



Dr Andy Revill has applied oil 'fingerprinting' techniques to find the source of oils contaminating little penguins at Phillip Island. He says 'chronic' oil inputs present a greater threat than 'acute' incidents such as accidental oil spills.



## Policing pollution

The ongoing pollution of a small Geelong beach has been testing the investigative skills of Victoria's Environmental Protection Authority for almost a decade. Rippleside Beach is often closed due to high levels of faecal contamination, apparently delivered to the beach by a stormwater drain.

On its way to the bayside beach, the drain cuts through four suburbs and many land uses (housing, light industry, a livestock saleyard and a golf course) each a potential source of contamination. Also crossing the catchment are sewers taking wastewater across Geelong to a treatment plant on Bass Strait.

A 1994 EPA study led to a tightening of waste control at the saleyard. Since then the pollution of Rippleside has been less frequent, but high levels of contamination still occur. To track the source of this contamination, a clue to its origin was clearly needed.

But traditional analytical techniques, which rely on bacterial indicators such as *E. coli*, can only indicate the level of faecal contamination, not the faecal type.

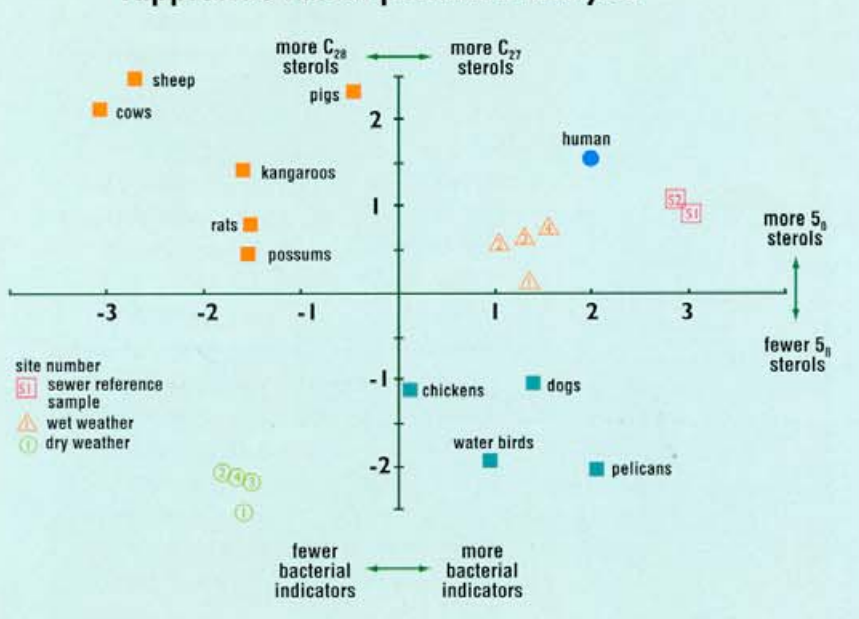
For Dr Rhys Leeming and his team at CSIRO Marine Research, the contamination at Rippleside offered an ideal opportunity to field test techniques developed at their Hobart laboratory for identifying the sources of faecal contamination. In particular, the team is pioneering the application of inert chemical markers called faecal sterols to distinguish between human and animal waste.

Faecal sterols are lipids that can be used to indicate the source of faecal matter because they vary in type and quantity according to diet (carnivorous, herbivorous and omnivorous) and the microbiota in the digestive tract.

In humans cholesterol is converted to  $5\beta$ -coprostanol in the digestive tract by anaerobic bacteria. In cows and sheep, 24-ethylcoprostanol is the predominant faecal sterol. Dogs do not have the necessary gut biota to reduce sterols to stanols: their identifying feature appears to be high levels of cholesterol only and uncommonly high levels of *C. perfringens* spores in their faeces. Such differences in the sterol and bacterial profile can help distinguish the source of faecal contamination.

