Sources of organic matter in Wallis Lake

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A study of lipid biomarkers at Wallis Lake has revealed sources of organic matter in sediment samples collected at five different sites. Lipid biomarkers are molecules derived from the cells of organisms that can be identified in sediments and related back to their sources. These sources include algae, bacteria and terrestrial plants, along with a signal for faecal contamination. Site comparisons reveal distinctive biomarker signals associated with terrestrial plant inputs at two river mouth sites. These sites also exhibit decreases in algal signals and the highest levels of faecal contamination. Analysis of down-core biomarker distributions indicates that biomarker signals become increasingly similar with depth. This is related to diagenesis and the more rapid degradation of algal tissues. A statistical approach has been used to interrogate the data and illustrate differences between and within sediment cores.

Wallis Lake (figure 1) is one of Australia's largest coastal lakes with an area of about 90 square kilometres. Sediment facies in the lake include a fluvial bay head delta near the entry points of the major rivers (Coolongolook and Wallamba Rivers), a shallow muddy central basin forming the southern part of the lake, and a marine tidal complex at the mouth of the estuary. Because of training walls, the estuary mouth is permanently open. The Wang Wauk/Coolongolook River and Wallamba Rivers are the main catchment tributaries. Much of the area traversed by these rivers and tributaries consists of cleared and partially cleared agricultural land.

A previous environmental study identified the presence of nutrient, animal and human faecal pollution, and other urban runoff pollutants from the Forster–Tuncurry area as the principal sources of pollution in the estuary.[1] But available data indicate that under dry conditions, estuary water quality complies with Australian guidelines.[1] Data also show that under low to moderate wet conditions, the lake waters contain high sediment and nutrient concentrations. As well, data show some high bacterial counts, particularly in the tributary rivers and around the urban drainage outlets.

Water-quality sampling of biological parameters in estuaries requires both intensive spatial and temporal sampling programs. The purpose of such surveys needs to be clearly defined so that the sampling program can be designed to address the appropriate management questions. Alternatively, various water-quality issues can be addressed by surveys of sediment and plant material. These surveys rely on the large spatial distribution of sediments and plant material, and the ability of sediments to integrate and record water-column characteristics.

This paper outlines an investigation of lipid biomarker compositions of sediment in Wallis Lake to determine the relative importance of urban and rural inputs. Lipid biomarkers were chosen because they can identify organic inputs to sediments and help trace the dispersal of these inputs through the estuary. Biomarkers are compounds of biological origin produced in cell walls and fat storage areas. They can often be related to sources such as phytoplankton, terrestrial plants and bacteria. Certain biomarkers are also specific to particular groups of organisms; for example, diatoms, dinoflagellates or cyanobacteria. Furthermore, biomarkers may help identify forms of environmental contamination, such as petroleum and sewage.

