Sedimentary records of sewage pollution using faecal markers in contrasting peri-urban shallow lakes.

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Keywords: Attenborough; Coprostanol; Gravel Pit; Pb-210; Sewage; Stable Isotopes

ABSTRACT**:** Sewage contamination in shallow lake sediments is of concern because the pathogens, organic matter and nutrients contribute to the deterioration of the water-bodies health and ecology. Sediment cores from three shallow lakes (Coneries, Church and Clifton Ponds) within Attenborough nature reserve located downstream of sewage treatment works were analysed for TOC, C/N, $\delta^{13}C$, $\delta^{15}N$, bacterial coliforms and faecal sterols. ^{210}Pb and ^{137}Cs activities were used to date the sediments. Elemental analysis suggest that the source of organic matter was algal and down profile changes in $\delta^{13}C$ indicate a possible decrease in productivity with time which could be due to improvements in sewage treatment. $\delta^{15}N$ for Coneries Pond are slightly higher than those observed in Church or Clifton and are consistent with a sewage-derived nitrate source which has been diluted by non-sewage sources of N. The similarity in $\delta^{15}N$ values (+12‰ to +10‰) indicate that the three ponds were not entirely hydrologically isolated. Analysis by Gas-Chromatography Mass-Spectrometry (GC/MS) reveal that Coneries Pond had sterol concentrations in the range 20 to 30 µg/g (dry wt.), whereas, those from Clifton and Church Ponds were lower. The highest concentrations of the human-sourced sewage marker 5β–coprostanol were observed in the top 40 cm of Coneries Pond with values of up to 2.2 µg/g. In contrast, Church and Clifton Pond sediments contain only trace amounts throughout. Down-profile comparison of 5β−coprostanol/cholesterol, 5β−coprostanol/(5β−coprostanol+5α−cholestanol) and 5β-epicoprostanol/coprostanol as well as 5α−cholestanol/cholesterol suggest that Coneries Pond has received appreciable amounts of faecal contamination. Examination of 5β-stigmastanol, (marker for herbivorous / ruminant animals), down core concentrations suggest a recent decrease in manure slurry input to Coneries Pond. The greater concentration of β-sitosterol in sediments from Church and Clifton Ponds as compared to Coneries is attributed in part to their greater diversity and extent of aquatic plants and avian faeces.

1. Introduction

Sewage pollution is a major cause of decreasing water quality in rivers and lakes within the UK and throughout the world. The presence of human and animal faecal matter at elevated concentrations in waters and surface sediments in shallow lakes is of widespread concern for two reasons. Firstly, the complex chemical mixture causes nutrient enrichment, eutrophication, toxic algal blooms and water column anoxia which in turn can lead to a reduction in species diversity and ecosystem instability. Secondly, untreated sewage can, under specific conditions, provide a growth medium for bacterial and viral pathogens that if ingested by humans leads to diseases such as Salmonella, Cholera, Diarrhoea, Typhoid, Gastroenteritis and Hepatitis A (Mudge and Ball, 2006).

Estimation of sewage pollution is normally elicited from the quantification of total coliforms, faecal coliforms and faecal *Streptococci*, which, although not pathogenic, serve as proxies for pathogenic bacteria and total sewage input. However, the use of these indicator organisms provides little information concerning the source or age of the faecal material and requires that waters and sediments be analysed soon after collection. A complementary approach to evaluate sewage discharge and accumulation in rivers, lakes, estuaries and marine environments is to characterise specific groups of molecules contained within the sewage such as faecal sterols (Bull et al., 2002; Leeming et al., 1996; Mudge et al., 1999; Mudge and Duce, 2005; Peng et al., 2005; Readman et al., 2005; Seguel et al., 2001).

Previous investigations of faecal sterol contents in waters and sediments have tracked concentrations of between five to seventeen sterols including coprostanone, coprostanol, epicoprostanol, cholesterol, cholestanol, campestrol, stigmasterol, β-sitosterol, fucosterol and stigmastanol (Isobe et al., 2002; Leeming et al., 1996; Shah et al., 2006). The abundance and distribution of faecal sterols in excreta is controlled in part by an animal's diet as well as bacterially-mediated reductive modifications in the gut and also endogenous production of sterols such as cholesterol (Leeming et al., 1996). For example, in the intestinal tracts of many higher mammals, the biological precursor compound cholesterol is converted to 5β-stanols via biohydrogenation of the Δ^5 double bond to give 5β(H) stereoisomers. Similarly, cholesterol is converted in the gut of higher mammals to coprostanol via various intermediates by oxidation of the OH group at the C-3 position (Bull et al., 2002). Once in the aquatic or terrestrial environment compounds such as coprostanol can undergo further microbial reduction to yield the product epicoprostanol (Bull et al., 2002).

The Attenborough Ponds Nature Reserve (52º 53'58''N, 1º 14'09''W) is located within the conurbation of Nottingham, UK, and is designated a site of special scientific interest (SSSI) primarily because of the wide diversity of birds. The ponds are a series of ex-gravel pits covering an area of about 1.67 km^2 located on the floodplain of the River Trent. Excavation of Church Pond occurred from 1962-1965, Coneries Pond 1966-1968 and Clifton Pond 1964-68. Their location, similar size (0.49 to 0.1 km^2), depth (~3m) and mode of formation make them ideally suited to ecosystem-scale comparisons. The ponds (including Church, Clifton and Coneries Ponds) have varying histories of connectivity to the polluted Erewash, which drains a heavily urbanised catchment and receives effluent from seven sewage treatment plants. The first discharge information for the sewage treatment works were issued between 1981 and 1990 (Severn Trent Water, unpublished.). Methods of sewage treatment for works discharging into the river prior to this time are unavailable. In 1972, the course of the Erewash was diverted directly into Coneries Pond, which was at that time hydrologically connected to Church and Clifton Pond during periods of high water level. In 1981, engineering works isolated Clifton from Coneries Pond, resulting in the system that exists today where Church and Clifton Pond are isolated from the Erewash and Coneries Pond system in all but the most extreme flood events. Consequently Coneries waters are enriched in total P (TP; 540 μg/L) and total dissolved inorganic nitrogen (TDIN; 6 mg/L) and are turbid whereas Clifton (TP 73 μg/L, TDIN 0.2 mg/L), and Church (TP 184 μg/L, TDIN 0.2 mg/L) have much lower nutrient concentrations and clear water (mean concentrations between 2005-2008) (Cross, 2009).

The objectives of this work were three fold: (i) Identify the main sources of organic matter entering the Ponds; (ii) Establish whether Coneries, Church and Clifton Ponds had received the same amounts of faecal organic matter through time; and (iii) ascertain using sterol biomarker whether the source(s) of faecal organic matter had changed.

2. Sampling and methods

2.1 Sample Collection

Sediment cores were collected on 22 and 23rd May 2007 from Church Pond (SK 51600, 34150), Clifton Pond (SK 52300, 33697) and Coneries Pond (SK 51234, 33856) (Fig. 1). The deepest part of the lake was located for coring using a handheld echo sounder and position marked using a Garmin 12 GPS system. Cores were sampled using a wide-diameter (14 cm i.d.) Livingstone type corer specially designed for the retrieval of large volumes of sediment. The core tube was pushed into the sediment until an impenetrable layer (basal gravel and sands) assumed to mark the inception of the lakes was reached. After each successful deployment-retrieval cycle the core was transported back to shore, whereupon the core was extruded and sampled every 1 cm for coliform counting and every 2 cm for elemental and isotopic analyses. Sub-samples for faecal sterol and stanol concentrations were collected at 4 cm resolution (~10 g wet/wt) and were stored in polyethylene bags and transported back to the laboratory at ~4 ºC, then immediately frozen (–70 °C). Aside from the basal gravel/ sand layer none of the cores showed a clear sediment stratigraphy being comprised of a homogeneous mixture of dark organic rich silty clay.

2.2 ^{210}Pb and ^{137}Cs Chronology

Dried sediment samples from cores taken from Church Pond and Clifton Pond were analysed for ^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am by direct gamma assay in the Bloomsbury Environmental Isotope Facility (BEIF) at University College London, using ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector (Appleby et al., 1986). Lead-210 was determined via its gamma emissions at 46.5keV, and ^{226}Ra by the 295keV and 352keV gamma rays emitted by its daughter isotope ^{214}Pb following 3 weeks storage in sealed containers to allow radioactive equilibration. Cesium-137 and ^{241}Am were measured by their emissions at 662kev and 59.5kev (Appleby et al., 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self absorption of low energy gamma rays within the sample (Appleby et al., 1992).

