchment, the work has built on and complimented previous work on Rivulet bacterial studies by the Hobart City

Council and Blacklow (1995), and extensive Derwent estuary sedimentary studies by CSIRO Division of Marine Research (Leeming & Nichols, 1996a; Volkman *et al.*, 1988; and Volkman *et al.*, 1989).

This study has addressed and answered many of the questions initially raised, particularly in regard to source determination of faecal material and hydrocarbons in stormwater systems. However, care should be taken in the application of results to other cities, because catchment areas all have unique attributes in regard to rainfall patterns, land use, and the nature of sewerage and stormwater systems. All ot these factors have an affect on the amount and type of faecal material and other organic contaminants entering storm drains. Finally, this work demonstrated, through the use of chemical techniques combined with the more traditional bacterial techniques, a means by which faecal inputs in a complex system may better be understood.

6.2 RECOMMENDATIONS FOR FURTHER WORK

6.2.1 Specific investigations required for better understanding and management of the Derwent estuary system.

Further stormwater research is required in Hobart to obtain a more comprehensive understanding of the differences in pollutant loads and characteristics between catchments. A limitation of the current study was that resources were focussed primarily at one catchment, with data collected from a total of four stormwater catchments. This necessitated extrapolation, with its inherent uncertainties, when drawing conclusions on the nature of Hobart's stormwater as a whole.

To compliment this study, and to provide sound data on which management priorities could be based, further research to ascertain hydrocarbon inputs to the Derwent estuary from sewage plant discharge, atmospheric deposition and fuel storage depots, based on locally collected data, would be useful.

The extent to which stormwater drain sediments act as bacterial reservoirs and the effect these sediments have on the bacterial inoculation of passing waters requires further investigation. This question was raised in this study by the high bacterial counts in stormwater drains during dry weather and the lack of any evidence of direct faecal input, other than low level human input, to covered drainage systems during dry weather.

To compliment and build on the current study it would be valuable to conduct further research, using similar source elucidation techniques, to determine the extent and type of faecal pollution from the extensive rural catchment of the upper Derwent estuary.

6.2.2 General recommendations for investigations and management Australia- wide.

Given the demonstrated high relative contribution of faecal material and hydrocarbons by stormwater to receiving waters, management of the entry of these organic compounds into stormwater should be a future priority. This should include addressing illegal sewage connections to stormwater and control measures for management of pet faeces in public places. Source control methods for reducing stormwater pollution would place less pressure on the need for expensive stormwater treatment infrastructure. Some stormwater management practices for Australia are outlined in Appendix 4.

Further hydrocarbon studies should be undertaken elsewhere in Australia to determine the reproducability of results between locations and to justify or validate the extrapolations made from this study.

Given the high concentrations of contaminants currently carried by stormwater in Australia, studies aimed at an improved understanding of the environmental impacts of stormwater discharge on aquatic ecosystems should be a priority.

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APPENDIX 1 - Certified concentrations of aromatic hydrocarbons in SRM 1491. (National Institute of Standards and Technology).

Compound	**µg/g**
Naphthalene	10.30
1-Methylnaphthalene	12.40
Biphenyl	10.46
2,6-Dimethylnaphthalene	10.80
Acenaphthylene	10.40
Acenaphthene	10.89
2,3,5-Trimethylnaphthalene	9.90
Fluorene	10.87
Phenanthrene	10.48
Anthracene	11.69
1-Methylphenanthrene	10.40
Fluoranthene	8.84
Pyrene	8.81
Benz(a)anthracene	5.37
Chrysene	10.50
Benzo(b)fluoranthene	7.85
Benzo(k)fluoranthene	8.33
Benzo(e)pyrene	8.40
Benzo(a)pyrene	10.14
Perylene	10.65
Indeno(1,2,3-cd)pyrene	9.40
Dibenz(a,h)anthracene	7.74
Benzo(ghi)perylene	7.90

APPENDIX 2 - PAH response factors.