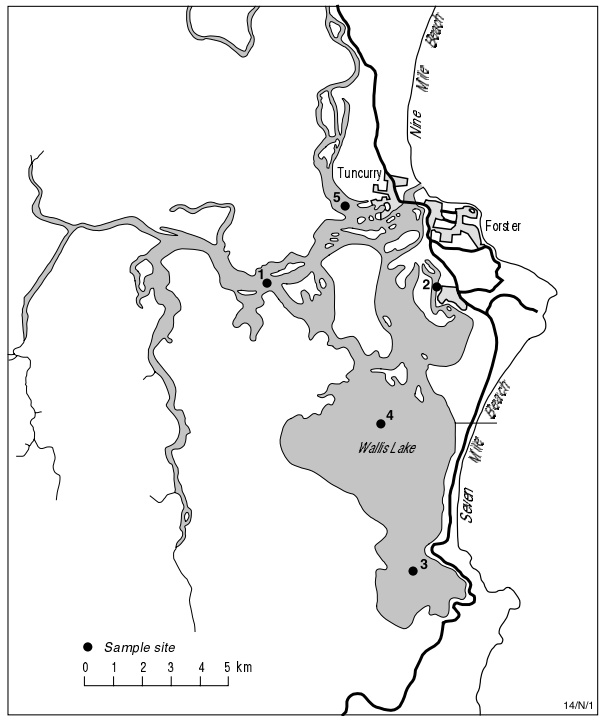

Figure 1. Map of Wallis Lake and sample locations

Sample collection and analysis

Sediment cores were collected at three sites around Wallis Lake. Two of these core sites were chosen to reflect potential end members for rural (Coolongolook River) and urban catchments (canal development at Forster Keys). The third site was selected to represent deposition within the lake system, remote from these end members. Two surface sediment sites were also chosen—one in the central basin area to reflect an integrated picture of inputs; the other at the Wallamba River mouth to examine rural inputs to the lake.

The details of sample collection and analysis are covered elsewhere.[2] Briefly, cores were collected using a hand-operated piston corer and sediments were freeze dried before grinding. Biomarkers were extracted by repeated sonication in mixtures of organic solvents (dichloromethane and methanol). The combined total extract was then analysed by gas-chromatography mass-spectrometry (GC-MS). This procedure separates complex mixtures of molecules for identification and quantitation. By a process called Target Compound Analysis, the GC-

MS computer software was trained to recognise and look for 69 biomarkers of interest. Each compound was quantified against the internal standard and then its abundance related to the amount present in each gram of sediment. Compound abundances were then used in the statistical software package 'Statistica' to perform principal component, cluster and cononical variation analyses.

Identification of specific organic matter inputs

The different sources of organic matter are recognised both on the basis of individual biomarkers and the presence of diagnostic patterns of multiple biomarkers. Specific sources are identified and discussed below. The importance of certain inputs is assessed through several ratios developed to examine the variation of different biomarkers (table 1). The use of these ratios allows inter-site comparison and helps in the interpretation of the statistical data.

Algae

Dinoflagellates are single-celled algae that live in oceans, estuaries, lakes and ponds. They can cause 'red tides', which kill fish or poison humans who eat the fish or shellfish affected by such algal blooms. The presence of dinoflagellates is recorded chemically in sediments by dinosterol, a biomarker specific to dinoflagellates.[3] It is present at all sites in Wallis Lake and throughout the sediment cores. This indicates that dinoflagellate production occurs across Wallis Lake and that dinoflagellates have been a component of the phytoplankton production over the entire depositional history of the cores (approximately 5000 years). Ratios of dinosterol to phytol can be used to estimate the comparative importance of dinoflagellates at each site. Phytol is derived from chlorophyll and is present in all photosynthetic plants. Dinosterol abundance ratios were lowest at sites 1 and 5 (1.8 and 0.5 respectively) compared with the other sites which ranged from 4.0 to 7.7. The percentage of dinosterol as part of the sum of all biomarkers quantified also indicates that abundances of dinosterol are highest away from the two river mouths (table 1). This suggests that dinoflagellate production was lower close to the river mouths.

The 'green algae' is the most diverse group of primarily aquatic algae, with more than 7000 species. The presence of green microalgae is indicated by C_{30} 1,15 alkandiol.[4] Again, this compound was found to be present at all sites and through each core. Generally speaking, this biomarker was most abundant and provided the highest ratios at sites 2, 3 and 4, away from the river influxes. For example, values for ratios of C_{30} 1,15 alkandiol to phytol are 0.5 and 1.4 for sites 5 and 1, and range between 2.1 to 7.0 for the sites 2 to 4. The percentage abundance of C_{30} 1,15 alkandiol is also higher away from the river mouths (table 1). As with the dinosterol data, this suggests that the algae group that produces C_{30} 1,15 alkandiol is most commonly found away from the river mouths.