2.3 %TOC, C/N, carbon and nitrogen isotope ratios

Total organic carbon and nitrogen from which we derive weight C/N ratios were analysed alongside carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios. %C, %N and $\delta^{13}C$ were measured on homogenized, acid-washed sediment while the $\delta^{15}N$ was measured on raw homogenized sediment. $^{13}C/^{12}C$ analyses were performed by combustion in a Costech Elemental Analyser (EA) on-line to a VG TripleTrap and Optima dual-inlet mass spectrometer, with $\delta^{13}C$ values calculated to the VPDB scale using within-run laboratory standards calibrated against NBS-18, NBS-19 and checked using NBS-22. Replicate analysis of well-mixed samples indicated a precision of $\pm$ <0.1‰ (1 SD). %TOC and C/N ratios were calibrated against an Acetanilide standard, with a precision of $\pm$ <0.1 for C/N. All C and N values in this current work are expressed on a weight ratio basis. $^{15}N/^{14}N$ analysis was performed on a ThermoFinnigan system comprising an elemental analyser linked under continuous flow with a Delta+XL mass spectrometer. Isotope ratios were calculated as $\delta^{15}N$ versus atmospheric N_2 by comparison with standards calibrated against IAEA N-1 and N-2. Analytical precision (1 S.D.) is typically <0.3‰.

2.4 Coliforms

In the laboratory two grams of sub-sample was transferred to a sterile universal container using aseptic technique. To each sub-sample 20 ml of sterile demineralised water was added and the contents centrifuged at 750 g for 10 minutes to disassociate bacterial cells from sediment samples (Furtado and Casper, 2000). The supernatant was then removed and used for microbial inoculations. The method used for enumeration studies was based on the standard method of membrane filtration (MF). The supernatant was filtered through a 0.45 µm cellulose nitrate filter (Gelman). Each filter was then placed onto a petri dish containing a pad saturated with Membrane Lauryl Tryptose Broth (Oxoid). The dishes were incubated for 24 hours at 35 ºC. Yellow colonies of between 1 mm and 3 mm were counted as presumptive coliform bacteria (total coliforms).

2.5 Faecal Steroid Preparation

Sediments were freeze-dried, sieved through a mesh aperture of 2 mm and ground to a fine powder in a ball mill (Retsch PM400). A 4-5 g aliquot of each powdered sediment was placed on a watch-glass and spiked with deuterated cholesterol (cholesterol-2,2,3,4,4,6-$_d$6) standard in toluene (5 ng/µl) (Sigma Chemical Co.). Thereafter, sediments were extracted with methanol/dichloromethane (MeOH/DCM) (1:1 v/v) using an accelerated solvent extraction system ASE 200 (Dionex) operated at a temperature of 100 °C and a pressure of 1500 psi. Activated copper powder (2 g) was added to remove elemental sulphur. The solvent was removed by evaporation using a turbovap system. The residue was reconstituted in 1 ml acetone, then transferred onto the surface of a silica gel column containing 5% H_2O deactivated silica (100-200 mesh) using a glass pipette. The silica column (1 × 9 cm) was first eluted with 20 ml hexane/DCM (3:1 v/v) then 40 ml DCM and finally with 30 ml acetone/DCM (3:7 v/v). The latter two fractions were combined, the solvent evaporated under a gentle stream of N_2 and the residue dissolved in 0.5 ml acetone prior to quantitative transfer to a glass vial (1.75 ml). Acetone was removed by evaporation with N_2 and the sample reconstituted in 0.9 ml of pyridine to which perylene-$_{d}$12 extraction efficiency standard in toluene was added. Prior to analysis, mixtures were silylated by heating in an oven at 50°C for 30 min with 50 µl of N, O *bis* (trimethylsilyl)trifluoroacetamide (BSTFA) with 1% TMCS (Sigma Chemical Co.) .

2.6 Gas Chromatography-Mass Spectrometry

Faecal sterols were analysed using a Varian CP3800 series gas chromatograph (GC) directly coupled with a Varian 1200L triple Quadropole MS/MS system (GC/MS). Sample injection (1.0 µl) was in splitless mode. Compounds were separated using a Varian Factor 4 VF-5MS column (30 m length × 0.25 mm i.d. × 0.25 µl film thickness). The oven temperature was programmed from 60 °C (1 min isothermal) to 250 °C at 20 °C min^{-1} then to 310 °C at 4 °C min^{-1} and held isothermally at 310 °C for 10 min. The mass spectrometer was operated at 70 eV with a mass range of m/z 30-550 (beam current 150 µA, source temperature 150 °C) with helium as carrier gas at a flow rate of 1 ml/min. Data acquisition was carried out using a Varian MS workstation v6.5. Peak assignments were made by comparison with published mass spectra and mass spectra and retention times of authentic standard compounds (Appendix 1). The limit of quantification for individual compounds ranged from 0.01-0.04 µg/g (dry wt), procedural blanks as well as reagent blanks contained no significant amounts of sterols.

2.7 Faecal Biomarker Nomenclature

Common compound names were used throughout this work to enable comparison with previous studies. The eleven faecal sterols measured were cholestane (5α-cholestane), coprostanol (5β-cholestan-3β-ol), 5β-epicoprostanol (5β-cholestan-3α-ol), cholesterol (cholest-5-en-3β-ol), 5α-cholestanol (5α-cholestan-3β-ol), coprostan-3-one (5β-cholestan-3-one), campesterol (24α-methyl-5-cholesten-3β-ol), stigmasterol (3β-hydroxy-24-ethyl-5,22-cholestadiene), fucosterol ((3β,24E)-stigmasta-5,24(28)-dien-3-ol), β-sitosterol (24-ethylcholest-5-en-3β-ol) and 5β-stigmastanol (24α-ethyl-5α-cholestan-3β-ol); chemical structures are presented in Appendix 1.

2.8 Statistical Analyses

Multi-variate ordination techniques were carried out using the vegan package in R (Oksanen et al., 2010; R Development Core Team, 2010) to explore the dominant patterns and inter-relationships in the sterol data. Following Detrended Correspondence Analysis (DCA), which indicated a linear response, Principal Component Analysis (PCA) was carried out to assess the main gradients of variation at each of the three sites. All data were down-weighted to reduce the influence of abundance sterols on the ordination outputs with samples containing no measureable sterol concentrations removed from the dataset. Sample depth 62 cm at Church Pond was also removed as an outlier in the ordination analyses, due to the high amount of coprostanol within the sample.

3. Results and Discussion

3.1 Lead-210 activity and artificial fallout radionuclides

Cores from Church and Clifton were first assessed using ^{137}Cs radionuclide and ^{210}Pb activity in order to develop a sediment chronology (Figs. 2 and 3). Lead-210 (half-life is 22.3 year) is a naturally-produced radionuclide, derived from atmospheric fallout (termed unsupported ^{210}Pb). Caesium-137 (half-life is 30 years) and ^{241}Am are artificially produced radionuclides, introduced to the study area by atmospheric fallout from nuclear weapons testing deposition (maximal ~1963) and nuclear reactor accidents (e.g. Chernobyl, Ukraine 1986). They have been extensively used in the dating of recent sediment in lakes and estuaries (Appleby, 2001; Vane et al., 2009).

The ^{137}Cs activity versus depth profile for Church Pond reveals a broad peak between 20 and 44 cm, suggesting 1963 occurs between these depths (Fig. 2). Unsupported ^{210}Pb activity, calculated by subtracting ^{226}Ra activity from total ^{210}Pb activity, declines more or less exponentially with depth in the top 20 cm (Fig. 2). Deeper than 20 cm, unsupported ^{210}Pb activities show non-monotonic features, with relatively low unsupported ^{210}Pb at 29.5 cm. The inventory of unsupported ^{210}Pb yields a mean unsupported ^{210}Pb flux to the sediments at 137 Bq m^{-2} yr^{-1}, which is at a similar level of deposition in the region. Lead-210 chronologies were calculated using the CRS dating model (Appleby and Oldfield, 1978). The raw CRS dating model places the 1963 layer at 40 cm, which is in between 20 and 44 cm suggested by the ^{137}Cs record. The average sedimentation rate of the core was about 0.28 g cm^{-2} yr^{-1}. Sedimentation rates calculated by unsupported ^{210}Pb data were relatively uniform but with a sharp peak, suggesting rapid accumulation in the late 1970s and may represent disturbance of lake bed material associated with blockage of the lake from the Coneries chain ca. 1980. Prior to isolation, variable river flows would have resulted in high sedimentation rates and a greater degree of sediment disturbance. Once isolated, the monotonic decline in Pb-210 activity is consistent with a more stable depositional environment. Historical records date the time of last gravel extraction and presumably the on-set of sedimentation in Church Pond at 1964 and in 1967 at Clifton Pond.