Compound	Response factor
(2rings)	
naphthalene	2.361
1-methylnaphthalene	1.381
2,6-dimethylnaphthalene	1.195
2,3,5-trimethylnaphthalene	1.088
(3 rings)	
acenaphthylene	1.925
acenaphthene	0.911
fluorene	1.487
phenanthrene	2.910
anthracene	1.971
1-methylphenanthrene	1.801
(4 rings)	
fluoranthene	4.776
pyrene	4.665
benz(a)anthracene	3.319
chrysene	3.630
(5 rings)	
benzo(b)fluoranthene	2.984
benzo(k)fluoranthene	3.406
benzo(e)pyrene	2.961
benzo(a)pyrene	2.273
perylene	2.613
indeno (123)	2.340
dibenz (ah)	2.395
(6 rings)	
benzo (ghi)	2.994

ng/µl compound = (peak area of compound) x (ng/µl D-std) / (peak area of D-std) x response factor

APPENDIX 3 - Content and composition of sterols from stormwater samples collected at the Prince of Wales Bay outfall of the Derwent Park catchment.

Storm event 1 - 4th January 1995

	powb 2		powb 3		powb 4	
	just before flush		flush		15 min	
Sterol	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.55	**7.3**	1.53	**6.2**	0.67	**7.1**
cholesterol	3.09	**41.5**	10.87	**44.2**	3.87	**41.3**
cholestanol	0.16	**2.1**	0.73	**3.0**	0.21	**2.2**
brassicasterol	0.35	**4.7**	1.02	**4.2**	0.41	**4.4**
24-methylenecholesterol	0.31	**4.1**	0.91	**3.7**	0.50	**5.3**
campesterol	0.32	**4.3**	1.55	**6.3**	0.47	**5.1**
stigmasterol	0.82	**11.1**	2.34	**9.5**	1.07	**11.4**
sitosterol	1.65	**22.1**	5.36	**21.8**	2.10	**22.4**
sitostanol	0.20	**2.7**	0.31	**1.2**	0.08	**0.9**
Total sterols (ug/l)	**7.44**		**24.60**		**9.38**	

Storm event 2 - 5th January 1995

	powb 5		powb 6		powb 7		powb 8	
	first flush		15 min		30 min		14 hrs	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	1.68	**9.2**	1.19	**11.9**	0.92	**6.3**	0.16	**7.6**
cholesterol	7.85	**42.8**	4.34	**43.3**	5.34	**36.6**	0.85	**39.2**
cholestanol	0.70	**3.8**	0.28	**2.8**	0.25	**1.7**	0.05	**2.4**
brassicasterol	0.91	**5.0**	0.36	**3.6**	0.63	**4.3**	0.08	**3.5**
24-methylenecholesterol	0.75	**4.1**	0.38	**3.8**	0.57	**3.9**	0.05	**2.2**
campesterol	0.95	**5.2**	0.46	**4.6**	0.86	**5.9**	0.10	**4.7**
stigmasterol	1.41	**7.7**	1.15	**11.4**	1.95	**13.3**	0.29	**13.5**
sitosterol	3.81	**20.8**	1.76	**17.6**	3.96	**27.1**	0.55	**25.4**
sitostanol	0.28	**1.5**	0.09	**0.9**	0.13	**0.9**	0.03	**1.5**
Total sterols (ug/l)	**18.33**		**10.01**		**14.60**		**2.17**	

Storm event 3 - 20th January 1995

	powb 10		powb 11		powb 12		powb 13	
	2hr after ff		1 hr later		2 hr later		11 hr later	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.07	**0.8**	0.10	**1.2**	0.07	**0.8**	0.13	**3.3**
cholesterol	1.03	**13.0**	1.47	**18.3**	0.91	**10.6**	0.99	**25.2**
cholestanol	0.03	**0.4**	0.06	**0.8**	0.04	**0.4**	0.05	**1.2**
brassicasterol	0.22	**2.8**	0.35	**4.4**	0.21	**2.4**	0.16	**4.0**
24-methylenecholesterol	0.07	**0.8**	0.19	**2.4**	0.10	**1.2**	0.28	**7.2**
campesterol	0.28	**3.6**	0.24	**3.1**	0.25	**2.9**	0.13	**3.2**
stigmasterol	4.74	**60.3**	3.66	**45.8**	5.24	**61.3**	1.03	**26.1**
sitosterol	1.26	**16.0**	1.75	**21.9**	1.66	**19.4**	1.08	**27.5**
sitostanol	0.17	**2.2**	0.18	**2.2**	0.08	**0.9**	0.09	**2.4**
Total sterols (ug/l)	**7.86**		**8.00**		**8.56**		**3.93**	