Diatoms are unicellular algae with cell walls that contain silica. They generally form an important component of photosynthetic production within estuaries, and often occur as a spring bloom. This happens when the concentration of silica and nutrients in combination with increased light and water temperatures allow for rapid growth. Different diatom species can produce a range of sterols, of these 24-methylcholesta-5,24(28)-dien-3β-ol and 24-methylcholesta-5,22E-dien-3β-ol are thought to be most diagnostic.[5] However, certain sterols such as cholesterol, also known to be produced by this group, have other potential sources. Although cholesterol is abundant in animals, it may be predominantly derived from diatoms in Wallis Lake. This is supported by the cluster analysis finding that cholesterol groups with compounds related to algal sources. Ratios of cholesterol to phytol and percentages of cholesterol as a function of total quantified lipids do not show marked variation with site (table 1). Except for site 1, cholesterol is the most abundant of all sterols in every sample. Most of the other diatom-related sterols[5] have been identified at each site, indicating that diatoms are also an important component of the phytoplankton.

Bacteria

Cyanobacteria is the scientific name for blue-green algae—plant-like bacteria that can fix nitrogen and contain cyanobacterial toxins. Cyanobacteria commonly bloom in shallow, warm, slow-moving or still water and can cause a health risk in areas of human activity. They are more prevalent in fresh water than in brackish or marine conditions. Cyanobacteria, along with other bacterial groups, produce a group of compounds called hopanoids. The presence of 2-methyl-hopanoids is now thought to be diagnostic for cyanobacteria,[6] although not all species in the group make this biomarker. C_{32} hopanol is detected in the surface sediments at all sites, although in very low concentrations. However, no 2-methyl-hopanoids are detected at any of the sites. This suggests that if cyanobacteria are present, they are not significant contributors to the sedimentary organic matter. The analysis of these biomarkers is problematic so this conclusion is tentative. Further work using more specific chemical techniques would be required to assess the composition and distribution of the hopanoids to address the nature of the sources of these compounds.

Sulfate-reducing bacteria are also present in all samples. In particular, iso- and anteiso-branched fatty acids, as well as 10-methyl hexadecanoic acid are diagnostic of this group of

		Dinoflagellates		Green algae		Diatoms		Land plants		Herbivore faecal sterol ratio
Site		dinosterol/ phytol	% dinosterol	alkandiol/ phytol	% alkandiol	cholesterol/ phytol	% cholesterol	triterpenoids/ phytol	% triterpenoids	24ethylcoprostanol/ 24ethylcholestanol
1	river mouth	1.8	1.4	1.4	1.1	7.1	5.6	12.0	9.4	1.3
2	canal development	4.9	2.4	4.1	2.0	10.8	5.3	<0.1	<0.1	0.6
3	southern basin	7.7	2.6	7.0	2.3	14.1	4.7	<0.1	<0.1	0.4
4	central basin	4.0	2.3	2.1	1.2	7.8	4.4	1.9	1.1	0.5
5	river mouth	0.5	1.7	0.5	1.6	1.7	5.4	1.3	4.2	1.6

Table 1. Ratios of compounds to phytol based on quantified data from GC-MS analysis. The percentages are based on the abundance of each compound, as a function of the total of all lipids quantified. The triterpenoids are derived from a sum of β-amyrin, friedelin, oleanoic and ursolic acids.

bacteria. These fatty acids are a common feature of almost all sediments and reflect part of a community of bacteria involved in the decay of the organic matter.

Land plant

Terrestrial plant input is recognised by the presence of leaf waxes in all the sediments. Leaf waxes are composed of a series of *n*-alkanes, *n*-alcohols and *n*-acids with carbon chain lengths ranging between C_{22} and C_{32}. The acid and alcohol series is dominated by even carbon numbered compounds, and the alkane series is dominated by odd carbon numbered compounds. Specific higher plant biomarkers called triterpenoids are also common in the sediments of each core. Sites 1 and 5 have a group of triterpenoid biomarkers (including friedelin, oleanoic and ursanolic acids) that are not present at any of the other sites. This suggests there is a specific terrestrial input in the sediments close to the river catchments, that is not present at other locations further from the river mouths.