The Clifton Pond ^{137}Cs profile is poorly defined (Fig. 3) and consequently radionuclide depositional events such as Chernobyl and maximal emissions from atomic weapons testing are not identifiable. The relatively constant ^{137}Cs activities below 19 cm may be due to variable deposition of material transported into the lake while it was connected to the main lake chain. There is an irregular decline in unsupported ^{210}Pb in the top 20 cm of the core, but little net decline in unsupported ^{210}Pb activities below this with low unsupported ^{210}Pb activities suggesting relatively high and variable sedimentation rates, with sediment disturbance. Mean unsupported ^{210}Pb flux to the sediments was calculated at 148 Bq m^{-2} yr^{-1}. ^{210}Pb chronologies were calculated using the CRS dating model. Mean sedimentation rate was about 0.29 g cm^{-2} yr^{-1} and was higher between the 1960s and 1980s. The ^{210}Pb profile is consistent with greater disturbance below ca. 20cm core depth (1980s), suggesting that the isolation of Clifton Pond in 1981 resulted in more uniform sediment deposition and less sediment disturbance (Sayer and Roberts, 2001). As for Church Pond, the lake inception date of 1964 would infer more rapid sedimentation in the lower part of the core than calculated by the CRS model.

3.2 %TOC, C/N and carbon isotope ratios

These data are presented in Figure 4. Downcore %TOC profiles for the three ponds show a systematic decrease in organic carbon content from the surface to the base (approximately 75 cm). The organic carbon contents range from *ca.* 2 to 6% in Coneries, 2 to 5% in Church, and 4 to 5% in Clifton. These concentrations are fairly typical of modern shallow lakes located in a peri-urban environment accumulating decaying vegetation from a variety of sources, as well as atmospheric and waterborne anthropogenic pollution. C/N ratios are widely used as source indicators for organic matter (Meyers, 1997) and tend to range 3-9 (in aquatic; protein rich plants), 10-20 (in aquatic/terrestrial plants) and > 20 (in terrestrial biomass; protein poor plants) and are thus used as an indicator of changes in allochthonous and autochthonous organic matter in freshwater systems (Meyers and Teranes, 2001). In the Attenborough Ponds, C/N ratios are fairly constant at between 8-10; values which tend to be indicative organic matter of aquatic origin. From the work of (Cross, 2009) this most likely represents phytoplankton (*Aulacoseira*, *Asterionella*, *Synedra*), cryptophyceae (*Cryptomonas*, *Rhodomonas*), and chlorophyceae (*Ankyra*, *Chalmydomonas*, *Tetradon*, *Tetrastrum*). Cross (2009) reported elevated concentrations of the latter class of green algae, ranging from 73 µg/L in Coneries Pond to 13 µg/L in Clifton Pond.

Most types of algae produce organic matter with $\delta^{13}C$ values about 20‰ lower than the value for dissolved bicarbonate (HCO_3^-), the main reservoir of inorganic carbon (Leng et al., 2005). On this basis the $\delta^{13}C$ values of –29 to –25‰ typical of the ponds' sediments would suggest a bicarbonate source with $\delta^{13}C_{bicarbonate}$ within the range –9 to –5‰; values that are higher than those typical of UK groundwaters (Andrews et al., 1993; Andrews et al., 1997), suggesting an additional source of inorganic carbon (e.g. atmospheric CO_2, through long residence time, or carbonate from limestone aquifers). $\delta^{13}C_{bicarbonate}$ values will also be influenced by organic productivity, with values increasing with production as the lighter isotopes are utilised by the algae and incorporated into the organic sediment. A reduction in the proportion of additional sources of inorganic carbon, or a decrease in productivity are therefore some of the factors which might cause changes in the ponds' organic $\delta^{13}C$ values (Leng et al., 2005). The upward decrease in core $\delta^{13}C$ values might for example reflect reduced limestone influence with the cessation of quarrying, or changes in nutrient inputs with changes in sewage management.

3.3 Nitrogen isotope ratios

In common with carbon, the isotope composition of sources of nitrogen and factors influencing productivity are amongst the most important controls on the $\delta^{15}N$ values of organic matter depositing to sediment in these shallow, well-mixed lakes (Leng et al., 2005). A comparison of the up-core changes in $\delta^{15}N$ and $\delta^{13}C$ values in fact reveals some co-incidence: the largest changes for both values are observed for Coneries between 70 to 60 cm depth, and for Church between 50 to 0 cm depth. However, the fact that the changes are in the opposite direction ($\delta^{15}N$ increasing and $\delta^{13}C$ decreasing upwards) tends to argue against a change in organic productivity as the dominant cause. We therefore consider the potential sources of N.

Sewage is clearly a major potential source of nutrient N in some of the Attenborough ponds. In many cases it has high $\delta^{15}N$ values, above +10‰ (Heaton, 1986; Kendall et al., 2007), and this is confirmed for our study by a single analysis of Erewash river nitrate from close to the Toton sewage works in April 2007, which gave a $\delta^{15}N$ value of +14.2‰. In contrast, most other sources of N (atmospheric deposition, fertilisers, soils, etc.) tend to have $\delta^{15}N$ values well below +10‰ (Heaton, 1986; Kendall et al., 2007), and where N-fixing cyanobacteria contribute to sediment (as they may do in Church and Clifton Ponds (Cross, 2009)), this will also reduce $\delta^{15}N$ values. Sediments in lakes in remote regions of the UK, for example, typically have $\delta^{15}N$ values below +5‰ (Jones et al., 2004). In simple source terms we would therefore expect $\delta^{15}N$ values to be most affected by the relative proportions of sewage and non-sewage inputs of N to the different ponds; and increases in macrophyte $\delta^{15}N$ in response to increased sewage inputs have been well documented elsewhere (Cole et al., 2004).

It is therefore perhaps surprising that the $\delta^{15}N$ values of recent sediments in the Church, Clifton and Coneries Ponds are so similar: the uppermost sections of the cores all have $\delta^{15}N$ values between +10 to +12‰ (Figure 4), and sediment traps have also yielded $\delta^{15}N$ values above +10‰ for all three ponds. These values may be expected for Coneries Pond, which is directly supplied by the Erewash. The fact that Church and Clifton Ponds have similarly high $\delta^{15}N$ values suggests that they may also derive much of their nutrient N from a similar sewage source, albeit at a lower concentration. This could occur as leakage of Erewash water through the gravel banks surrounding Church and Clifton Ponds.

3.4 Coliform counts

Total coliform counts in sediment cores from Clifton, Church and Coneries Ponds are presented in Figure 5. Bacterial coliform numbers were highest between 0-10 cm for all three cores and no coliforms were detected at depths >64 cm at any of the sites. Coliform numbers in Church and Coneries were maximal at ~400 colony forming units (CFU)/g sediment, whereas the coliform counts for Church remained at <200 CFU/g sediment throughout (Fig. 5). Comparison of the depth profiles reveals that Clifton and Church were somewhat similar, with the greatest coliform numbers just beneath the sediment surface and no detectable coliforms observed between 30-40 cm and 50-60 cm. In contrast, relatively high numbers of coliforms were detected between 20-30 cm and 30-40 cm as well as 50-60 cm at Coneries (Fig. 5). Research on faecal coliforms in sediments and waters has shown extended survival in the former due in part to factors such as organic matter content and sorption which provides protection against bacteriophage (Burton et al., 1987; Stenstrom and Carlander, 2001). However, survival of viable enteric bacteria (*Pseudomnas aeruginosa*, *Salmonella Newport*, *Escherichia coli* and *Klebsiella pneumoniae*) in two lake and two river sediments in USA extended to no more than a few months (Burton et al., 1987). Similarly, T50- values of *E. coli*, faecal enterococci, *Clostridium* and coliphages in constructed wetland sediments ranged from 27-370 days (Stenstrom and Carlander, 2001). Sediments act as a reservoir for bacteria and can via the mechanism of sediment re-suspension contribute to bacterial numbers in overlying surface waters (Obiri-Danso and Jones, 2000). In light that indictor organism tests such as total coliform counting require viable bacteria, and that the literature suggest that these are not particularly long lived, it is not unexpected that the highest number of coliforms were observed in the uppermost interval of the sediment cores. The frequent occurrence of detectable coliforms down core at Coneries maybe attributed to the combined effect of either: Coneries receiving a greater amount and more regular supply of faecal matter than either Clifton or Church; or bioturbation of the sediment column.