Storm event 4 - 16th February 1995

	powb		powb		powb		powb 20		powb	
	onset of storm flow		first flush		10		30 min		50 min	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.46	**8.7**	0.33	**2.2**	0.1	**1.8**	tr		tr	
cholesterol	1.6	**32.2**	7.2	**49.4**	3.1	**45.3**	1.4	**41.8**	0.75	**42.3**
cholestanol	0.30	**5.6**	0.2	**1.4**	0.1	**1.9**	0.1	**3.7**	0.04	**2.5**
brassicasterol	0.34	**6.5**	0.85	**5.8**	0.30	**4.3**	0.2	**6.1**	0.06	**3.4**
24-methylenecholesterol	0.22	**4.2**	0.78	**5.3**	0.36	**5.2**	0.23	**6.7**	0.06	**3.6**
campesterol	0.37	**7.0**	0.90	**6.2**	0.57	**8.1**	0.29	**8.7**	0.1	**7.7**
stigmasterol	0.67	**12.8**	0.99	**6.8**	0.45	**6.4**	0.50	**14.8**	0.22	**12.2**
sitosterol	0.84	**16.0**	3.04	**20.9**	1.7	**25.0**	0.55	**16.1**	0.39	**22.2**
sitostanol	0.36	**6.9**	0.28	**1.9**	0.1	**2.0**	0.07	**2.2**	0.1	**6.1**
Total sterols (ug/l)	**5.25**		**14.59**		**7.03**		**3.40**		**1.77**	

Storm event 5 - 27th June 1995

	powb 23		powb 24		powb 25		powb 26	
	27/6/95 steady light rain		30 min		1 hr		1 hr 30	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.30	**6.2**	0.38	**28.0**	0.49	**18.5**	0.13	**6.0**
cholesterol	1.26	**26.2**	0.48	**35.2**	0.79	**29.7**	0.93	**44.5**
cholestanol	0.24	**5.1**	0.09	**6.6**	0.13	**4.8**	0.09	**4.5**
brassicasterol	0.37	**7.8**	0.03	**2.2**	0.08	**3.1**	0.08	**4.0**
24-methylenecholesterol	0.13	**2.8**	0.09	**6.6**	0.18	**6.8**	0.04	**2.0**
campesterol	0.23	**4.7**	0.03	**2.3**	0.16	**6.1**	0.13	**6.1**
stigmasterol	0.46	**9.6**	0.04	**3.2**	0.12	**4.7**	0.12	**5.8**
sitosterol	1.37	**28.5**	0.18	**13.5**	0.53	**19.8**	0.46	**22.1**
sitostanol	0.44	**9.1**	0.03	**2.4**	0.17	**6.5**	0.11	**5.1**
Total sterols (ug/l)	**4.81**		**1.36**		**2.66**		**2.10**	