Anthropogenic inputs

Urban sources of organic matter can include human sewage, outlined below, and also petroleum products. These can be from spillage, combustion of fossil fuels, and run-off from bitumen sealed roads. None of these sources could be identified in the sediments from site 2, chosen as the urban catchment end-member, nor were they found at any other sites. The impact of urban processes, therefore, is not significantly reflected in the biomarkers deposited at any of the chosen sites.

A group of sterols has been found to be diagnostic of faecal inputs.[7] Human sewage contains a compound called 'coprostanol'—a biomarker that can trace sewage in the environment. None of the sites examined contains detectable concentrations of this biomarker. Even site 2, which was selected to represent an urban catchment, does not appear to be affected by human faecal contamination. In contrast, the biomarker for herbivore faecal inputs, 24-ethyl-coprostanol is detected at all sites. The presence of this marker is not in itself a direct indicator of faecal contamination, since it can form in sediments through natural processes. However, a ratio of this sterol to 24-ethyl-5β-cholestan-3β-ol with values greater than one is evidence for herbivore faecal inputs to an environment. Both site 1 and site 5 have sterol ratios greater than one (1.3 and 1.6 respectively), indicative of contamination by herbivores. In contrast, sites 2, 3 and 4 have values between 0.4 and 0.6 (table 1), which fall close to values expected naturally in anaerobic muds. These results suggest that contaminated run-off from rural catchments is affecting sites 1 and 5, but sites 2, 3 and 4 are probably not strongly affected by this form of contamination. Furthermore, the absence of 24-ethyl-*epi*-coprostanol (which is an important sterol in sheep faeces[7]) suggests that the herbivore faecal contamination at sites 1 and 5 is most likely from cattle.

Inter-site comparison

Inputs to surface sediments at sites 1 and 5 are clearly distinguished by a range of terrestrial biomarkers. These include several plant triterpenoids, such as oleanoic and ursolic acids, and the abundance of lipids derived from plant leaf waxes. Furthermore, these sites have lower abundances of biomarkers associated with green microalgae and dinoflagellates (table 1). Sites 2, 3 and 4 have similar biomarker compositions to one another and include terrestrial, planktonic and bacterial sources. Bacterial and phytoplankton sources can also be recognised at sites 1 and 5; however, these sites are dominated by biomarkers of land-plant origin. Significantly these sites also have strong signals derived from herbivore faecal contamination (table 1). This is an important finding, because it suggests that run-off from the rural catchment brings with it faecal material that may present a problem for water quality. The absence of coprostanol, the biomarker associated with human faecal contamination, indicates that sewage from urban sources is not significantly affecting Wallis Lake.

Multi-variate statistical analysis of biomarker data

Lipid biomarker analysis provides concentration data on some 69 separate compounds including fatty acids, alcohols, alkanes, sterols and triterpenoids. Multi-variate data sets of this size are often difficult to interpret because of the large number of permutations of variables that need to be considered. Principal Components Analysis (PCA) was used to investigate the basic structure of the data and compositional differences between cores at sites 1, 2 and 3. PCA is a method of reducing the number of variables in a data set to a minimum by creating principal components from the original, highly correlated parameters. The principal components are selected so they are parallel to the major variation in the data. The first principal component or axis is parallel to the greatest variation in data (maximum variance), and the second axis is chosen to be parallel to the next greatest variation in the data.

PCA was carried out using the 'Statistica' software package produced by StaSoft. PCA within the Statistica package can only be performed where the number of analyses (cases) is less than the number of samples. Data were therefore broken down into two broad groups. The first group (group 1) contained sterol and triterpenoid compounds along with other compounds related to leaf waxes. It is most likely to differentiate variations in phytoplankton and terrestrial sources. The second group of compounds (group 2) contained short chain fatty acids, alcohols and *n*-alkanes, ranging between C_{14} and C_{20}. These compounds are common to a greater number of sources (higher plant tissues, phytoplankton and bacteria), and therefore the group has a lower degree of specificity.