3.5 Sterol and Stanol Concentrations

Total sterol concentrations decrease down profile in all three shallow lake sediment cores. Church and Clifton are broadly similar, with the highest values observed at the surface (0-4 cm) and a gradual fall in sterol concentration to <10 µg/g at 12-16 cm (Fig. 6). Small changes in sterol concentrations occur from 12-16 to 56-60 cm and an increase in sterol values occurs in Clifton at 58-68 cm and Church at 60-64 cm (Fig. 6). In contrast, Coneries total sterol depth-profile shows higher concentrations in the range of 20 to 30 µg/g at the surface to 36-40 cm depth; thereafter, there is a progressive decrease in concentrations to the base of the core at 74 cm.

Ten of the eleven individual sterols were observed in this study; the compound 5α-cholestane was not detected in any of the sediments. As expected cholesterol is the most abundant sterol in the surface sediments at Coneries with concentrations in the range of 4.1 to 8.5 µg/g whereas, β-sitosterol is the principal sterol at the surface in Church and Clifton with concentrations in the range of 6.5 and 16.1 µg/g (Fig. 6). β-sitosterol is known to be derived from vascular plants and together with cholesterol, stigmasterol and campesterol are the main sterols which undergo reduction by enteric bacteria to yield 5β-stanols (Leeming et al., 1996). The greater concentration of β-sitosterol in the surface sediment at Church and Clifton as compared to Coneries may be due to the diverse range of aquatic plants whereas Coneries is devoid of aquatic plants. However, sterols from avian faeces are reported to be mainly comprised of β-sitosterol with lower amounts of cholesterol as well as 24-ethylepicoprostanol and trace quantities of other sterols (Shah et al., 2007). Therefore, the predominance of β-sitosterol in the surface sediments at Clifton and Church could be due to direct input from higher plants or possibly from the accumulation of avian derived faecal matter or a combination of the two. The latter multiple-source explanation is probably most plausible because Attenborough Ponds is an important wildlife refuge for ~80 species of birds and the shallow gravel-pit lakes are vegetated with *Phragmites communis* (Reed) *Typha latifolia* (Bull-rush) *and Spargamium erectum (Bur-reed)* and the banks are populated with willow, ash and alder trees. Although this supposition appears to contradict the TOC, C/N and δ^{13}C interpretation (section 3.2) which suggests that all three ponds mainly accumulated organic carbon from algal sources, it should be borne in mind that molecular biomarkers including sterols presented herein only represent a small fraction of the bulk organic matter that maybe undetected in the bulk measurements.

A different situation occurs in Coneries Pond where 5β-stigmastanol is present at relatively high concentrations of up to 14.5 μg/g at 32-36 cm depth (Fig. 6). Previous studies have reported that 5β-stigmastanol is derived from the intestinal microbial reduction of the plant derived marker sitosterol (Grimalt et al., 1990). Therefore, the presence of 5β-stigmastanol at elevated concentrations relative to other sterols could indicate faecal matter from herbivorous animals and, particularly, ruminant animals such as cows and sheep. Within Coneries Pond, the high proportion of 5β-stigmastanol relative to other sterol markers in the mid-portion of the core profile suggests a possible manure/slurry input and that the accumulation had recently decreased as evidenced by the decline in 5β-stigmastanol values at the near surface <10 cm (Fig. 6). Clear evidence that Coneries has also received input from plant matter is suggested by the presence of another sterol plant marker, campesterol, at concentrations up to 3.5 μg/g in 12 out of 18 sediment levels analysed (Reeves and Patton, 2005). The absence of aquatic plants in Coneries Pond confirm the notion that plant derived matter and associated sterols have been washed into the shallow lake. Comparison of the Clifton total sterol and 5β-stigmastanol profiles show a clear co-variance which suggests that the small increase in total sterol values at 58 cm depth was probably due to input of herbivore faecal matter.

Concentrations of coprostanol in the samples from Coneries range from 2.2 at surface to 0.04 μg/g (dry wt) at a depth of 70 cm with an average of 0.64 μg/g (Fig. 6). In contrast, coprostanol concentrations are low and declined rapidly at Church and Clifton with the exception of a single coprostanol peak at a depth of 62 cm in Church. In general, sediments with coprostanol concentrations of 0.5 μg/g are considered to have received appreciable amounts of sewage pollution (Readman et al., 2005). Thus, using this criteria, almost the entire Coneries core is polluted with sewage, Church is contaminated at 2 cm and 62 cm depth and Clifton is not polluted, the highest coprostanol concentration being 0.4 µg/g at surface. This likely reflects the fact that although both Church and Clifton Ponds were connected to the Erewash system prior to 1981, they were less directly connected than Coneries, which lies directly between the main river inflow and the major outflow. Although treated sewage effluent is discharged into the Erewash in liquid form, the high abundance of coprostanol in Coneries as compared to Church and Clifton supports the view that faecal sterols rapidly partition into the solid phase (Mudge and Ball, 2006) and their accumulation is then controlled by fluvial/lacustrine sedimentation processes.

3.3 Application of sewage indicator ratios to source apportionment

The ratio 5β-coprostanol to total sterol provides one measure of human derived sewage input; it has also been demonstrated that human faecal contamination is indicated by coprostanol:cholesterol values >0.2. Raw untreated sewage typically has a 5β-coprostanol / cholesterol ratio of ~10, which decreases through a sewage treatment plant (STP) such that in the discharged liquid wastewaters the ratio is approximately 2; undiluted STP wastewaters may be identified by this high ratio. As the faecal matter is dispersed in the environment, the ratio will decrease as more (non-faecal) cholesterol from animals is encountered (Grimalt and Albaiges, 1990; Grimalt et al., 1990). In this study, coprostanol was detected in 16 out of 18 sediment levels in Coneries Pond, ranging from 2.2 ng/g at the surface to 0.1 ng/g at a depth of 62 cm (Fig. 6). Down core, coprostanol to cholesterol ratios are greater than or equal to the 0.2 threshold value in 14 of the 18 sediment depth intervals, confirming that Coneries Pond has been subject to human sourced faecal matter which had been treated (Fig. 7). One plausible explanation is that the sewage treatment plants on the Erewash had discharged into the river which flows into Coneries Pond. Lower ratios ranging from 0.1 to 1.9 occur in the three levels at the base of the sediment core suggesting lower contribution of sewage sourced faecal matter in the early 1970s. In contrast, sediments from Clifton or Church Ponds gave coprostanol:cholesterol ratios ranging from 0 to < 0.2, which suggests that the sediments have not been subject to a significant amount of human-sourced sewage pollution (Fig. 7). Taken together this suggests that earth embankments that separate the Ponds (Fig. 1) may act as filters for the particulate organic matter (including sewage).

Human sourced faecal contamination can be tracked in sediments using the proportion of coprostanol:(coprostanol+5α-cholestanol) (Grimalt et al., 1990; Grimalt and Albiages, 1990). 5α-cholestanol is formed naturally in the environment by bacteria and generally does not have a faecal origin. Sediments with coprostanol:(coprostanol+5α-cholestanol) values > 0.7 are considered contaminated with human faecal matter whereas those with values < 0.3 are categorised as uncontaminated. Sediments with ratios between these criteria can not be readily apportioned on the basis of this ratio alone. In this work, Coneries sediments at 54, 62, 66 and 70 cm depth gave ratios of 1.0 because no 5α-cholestanol was detected; at shallower depths, ratios varied in a non-systematic manner from 0.1 to 0.34 (Fig. 7). Examination of the Clifton coprostanol:(coprostanol+5α-cholestanol) profile showed low values indicating minimal human faecal pollution and similarly Church yielded ratios of between 0 to <0.3 with the exception of 62 cm depth which gave a value of 1.0 (Fig. 7). During sewage treatment, 5β-coprostanol may be converted to the 5β-cholestan-3α-ol form, epicoprostanol, and there is also a slow conversion of 5β-coprostanol to epi-coprostanol in the environment and so this ratio will indicate either the degree of treatment of sewage or its age in the environment (Mudge and Ball, 2006). In the current study Coneries 5β-coprostanol to epicoprostanol ratio varied from 0.2 to 0.6 and Clifton gave ratios of 1.1 and 0.6 at 2 and 6 cm depth respectively (Fig. 7). A cross-plot of the 5β-coprostanol / cholesterol ratio against the epi-coprostanol / 5β-coprostanol ratio can indicate both faecal contamination and treatment (Fig. 8). The sediments from Church and Clifton Pond indicate little sewage input and / or a high degree of treatment whereas sediments from Coneries Pond plot in an area indicative of greater sewage input.