Leeming's team began the Rippleside study by verifying the relationships between faecal sterols and the various bacterial indicators present in reference samples from the sewers and saleyard. These were then compared with wet and dry-weather samples

Rippleside faecal pollution analysis



This diagram shows the results of a principle components analysis of human, herbivore, bird and dog faeces in stormwater reference samples from Geelong's Rippleside beach. The main difference between components are the  $5\beta$ -stanol to sterol ratio, the carbon number of sterol precursors ( $C_{27}$ - $C_{29}$ ), and the concentration of bacterial indicators.



from the stormwater drain and the beach. Parameters compared included five sub groups of bacterial indicators and eight different sterols.

The principal components analysis (see diagram on page 24) shows how similar the wet weather samples were to both the reference samples and previously-analysed human faecal samples. The dry weather samples had much lower levels of faecal contamination and only small amounts of residual human faecal matter. These results suggested sewer overflow was the more likely source of contamination.

EPA Victoria South-west Region manager, Tony Robinson, says in situations where there are multiple potential sources of contamination in a catchment, faecal sterol analysis offers two benefits. 'Firstly, it helps us to identify where remedial action is needed, such as upstream sewage storage,' he says.

'Secondly, it can help to determine the health risk. If the contamination is caused by seagulls or cattle, the risk of disease is not as great as for human sewage. But we can't determine this risk from bacterial analysis alone.'

Leeming's pollutant detection techniques have also been applied at Wyong's Tuggerah Lakes, in Melbourne's Yarra River and at Lake Macquarie in Newcastle, NSW. The pilot Tuggerah Lakes study, in

1994-95, suggested 80% or more of faecal pollution after rain was from the prolific local sea birds.

The Yarra River study found that 60% of all faecal residue in a particular section of the river derived from humans, not from dog droppings carried by stormwater run-off. Faulty sewer pipes, domestic run-off from hard surfaces, sewer overflows, septic tanks and illegal connections to stormwater drains have been blamed as major sources of this faecal residue.

Between field studies, Leeming's team is busy gaining a better understanding of faecal sterols in work funded by the Water Services Association of Australia. 'At the moment we don't have specific chemical markers for dogs or birds,' Leeming says. 'It is possible that, with bacteria such as *E. coli* for example, we may be able to pick out a piece of genetic code that differs between different sources, such as humans, dogs and birds. Geographic variations of the DNA fragments within species might also offer a way of tracing the transmission of diseases caused by faecal contamination.'

Future laboratory work will focus on combining chemical marker technology with DNA fingerprinting techniques and novel classes of compounds. The ultimate aim of Leeming's research is to devise for environmental managers a portable and efficient

## Corpulent crabs crawl on paradise

IN a sheltered lagoon at Falmouth on Tasmania's east coast, the green crab (*Carcinus maenas*) has discovered Utopia. Untroubled by predators, nourished by native crustaceans, and nurtured by a near-perfect climate, the crabs here grow up to one third bigger than elsewhere in the world.

Craig Proctor of CSIRO's Centre for Research on Introduced Marine Pest species (CRIMP) says the green crab, a native of Europe, tolerates wide ranging environmental conditions, and can survive in water of high or low salinity in intertidal or subtidal habitats. It was introduced to Victoria in the mid-1800s, probably by ships lured to the Gold Rush, and also has invaded US, South African and Japanese waters.

In Tasmania, the spread of the green crab spread is cause for concern. 'We've recently received reports of crabs being found on Bruny Island, south of Hobart,' Proctor says. 'We are concerned the crab may reach Port Davey, or the Derwent Estuary, where it will add to the plunder of another invader, the Northern Pacific Seastar. The Derwent is already home to an estimated 27 million of these predatory starfish.'



One way to control pest species such as the green crab is to catch them.

Some 170 marine species are known to have been introduced to Australia, and many others have yet to be identified. Proctor and his colleagues are working with the University of Tasmania and overseas groups such as the US Smithsonian Environmental Research Center to study options for controlling a handful of introduced species, such as the green crab, which have the potential to become marine pests.

Those options extend from ballast-water treatments to prevent new introductions or the transfers of species within Australia,

examining natural biological controls, transgenic approaches to produce sterilised species, and even 'fish-downs' involving local and community groups to remove species by simply catching them.

CRIMP community liaison officer, Karen Parsons, is working with community networks as part of the Introduced Marine Pests program of Environment Australia's Coasts and Clean Seas initiative. She says people are encouraged to alert scientists to changes in marine environments and the appearance of unfamiliar species.