The PCA of the two groups shows that the data set is highly correlated, but in this paper only the first two axes for group one are considered. After PCA the biogeochemical reasons for the statistical variation must be assessed. This was partly done through examination of the PCA loadings (which for space reasons are not presented in this report). The variation in PC1 is related to depth but controlled by the concentration of compounds, which decreases down core. This is a normal feature of lipid biomarker analysis and reflects decay and early diagenesis of organic matter.[8,9] PC2 is controlled by the different sources of organic matter, with negative PC loadings related to stronger terrestrial biomarker signals and positive loadings reflecting greater inputs of algal biomass. These PC loadings are used as axes in figures 2 and 3. The cross-plots of PC factors 1 and 2 show that sites 2 and 3 plot closely together and have similar trends. All sites converge to a common location on the graph. This can be explained by selective preservation of terrestrial material, leading to increasing similarity of all cores with depth.

When the first principal component of group 1 and group 2 are plotted against depth within each core (figure 2), all sites appear to exhibit similar trends. At the sediment–water interface, compound concentrations are generally high. But they decrease rapidly within a few

centimetres of the surface, primarily as a result of bacterial degradation within the surface sediment. Although there is a general loss of biomarkers, selective degradation of certain compounds occurs. This process leads to a biasing of the preserved organic matter. (These effects will be discussed below.) Biomarker compositions at sites 2 and 3 become more similar to site 1 with increasing depth in the cores. Generally, algal material is more easily recycled by bacteria and degrades faster than organic matter from higher plants. This leads to an apparent increase in the influence of higher plant sources with increasing depth in the core.

Group 1 (sterols, triterpenoids and waxes)

All sites show decreasing compound concentration with depth, except site 3. At 40-centimetres depth, site 3 had markedly higher biomarker concentrations. This difference is identified within the PCA, and illustrated in the PC factors plotted against depth (figure 2). This particular sample has a high concentration of leaf-wax biomarkers and a total organic carbon content of 12 per cent. The high organic carbon content and high concentration of leaf-wax compounds suggest that this horizon received an unusually high input of terrestrial plant material. Analysis of the fossil pollen record in the sediments at site 3 confirms the presence of a terrestrial wetland/swamp at this depth.[2,10]