It has been previously reported that in sediments, bacteria preferentially produce 5α-cholestan-3β-ol (5α-cholestanol) from cholesterol rather than the 5β isomer (Bull et al., 2003; Bull et al., 2002). This reaction occurs principally in anaerobic reducing sediments and the 5α-cholestanol / cholesterol ratio may be used as a secondary (process) biomarker for such conditions. No cut-off values have been suggested for this marker and so it is used in a relative sense; the greater the ratio, the more reducing the environment. The 5α-cholestanol / cholesterol vertical profiles are presented in Figure 9. Clifton sediments show a rather constant value of ~1, Church sediments were maximal at 2.8, whereas Coneries values range from 0.5 to 6.1 indicating a possibly more reducing environment at 20 to 50 cm (Fig. 9). It is also plausible that the rise in 5α-cholestanol / cholesterol ratios between 50 to 25 cm depth in Coneries could be related to changing redox conditions in the sedimentary column and or varying sewage treatment practices such as the introduction of filter beds and activated sludge plants.

3.3 Principal component analysis

PCA indicates that a single variable at each lake is dominating the main patterns of variability in the sterol data with the first axis explaining 82.5%, 71.0% and 67.7% of the variability for Clifton, Church and Coneries Pond respectively (Figs. 10-11). Combining the datasets together indicates that that this variable is constant across all sites (PCA axis 1 eigenvalue = 0.613) and when combining sites individually (Fig. 10 Clifton and Church). The first axis is dominated by sterol characteristic of faecal matter such as β-sitosterol and and the by product of sewage treatment epicoprostanol.

The presence of faecal sterols strongly aligned to the first PCA axis that are representative of increasing faecal matter content suggests that the first PCA axis represents a sewage gradient, reinforcing our interpretation that the three lakes have been strongly dominated by changes in influx over their history. The observation that the uppermost samples in each lake are increasing aligned and to the right of PCA axis 1 (increasing faecal matter input) further suggest that inputs have significant increased in recent time. Conversely, the cluster of sample depth below c. 30 cm to the centre and left of the axis suggest that another unknown variable is controlling sterol input.

4. Conclusions

The application of bulk geochemical and isotopic (TOC, C/N, $\delta^{13}C$, $\delta^{15}N$), total coliforms and eleven faecal sterol biomarkers in sediment cores from three shallow lakes have proved useful for several reasons.

1) In a system of ponds where some are supposedly isolated the $\delta^{13}C$ and $\delta^{15}N$ show significant inter-lake consistency. Taken together, C/N and $\delta^{13}C$ values indicate that

the main source of organic matter in the sediments was of algal origin possibly augmented by minor contributions of vegetation and that the productivity of the lakes varied temporally. One plausible explanation is that the changes in productivity were driven by improvements in sewage treatment works (which remove nutrients other than N, utilised by phytoplankton) located upstream of the ponds and nitrate pollution does not decrease with time. $\delta^{15}N$ values of up to +12‰ suggest that Coneries Pond received a greater amount of sewage pollution than the other ponds. The relatively elevated $\delta^{15}N$ of +10‰ at Church and Clifton implies that they also receive treated sewage effluent from the Erewash and connected Coneries Pond.

2) Molecular level characterisation of sterol and stanol content of sediment cores from three shallow peri-urban lakes (ex-gravel pits) reveal that Coneries Pond had received a greater input of sewage than Church or Clifton Ponds. Using the coprostanol / cholesterol criteria of >0.2 to indicate sewage pollution it is possible to infer that Coneries has continually received and accumulated sewage since it's excavation in 1968. We hypothesise that the greater amounts of treated human sourced faecal matter in Coneries as compared to Church or Clifton is a function of partitioning of faecal sterols to the particulate phase and, the sinking of these particulates when the Erewash current slows as it enters Coneries Pond.

3) This study also demonstrates the utility of a molecular approach to understanding the shallow lake sediments in that the dominance of β-sitosterol indicated vegetation sourced organic matter in Clifton and Church Pond even when bulk geochemical and isotope do not. Furthermore, the concentration of specific biomarkers such as 5β-coprostanol and ratio of coprostanol to cholesterol ratios clearly distinguish human from ruminant sources at Coneries Pond. However, application of epicoprostanol to

5β-coprostanol ratios and 5β-coprostanol to cholesterol values to infer the degree of treatment of sewage or its age in the environment gave results which were ambiguous.

Overall, the extraction and analysis of sewage biomarkers in sediments provides environmental forensic information that complements bulk geochemical and isotopic data and supplements traditional microbiological methods used in the study of lakes.

4. Acknowledgements

The authors would like to thank Dr Thomas Davidson and colleagues of the University College London for assistance in coring Attenborough Ponds, Iain Cross and Teresa Needham (University of Nottingham) for help with the field and laboratory work, Cemex and Nottinghamshire Wildlife Trust for permitting access to the site and Sandy Aitken for providing historical information about the formation of the ponds. We are also grateful to three anonymous reviewers for constructive comments. Severn Trent Water Ltd. provided information about discharge consents into the River Erewash. The fieldwork was funded by the School of Geography, University of Nottingham. This paper is published by permission of the Executive Director, British Geological Survey.

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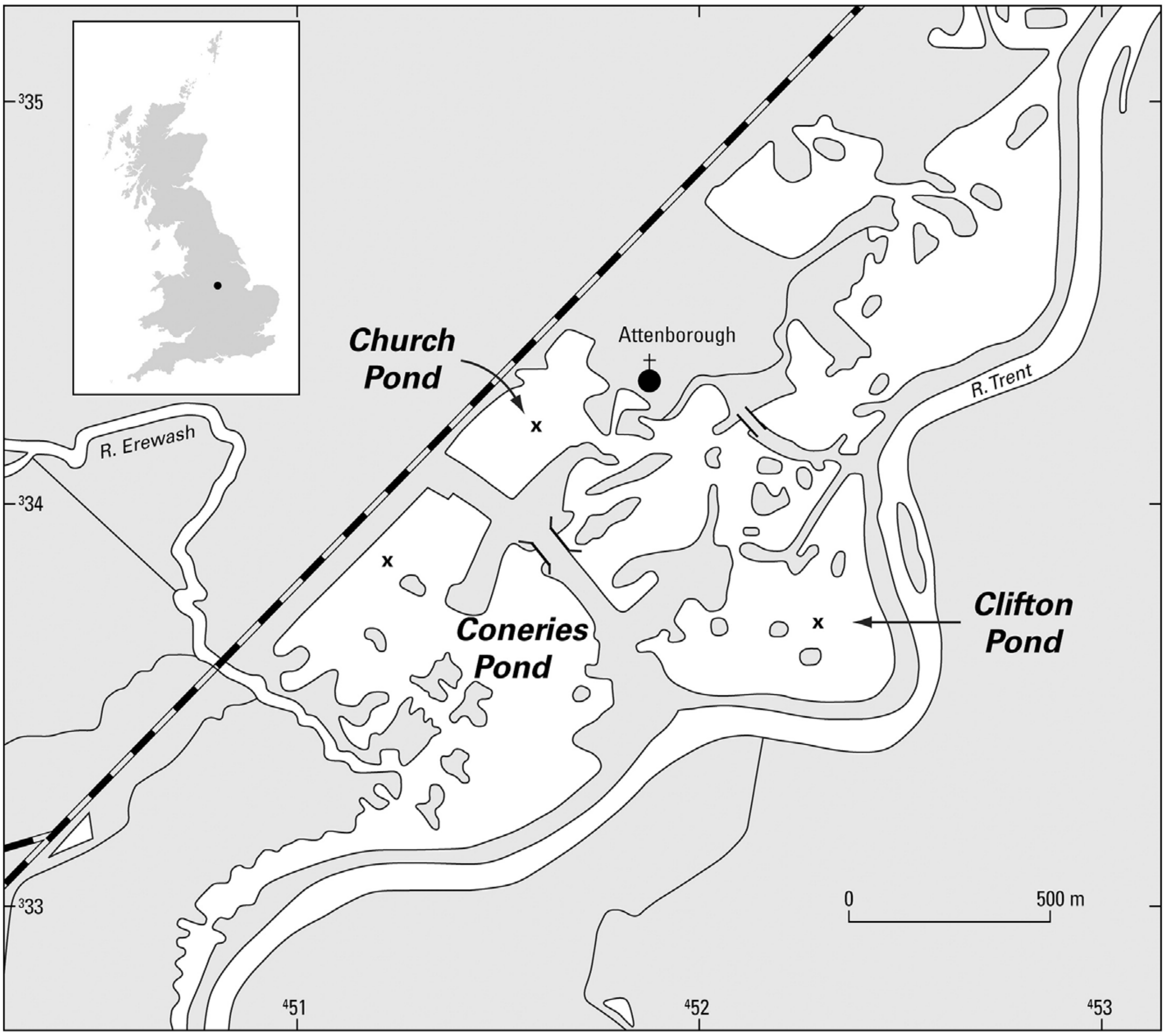