Storm event 6 - 17th August 1995

	powb 29		powb 30		powb 31		powb 32		powb 33		powb 34		powb 35	
	steady rain 8:00 17/8/95		steady rain 8:30 am		steady rain 9:00 am		steady rain 10:00 am		11:00 am		12 noon		rain stopped 2:40 pm (rain ende	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.06	**6.2**	0.09	**2.8**	0.10	**2.5**	0.04	**0.8**	0.02	**0.8**	0.02	**1.0**	0.02	**0.7**
cholesterol	0.32	**35.9**	1.51	**47.7**	1.58	**39.3**	1.53	**34.9**	1.00	**37.6**	0.69	**34.9**	1.14	**35.8**
cholestanol	0.02	**2.6**	0.06	**1.9**	0.09	**2.3**	0.11	**2.5**	0.07	**2.7**	0.06	**3.1**	0.08	**2.5**
brassicasterol	0.04	**4.9**	0.17	**5.4**	0.25	**6.1**	0.27	**6.2**	0.17	**6.5**	0.13	**6.6**	0.19	**5.9**
24-methylenecholesterol	nd		nd		nd		0.05	**1.2**	nd		nd		nd	
campesterol	0.11	**12.6**	0.20	**6.3**	0.30	**7.4**	0.34	**7.8**	0.23	**8.7**	0.21	**10.4**	0.28	**8.7**
stigmasterol	0.08	**8.8**	0.23	**7.1**	0.35	**8.6**	0.41	**9.3**	0.26	**9.8**	0.19	**9.5**	0.35	**10.8**
sitosterol	0.24	**26.2**	0.84	**26.6**	1.22	**30.2**	1.49	**34.0**	0.82	**30.9**	0.61	**30.7**	1.03	**32.4**
sitostanol	0.03	**2.9**	0.07	**2.2**	0.14	**3.6**	0.15	**3.3**	0.08	**3.1**	0.08	**3.8**	0.10	**3.1**
Total sterols (ug/l	**0.90**		**3.16**		**4.03**		**4.38**		**2.65**		**1.98**		**3.19**	

Storm event 7 - 23rd September 1995

	powb 36		powb 37		powb 38		powb 39		powb 40		powb 41	
	base flow 11:10 am		flush start 1:36		flush 1:39				1:43		1:45	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.202	9.4	0.55	13.	0.18	2.5	0.15	2.8	0.27	2.6	0.40	6.3
cholesterol	1.229	57.2	2.08	49.6	2.51	36.0	2.35	44.4	4.40	42.2	2.73	43.0
cholestanol	0.069	3.2	0.22	5.3	0.28	4.1	0.18	3.3	0.26	2.5	0.27	4.2
brassicasterol	0.039	1.8	0.08	1.9	0.25	3.6	0.23	4.3	0.23	2.2	0.23	3.7
24-methylenecholesterol	0.029	1.4	0.14	3.2	0.35	5.	0.19	3.5	0.54	5.2	0.24	3.8
campesterol	0.073	3.4	0.19	4.6	0.85	12.	0.32	6.1	0.58	5.6	0.38	5.9
stigmasterol	0.055	2.5	0.18	4.2	0.53	7.6	0.30	5.7	0.61	5.9	0.37	5.9
sitosterol	0.41	19.	0.65	15.4	1.9	27.3	1.47	27.7	3.38	32.4	1.63	25.6
sitostanol	0.043	2.0	0.1	2.6	0.12	1.	0.12	2.3	0.15	1.4	0.10	1.
Total sterols (ug/l)	**2.15**		**4.20**		**6.98**		**5.30**		**10.43**		**6.35**	

	powb 42		powb 43		powb 44		powb 45		powb 46		powb 47	
	1:47		1:50		1:54		2:01		2:10		2:20	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.26	4.2	0.22	5.4	0.13	3.6	0.07	2.0	tr		n.d.	
cholesterol	2.22	35.8	1.67	41.2	1.39	38.6	1.30	38.0	1.05	35.3	1.11	25.2
cholestanol	0.28	4.4	0.21	5.	0.21	5.8	0.20	5.9	0.14	4.7	0.11	2.5
brassicasterol	0.20	3.2	0.17	4.3	0.16	4.5	0.15	4.5	0.08	2.8	tr	
24-methylenecholesterol	0.16	2.6	0.13	3.2	0.16	4.3	0.10	2.9	0.10	3.3	0.202	4.6
campesterol	0.31	5.0	0.20	4.9	0.15	4.0	0.18	5.2	0.26	8.9	0.51	11.
stigmasterol	0.71	11.	0.37	9.1	0.27	7.4	0.23	6.7	0.26	8.6	0.287	6.5
sitosterol	1.92	31.0	1.04	25.7	0.99	27.6	1.07	31.4	0.95	32.0	2.187	49.5
sitostanol	0.14	2.2	0.04	0.9	0.15	4.1	0.12	3.4	0.13	4.4	tr	
Total sterols (ug/l)	**6.19**		**4.05**		**3.60**		**3.41**		**2.96**		**4.42**	