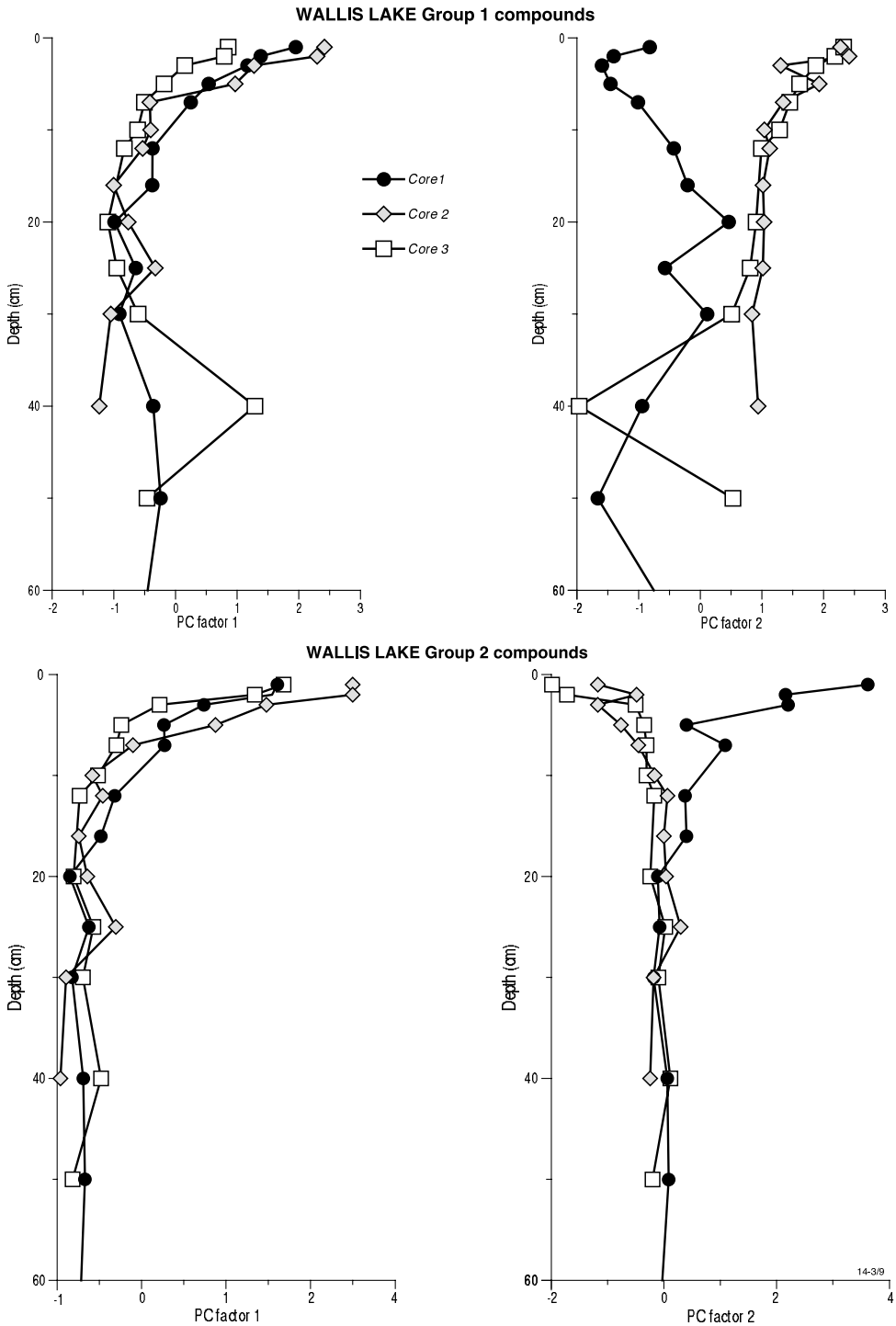

Figure 2. Principal components 1 and 2 plotted against depth. PC factor 1 for both compound groups is controlled by concentration/ diagenesis. PC factor 2 contains greater source information.

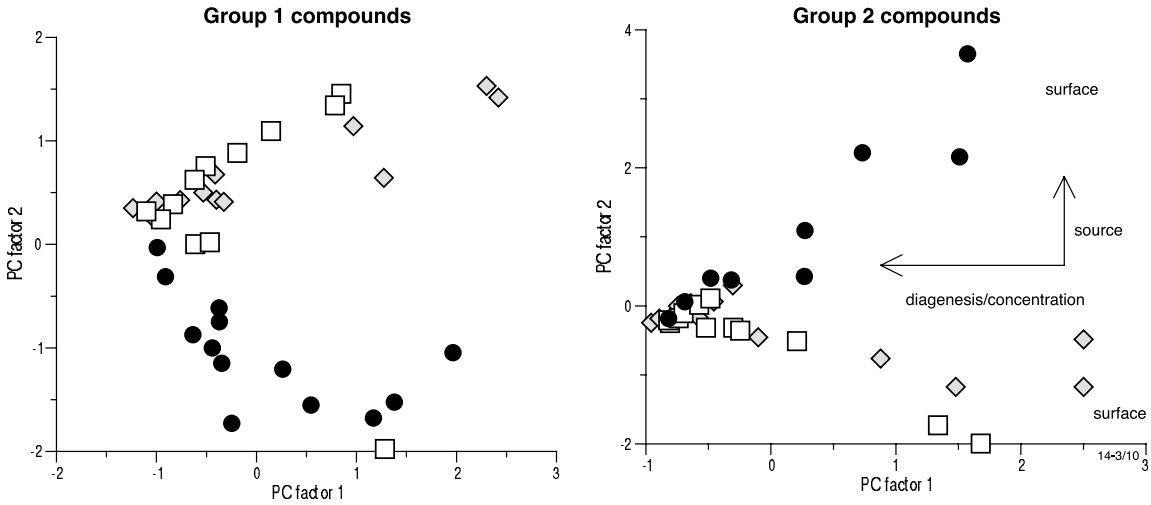

Figure 3. Cross-plots of principal components 1 and 2 for both compound groups illustrate that the two main controls on each grouping are the same.