Fig 1. The Attenborough Nature Reserve Nottinghamshire, UK containing the Coneries, Clifton and Church Ponds.

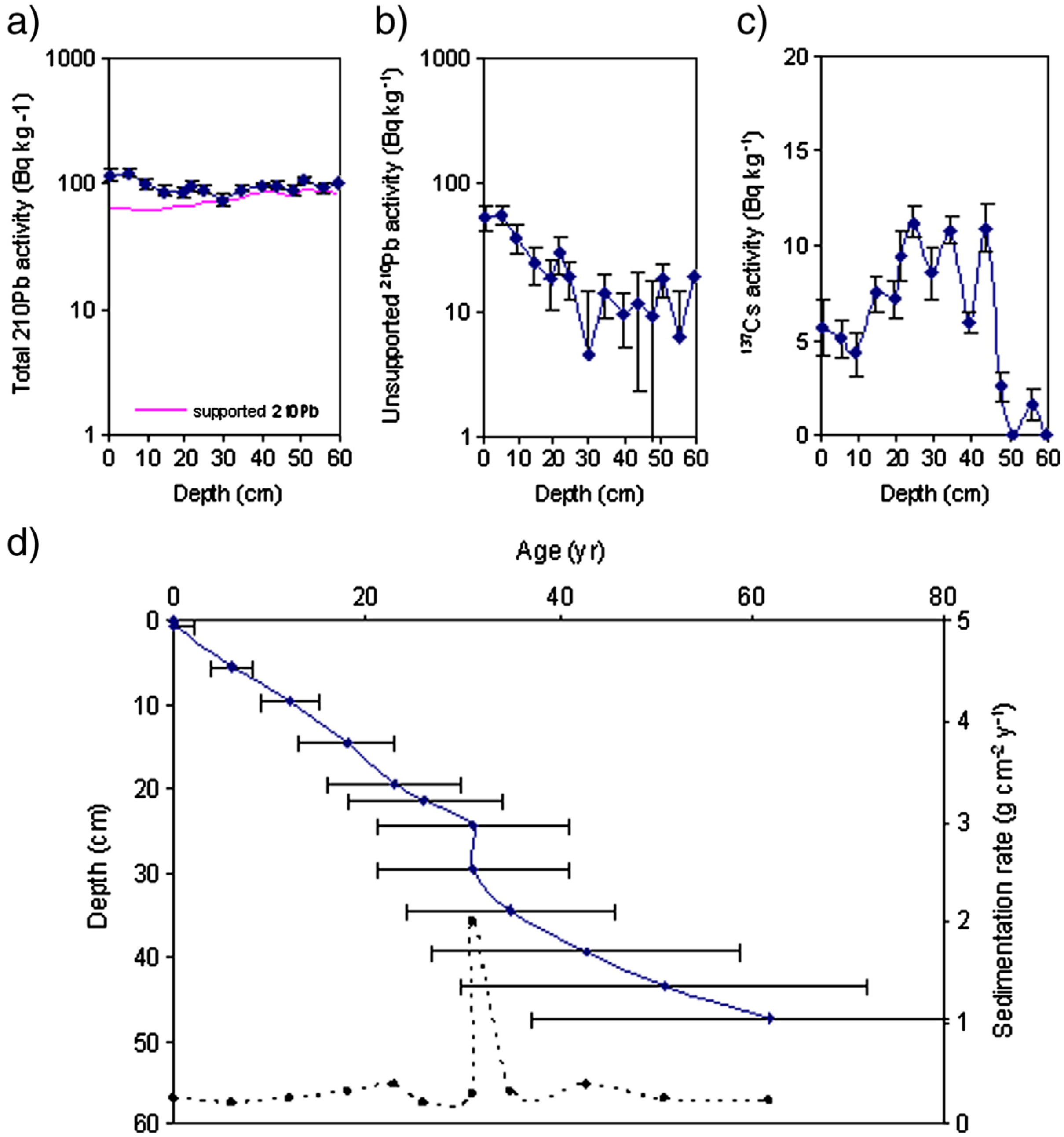


Fig 2. Fallout radionuclide concentrations in Church Pond core showing (a) total ^{210}Pb, (b) unsupported ^{210}Pb, (c) ^{137}Cs concentrations versus depth, and (d) radiometric chronology showing the CRS model ^{210}Pb dates.