'toolbox' of chemical marker techniques for characterising the sources and loads of faecal contamination. Once that is accomplished, only those tools necessary for long-term monitoring need be used to monitor remediation efforts.

### Slick work

'Fingerprinting' of another kind is used by marine scientists and environmental authorities to discover the sources, fates and effects of oils in Australia's marine environment. This is the aim of research by CSIRO's Dr Andy Revill.

Revill says oils contain many distinctive compounds derived from organisms deposited in sediments millions of years ago. In fact, every one of the world's oil fields has a characteristic molecular profile. Exploration geologists use these profiles to decipher the geological history of wells, and to gain pointers about where to concentrate future drilling.

The oil fraction of water or sediment samples is analysed by gas chromatography and mass spectrometry. The gas chromatograph contains a capillary column that separates various components on the basis of their volatility. When the sample is heated, helium gas carries the volatilised material from the end of the column into the mass spectrometer. Here the molecules are bombarded by a stream of electrons which ionises them and splits them into fragments according to their mass.

If two oils appear similar, another fingerprint can be used: the isotopic signature. Carbon exists as two stable isotopes: carbon 12 (99% of the total carbon) and carbon 13. This natural ratio is modified by organisms, depending on the mechanism by which they take up carbon, as well as by environmental effects.

Organic matter deposited in sediments can have a distinctive isotope ratio, as can the oil it produces when the sediment is buried and heated. The isotope ratio provides an added signature for distinguishing otherwise similar oils and for differentiating between hydrocarbons of natural and pollutant origin.

In 1996, Revill suggested applying these techniques to discover the source of oils contaminating little penguins at Victoria's Phillip Island Nature Park, or at least to rule out potential sources such as nearby Bass

Strait oil rigs. Oily water is a hazard for the penguins which forage in heavily-trafficked sea lanes off Australia's south-east coast. Since 1994, the number of oiled little penguins treated at Phillip Island has ranged from 30 to 120 per year, but these probably represent a small fraction of birds oiled at sea.

Revill's ongoing analysis of the oils has identified their source as ships' fuels and cargo. This finding highlights the problem of 'chronic' oil inputs from urban settlements and shipping, which he believes add up to a greater threat than 'acute' incidents such as accidental oil spills. A major chronic input is oil sludge, the by-product of fuel use, which must be discarded periodically, much like a grease-and-oil change in a car.

Under the Commonwealth Protection of the Sea Act (1983), ships are banned from discharging oil or oily water into Australia's oceans. Most ports provide facilities for sludge removal, but some ships, to save time and money, discharge it illegally at sea.

Some 300 reports of illegal oil discharge are received each year by the Australian Maritime Safety Authority. Few of these can be acted upon, due to a lack of evidence linking the ship to the slick. Since the beginning of 1990, the authority has mounted only 74 successful prosecutions. Many of these cases relied on hydrocarbon fingerprinting carried out by the Australian Government Analytical Laboratory at Perth.

As well as exposing unlawful activities of ships, oil fingerprinting can identify the sources of oils discharged from land. 'Some 30% of petroleum hydrocarbons enters marine systems through stormwater systems and drains,' Revill says. 'These can be traced to lubricating oils used by motor vehicles, to industries, or to the dumping of oils. Other sources of oil are bushfires, sugar cane burn-off, vehicle exhausts and combustion products in factory emissions.'

A 1996/97 CSIRO-Tasmanian Department of Environment and Land Management study of hydrocarbon sources in urban run-off conservatively estimated that about 8000 tonnes of oils are discharged annually into Australian coastal waters from run-off. This figure could be much higher, possibly up to 20 000 tonnes, adding each year to the build up of oils in estuarine sediments.

*Scientists are working with the Atlantic salmon industry to investigate the effects of waste organic matter from fish cages on sediments beneath salmon cages. The findings will help farm managers develop following options for sea-floor maintenance beneath salmon production cages, in the same way as primary producers leave land to fallow. Methods of speeding the decay of waste, without reducing water quality, are also being developed. These outcomes will benefit other aquaculturists too, such as those farming tuna off South Australia.*