Storm event 8 - 12th October 1995

	powb 48		powb 49		powb 50		powb		powb 52	
	base flow		flush start		light rain		v. light rain		easing off	
	8:10 pm		8:35 pm		8:40 pm		8:50 pm		9:05 pm	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.03	**6.1**	0.06	**7.5**	0.1	**10.0**	0.49	**16.4**	0.1	**6.1**
cholesterol	0.23	**55.4**	0.36	**48.4**	0.62	**42.4**	1.2	**41.6**	1.0	**44.4**
cholestanol	tr		0.0	**1.4**	0.03	**2.3**	0.06	**2.1**	0.03	**1.4**
brassicasterol	nd		0.0	**1.7**	0.06	**4.2**	0.09	**2.9**	0.07	**3.2**
24-methylenecholesterol	nd		0.0	**1.8**	0.06	**3.9**	0.07	**2.3**	0.06	
campesterol	0.04	**10.1**	0.07	**9.8**	0.1	**10.0**	0.25	**8.3**	0.1	**6.8**
stigmasterol	0.02	**4.8**	0.03	**4.5**	0.06	**3.8**	0.1	**3.3**	0.07	**3.0**
sitosterol	0.1	**23.6**	0.1	**24.1**	0.3	**21.3**	0.63	**21.2**	0.74	**31.6**
sitostanol	nd		0.0	**0.9**	0.03	**2.1**	0.05	**1.8**	0.02	**1.0**
Total sterols (ug/l)	**0.42**		**0.75**		**1.46**		**2.97**		**2.33**	

Storm event 9 - 18th December 1995

	powb 53		powb 54		powb 55		powb 56		powb 57	
	light rain		light rain		rain		rain		heavy rain	
	10:30		11		1am		3 pm		5:30 pm	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.01	**6.0**	tr		tr		nd		0.0	**2.3**
cholesterol	0.089	**40.0**	0.04	**23.7**	0.03	**28.4**	0.04	**28.5**	0.1	**35.8**
cholestanol	0.005	**2.3**	tr		tr		0.0	**3.8**	0.0	**2.6**
brassicasterol	0.027	**12.3**	0.04	**25.8**	0.02	**20.6**	0.03	**22.9**	0.05	**11.7**
24-methylenecholesterol	0.005	**2.2**	0.0	**4.8**	0.0	**6.6**	0.00	**3.5**	0.0	**3.0**
campesterol	0.024	**10.6**	0.03	**14.5**	0.02	**23.1**	0.02	**16.2**	0.04	**9.4**
stigmasterol	0.023	**10.4**	0.03	**18.9**	0.0	**11.9**	0.0	**9.2**	0.08	**19.3**
sitosterol	0.025	**11.3**	0.02	**12.3**	0.0	**9.4**	0.02	**11.9**	0.05	**13.9**
sitostanol	0.01	**5.0**	nd		nd		0.0	**4.0**	0.0	**1.9**
Total sterols (ug/l)	**0.22**		**0.17**		**0.11**		**0.14**		**0.39**	

	powb 58		powb 59		powb 60		powb61		powb 62	
	rain		rain		solid rain		rain		light rain	
	10pm		6:30 am		10am		12:15p		3:15pm	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	tr		0.00	**5.6**	0.02	**11.0**	0.1	**48.4**	0.2	**46.7**
cholesterol	0.0	**20.0**	0.0	**15.7**	0.03	**18.5**	0.05	**12.9**	0.09	**19.6**
cholestanol	tr		0.00	**5.4**	0.0	**5.7**	0.02	**4.1**	0.02	**3.4**
brassicasterol	0.02	**34.9**	0.0	**13.8**	0.02	**11.3**	0.03	**7.9**	0.03	**5.7**
24-methylenecholesterol	tr		0.00	**4.3**	0.00	**3.4**	0.00	**0.8**	0.00	**0.7**
campesterol	0.0	**20.0**	0.0	**15.6**	0.02	**15.4**	0.04	**10.3**	0.05	**10.1**
stigmasterol	0.0	**13.7**	0.02	**20.3**	0.02	**15.9**	0.02	**6.2**	0.0	**2.6**
sitosterol	0.0	**11.5**	0.0	**13.8**	0.02	**16.4**	0.03	**6.9**	0.04	**8.8**
sitostanol	nd		0.00	**5.4**	0.00	**2.5**	0.0	**2.6**	0.0	**2.4**
Total sterols (ug/l)	**0.05**		**0.08**		**0.14**		**0.40**		**0.46**	