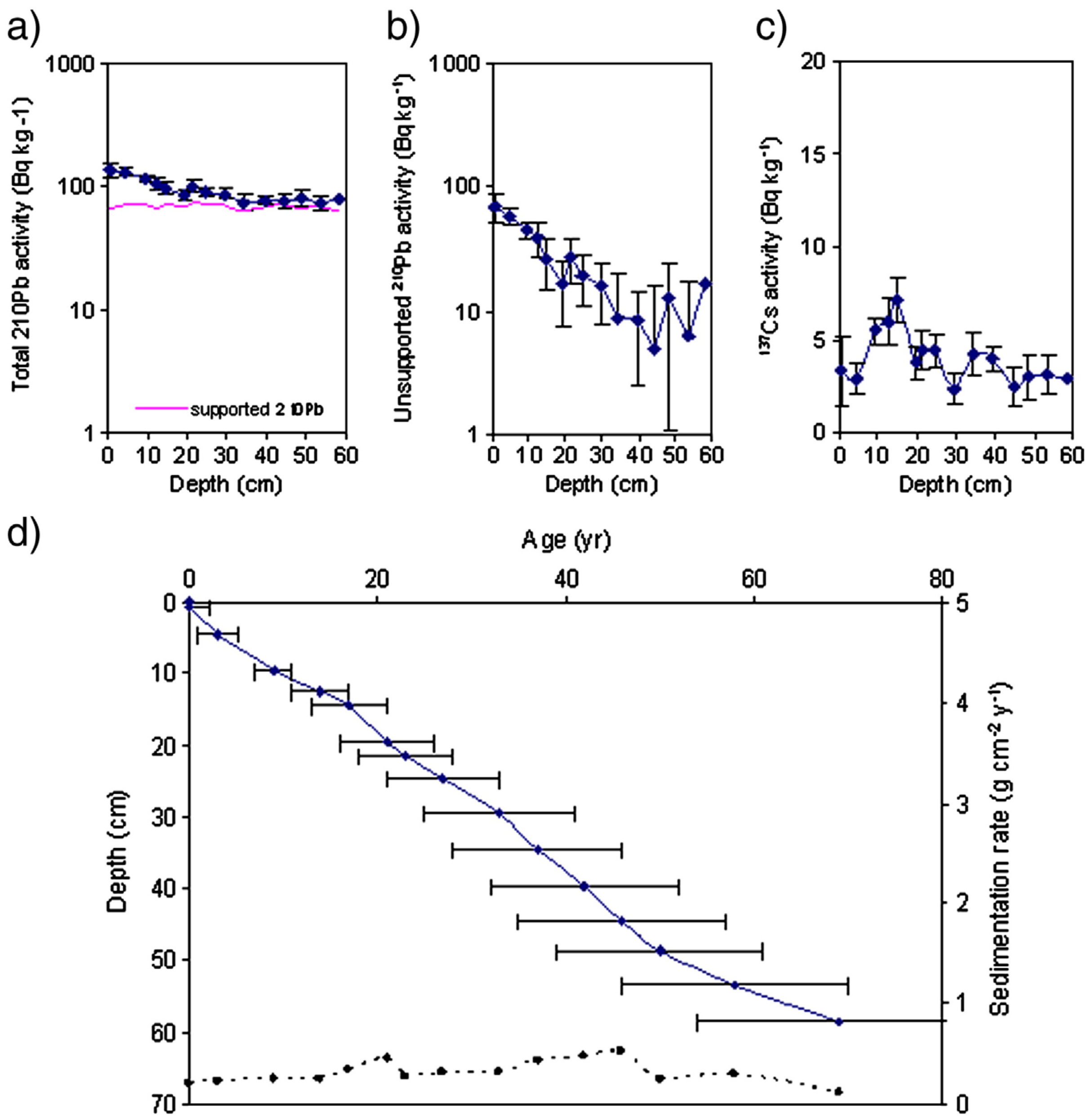


Fig 3. Fallout radionuclide concentrations in Clifton Pond core showing (a) total ^{210}Pb, (b) unsupported ^{210}Pb, (c) ^{137}Cs concentrations versus depth, and (d) radiometric chronology showing the CRS model ^{210}Pb dates.

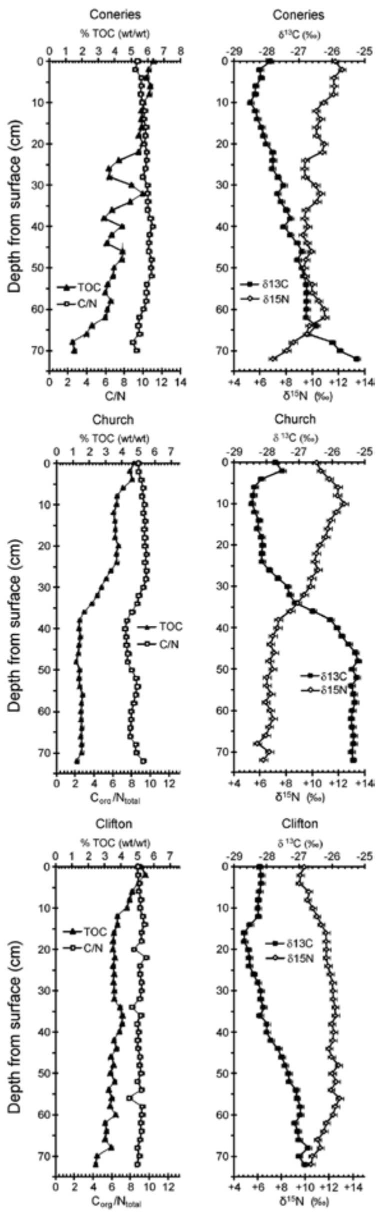


Fig 4. Comparison of organic carbon (TOC), organic carbon to nitrogen ratios (C/N), and organic $\delta^{13}C$ and $\delta^{15}N$ values in sediment cores from Attenborough Ponds, Nottinghamshire, U.K.

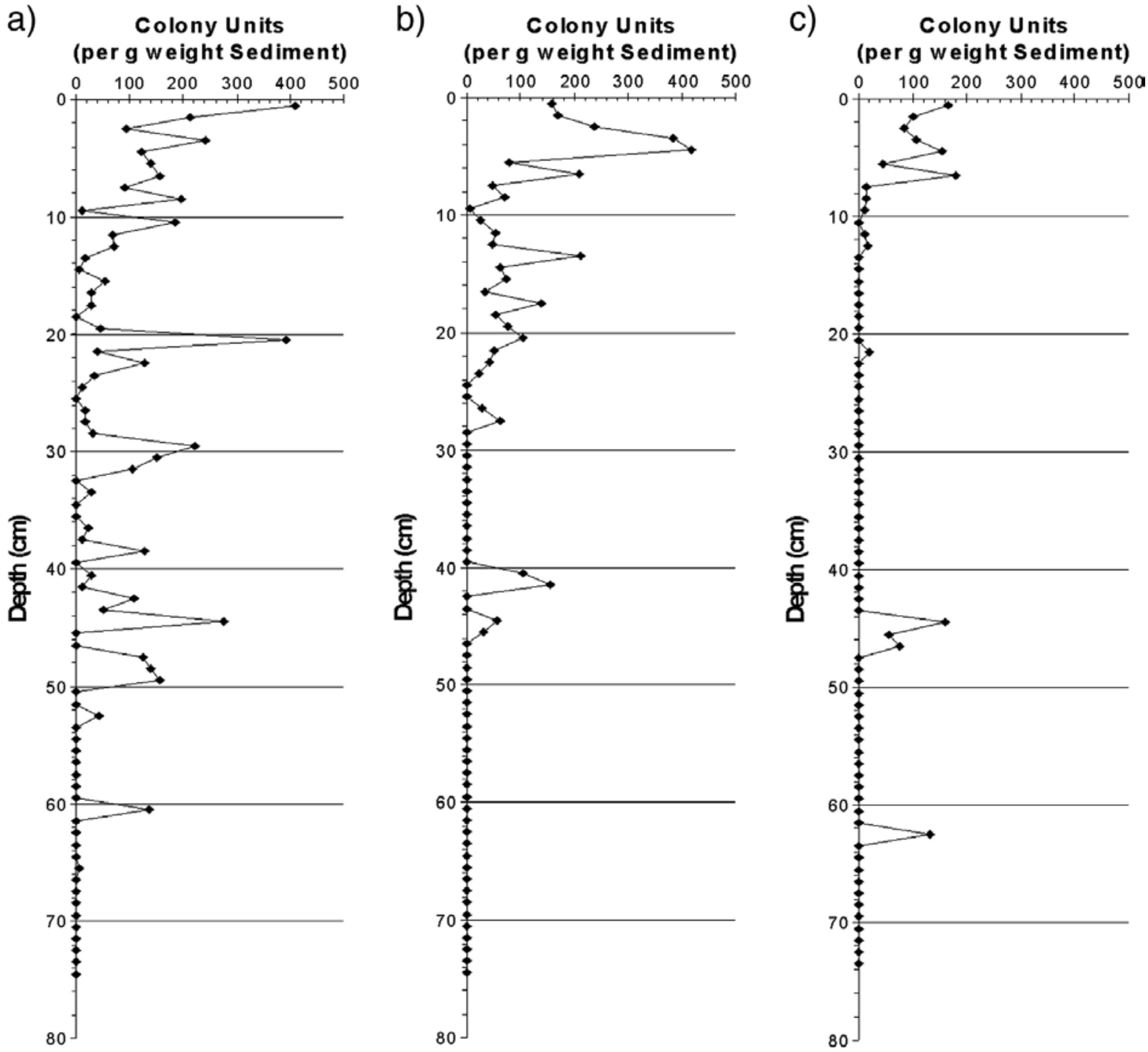


Fig 5. Comparison of total coliform counts in sediment cores from Attenborough Ponds, (a) Coneries Pond, (b) Church Pond and (c) Clifton Pond.