This observation supports the data provided by biomarker analysis and helps explain the increased amount of organic carbon, as well as its unusually strong terrestrial plant signal.

Plotting the second principal component (PC2) of group 1 against depth shows that there is a clear difference between site 1 and sites 2 and 3, particularly from the surface to 20 centimetres (figure 2). These data suggest that organic matter deposited at sites 2 and 3 has not varied significantly during sedimentation of the cores. In contrast, the value of PC2 varies throughout the core collected at site 1, suggesting a variable input of a substantially different composition. Examination of the biomarker concentration data shows that samples from site 1 contain a group of terrestrial plant triterpenoids not present at either of the other sites, and that the components related to leaf waxes are more abundant in samples from this site. The data indicate that site 1 receives inputs from a particular group or species of terrestrial plants that is either not present in the catchment areas of sites 2 and 3 or not transported to these sites. Again this interpretation has been supported by pollen and spore analysis, which indicates that site 1 has received the greatest input of dry sclerophyll forest and woodland pollen, mainly *Eucalyptus gummifera* type.[10] The similarity between sites 2 and 3 indicates that they are receiving similar algal and plant inputs. Since site 2 was chosen to study the effects of urban impact, the lack of variation compared with site 3 indicates that organic matter from the urban catchment is not significantly affecting the biomarker profiles at site 2.

Group 2 (fatty acids and compounds <C_{20})

Group 2 compounds include biomarkers for phytoplankton, sediment bacteria and some higher plant material. PCA of group 2 compounds provides a similar interpretation to the group 1 data set. The value of PC1 is controlled primarily by concentration/diagenesis, and decreases with depth (figure 2). PC2 shows clear differences among sites, particularly in the upper 10–15 centimetres. Below this depth all sites are relatively similar. Although compounds of group 2 are less specific to terrestrial sources, the PCA cross-plot of factors 1 and 2 illustrates a similar divergence between site 1 and sites 2 and 3 (figure 3). As with biomarkers within group 1, differences in the upper sediment samples are due mainly to variation in terrestrial plant sources between site 1 and sites 2 and 3. The biomarker signal converges with depth. This similarity at depth, results from the presence of common microbial processes and the preferential preservation of terrestrial plant material at all sites.

Summary

The key points of this study are as follows:

1. Phytoplankton biomarkers (dinoflagellates, diatoms and green microalgae) are present in all cores.
2. Terrestrial inputs can be recognised at every site. Furthermore, specific land plant inputs from the Wallamba and Coolongolook Rivers are suggested by distinct biomarker abundances at sites 1 and 5.
3. No evidence of human faecal contamination, based on biomarker techniques, is identified in sediments at any site. Also, a specific urban biomarker signature could not be identified at any site.
4. Biomarkers have identified herbivore (cattle) faecal contamination at the mouths of the Wallamba and Coolongolook Rivers.
5. Freshwater from rivers at sites 1 and 5 may affect algal productivity or populations, based on decreases in the ratios of biomarkers related to certain algae.
6. Diagenesis and concentration affect biomarker distributions down each core.
7. Statistical methods can be used to assess changes in sources of biomarkers within a large data set.
8. The similarity of biomarkers at depth is the result of common microbial processes and the preferential preservation of terrestrial plant material at all sites.
9. The selective preservation of terrestrial plant material implies there is a relatively more rapid turnover of algal organic matter. This material will be an important source of nutrients and may support further algal productivity within the water column.

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