Other samples

	powb 9		powb 14		powb 15		powb 16		powb 22		powb 27		powb 28	
	constant drizzle		end of wet period		light rain		small rain event		after heavy o/n rain		light rain		showers	
	16/1/95		29/1/95		29/1/95		30/1/95		7/4/95		29/7/95		7/8/95	
Sterol	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%	ug/l	%
coprostanol	0.10	0.5	0.16	5.2	0.04	1.8	0.37	5.5	nd		nd		0.07	5.3
cholesterol	3.55	18.3	1.61	53.2	1.19	57.8	3.96	58.3	0.90	42.6	0.285	49.5	0.50	40.6
cholestanol	0.08	0.4	0.06	2.0	0.04	1.8	0.09	1.3	0.07	3.3	0.032	5.5	0.07	5.5
brassicasterol	0.63	3.3	0.10	3.1	0.10	4.9	0.21	3.1	0.08	3.9	0.025	4.3	0.09	7.0
24-methylenecholesterol	0.68	3.5	0.03	1.1	0.03	1.6	0.65	9.6	0.08	3.9	nd		nd	
campesterol	0.23	1.2	0.21	6.8	0.09	4.5	0.66	9.7	0.08	3.9	0.032	5.5	0.12	9.7
stigmasterol	12.05	62.1	0.25	8.1	0.17	8.2	0.19	2.7	0.36	17.2	0.059	10.2	0.10	8.3
sitosterol	1.97	10.1	0.59	19.3	0.38	18.6	0.63	9.3	0.50	23.8	0.110	19.1	0.24	19.4
sitostanol	0.10	0.5	0.03	1.1	0.02	0.8	0.03	0.5	0.03	1.5	0.034	5.9	0.05	4.3
Total sterols (ug/l)	**19.39**		**3.03**		**2.06**		**6.79**		**2.12**		**0.58**		**1.24**	

APPENDIX 4 - Stormwater management practices

Best management practices for urban stormwater control in Australia have been reviewed by Lawrence and Phillips (1993) with recent studies conducted by Ellis (1993), Tilley *et al.* (1994), National Capital Planning Authority (1993), and Argue (1994). The most appropriate BMPs for Australian conditions must consider the major processes for the removal of priority pollutants from the runoff. These include adsorption, precipitation and complexation, volatilisation, biodegradation, and photolysis (Scholze *et al.*, 1993).

Stormwater treatment methods currently being employed or developed in Australia are summarised by Scott (1996). These methods include research into injection of stormwater into aquifers for future re-use. The concept of re-using stormwater is rapidly increasing in most Australian cities. This ranges from simple methods such as the collection of roof water in household rainwater tanks to collection and treatment of stormwater at centralised locations within a catchment (Scott, 1996). An overview of stormwater re-use in Australia is provided by Anderson (1995). On-site stormwater detention (OSD) involves the temporary storage of stormwater generated within the site which aims to alleviate downstream flood discharges. This system has been widely implemented throughout Sydney. There are a number of constructed wetlands currently in use in Australia including those at the Blue Mountains, Adelaide, Melbourne and Canberra.

The most effective stormwater quality management practices are summarised by Urbonas (1991) who concluded that the best controls are those that reduce runoff peak and volume, followed by those that mainly reduce runoff peak. Best management practices generally involve some form of infiltration (swales, filter strips, porous pavements, infiltration basins and trenches) and the absence of directly connected impervious areas. It was also concluded that storage practices tend to be less effective, however most obnoxious pollutants in urban runoff are settleable.

Additionally, Urbonas (1993) states structural best management practices (BMPs) which include the minimisation of directly connected impervious areas, incorporation of grass swales, grass buffer strips, porous pavements, percolation trenches, infiltration basins, sand filter basins, water quality inlets, extended detention basins, retention ponds, and wetland basins. Non-structural BMPs include public education and citizen involvement programs, street sweeping, local government rules and regulations, elimination of illicit discharges, organised collection of household chemicals, proper disposal of pet droppings, and controlled transport of chemicals and hazardous waste (Stahre, 1993).

APPENDIX 5 - List of acronyms used in the text

ACT	Australian Capital Territory
ANARE	Australian National Antarctic Research Expedition
ANZECC	Australia and New Zealand Environment and Conservation Council
BMP	Best management practice
CEPA	Commonwealth Environment Protection Agency
cfu	colony forming units
CPI	Carbon preference index
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAPA	Data acquisition processing and analysis
DELM	Department of Environment and Land Management
FC	Faecal coliforms
FID	Flame ionisation detector
FS	Faecal streptococci
GC	Gas chromatography
GC-FID	Gas chromatography - flame ionisation detector
GC-MS	Gas chromatography - mass spectrometry
HR	Hobart Rivulet
HPLC	High performance liquid chromatography
IASOS	Institute of Antarctic and Southern Ocean Studies
kW	Kilowatt
LAB	Linear alkylbenzene
MS	Mass spectrometry
NATA	National Association of Testing Authorities
NIST	National Institute of Standards and Technology
NRC	National Research Council
NSW	New South Wales
NTR	New Town Rivulet
PAH	Polycyclic aromatic hydrocarbon
POWB	Prince of Wales Bay
SAB	Special Antarctic Blend
SRM	Standard reference material
tic	Total ion current
TLC-FID	Thin layer chromatography with flame ionisation detector
TSS	Total suspended solids
UCM	Unresolved complex mixture
USA	United States of America
USEPA	United States Environmental Protection Authority
UV	Ultraviolet
UV-VIS	Ultraviolet and visible light
WC	Waimea catchment

APPENDIX 6 - Conferences, presentations and publications

i) Scientific Papers

Butler, E.C.V, Green, G.J., Higgins, H. W., Holdsworth, D. G., Leeming, R., Mackey, D. J, Morgan, P., Nichols, P. D., O'Sullivan, J. E., Plaschke, R. B., Revill,A. T. R., Volkman, J. K. and Watson, R. J (1996). Contaminants in the Derwent - 20 Years On. In: Proceedings 23rd Hydrology and Water Resources Symposium. pp 679-680, Institution of Engineers, Australia.

Green G.J. and Nichols, P.D. (1995). Hydrocarbons and sterols in marine sediments and soils at Davis Station, Antarctica: A survey for human derived contaminants. *Antarctic Science* 7, pp. 137-144.

ii) Scientific Reports (CSIRO Div. Oceanography)

Green, G. (June 1995). Hydrocarbons and faecal material in urban run-off from Hobart. Report prepared for DELM as part of proceedings from 1995 Derwent Scientific Forum.

Green, G. (November 1994). Hydrocarbon discharge into Prince of Wales Bay via the stormwater system. Report prepared for Glenorchy City Council.

iii) Presentations, conferences and courses

- Presented paper at "International Symposium on Environmental Chemistry and Toxicology Conference", Sydney, July 1996.
- Presented paper at "Australian Marine Science Association conference", Hobart, July 1996.
- Attended "Stormwater Management Course", Gold Coast, July 1996.
- Attended "Analysis of Petroleum Hydrocarbons workshop", Melb. December 1995.
- Attended scientific writing course at University of Tasmania, September 1995.
- Presented paper at stormwater meeting (AWWA), Hobart, July 1995.
- Attended Marine Pollution Monitoring Course, James Cook University, July 1995.
- Presented a paper at Derwent Scientific Forum, April 1995.