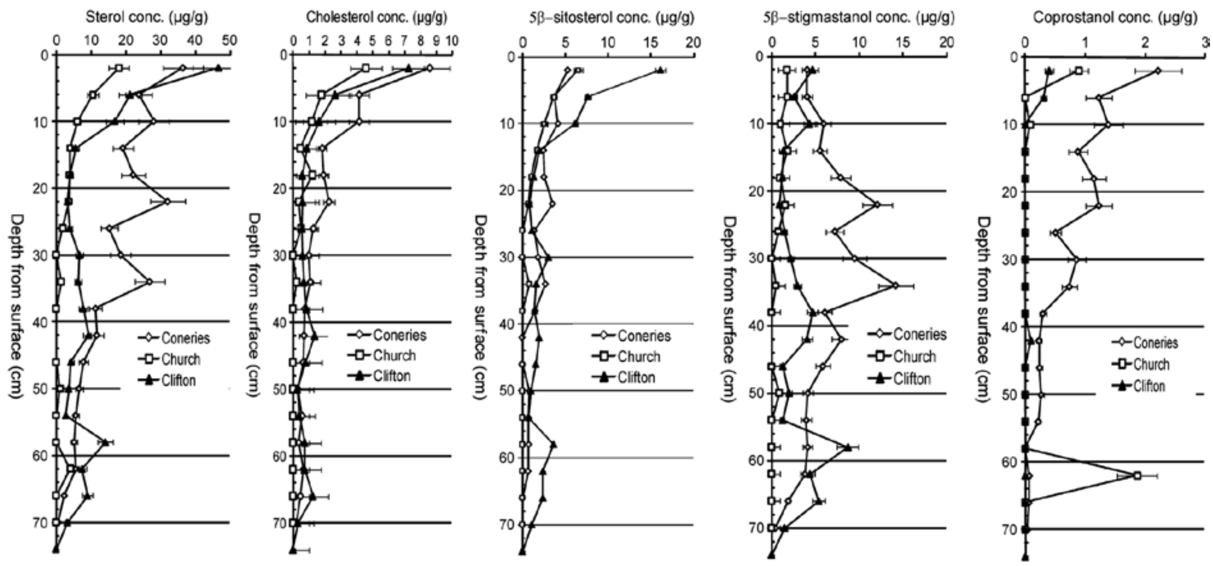


Fig 6. Variation in total sterol and faecal sterol concentrations in sediment cores from Attenborough Ponds. In general, 5β-sitosterol is derived from plants, 5β-stigmastanol is sourced from faecal matter from herbivorous animals and, coprostanol is from human sewage pollution (See section 3.2 for additional interpretations).

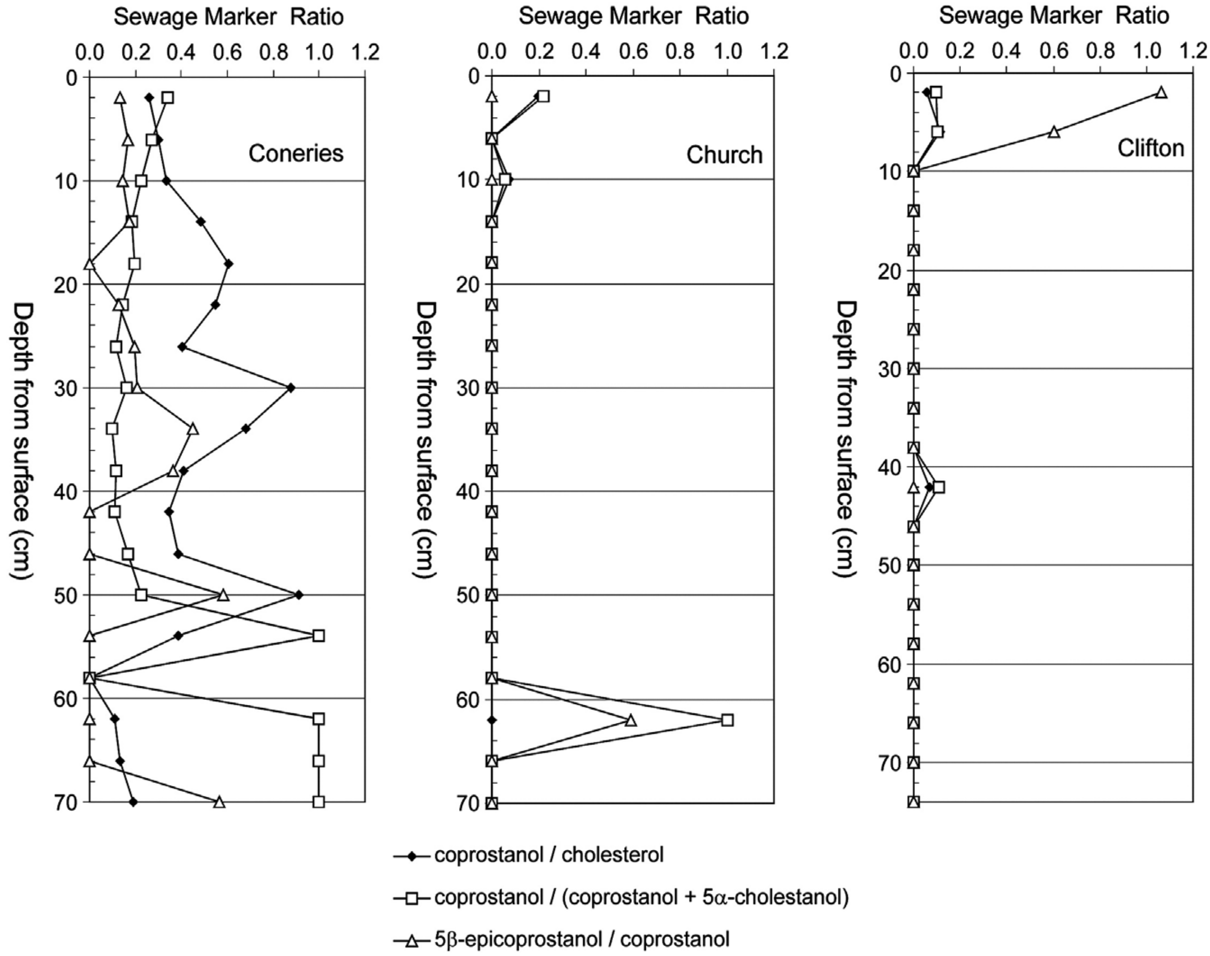


Fig 7. Ratios of sterol sewage marker compounds in sediment cores from Attenborough Ponds. Only Coneries Pond shows sustained sewage input.

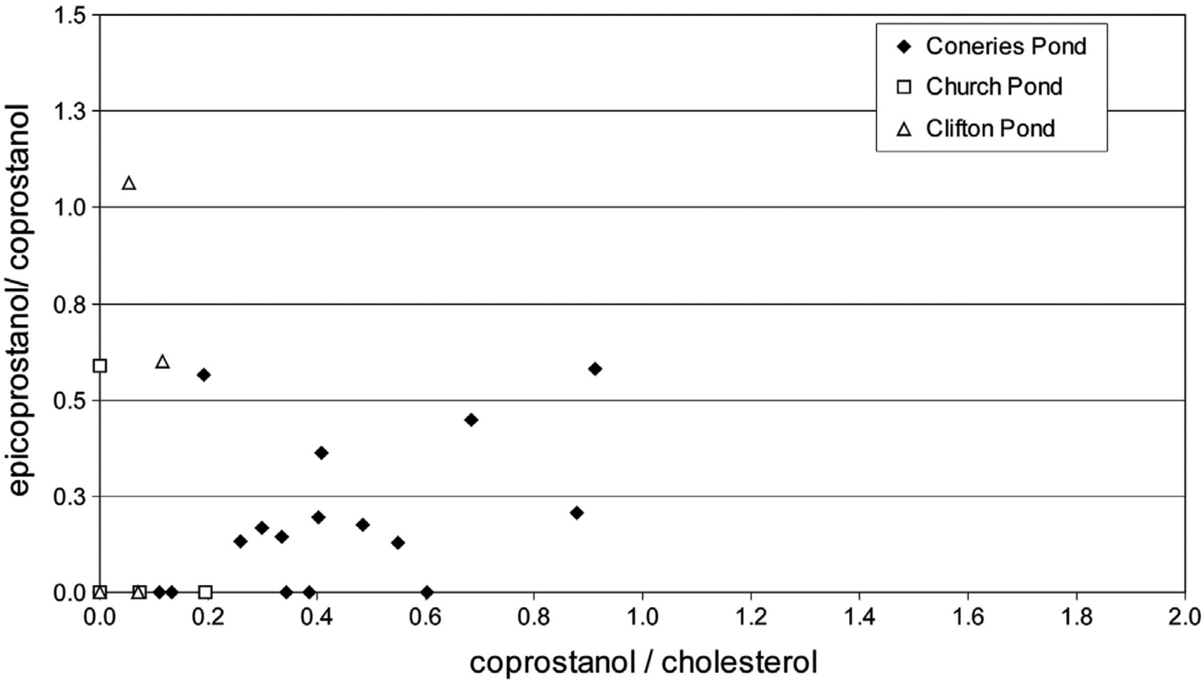


Fig 8. Cross-plot of epi-coprostanol/5β-coprostanol with 5β-coprostanol/cholesterol.

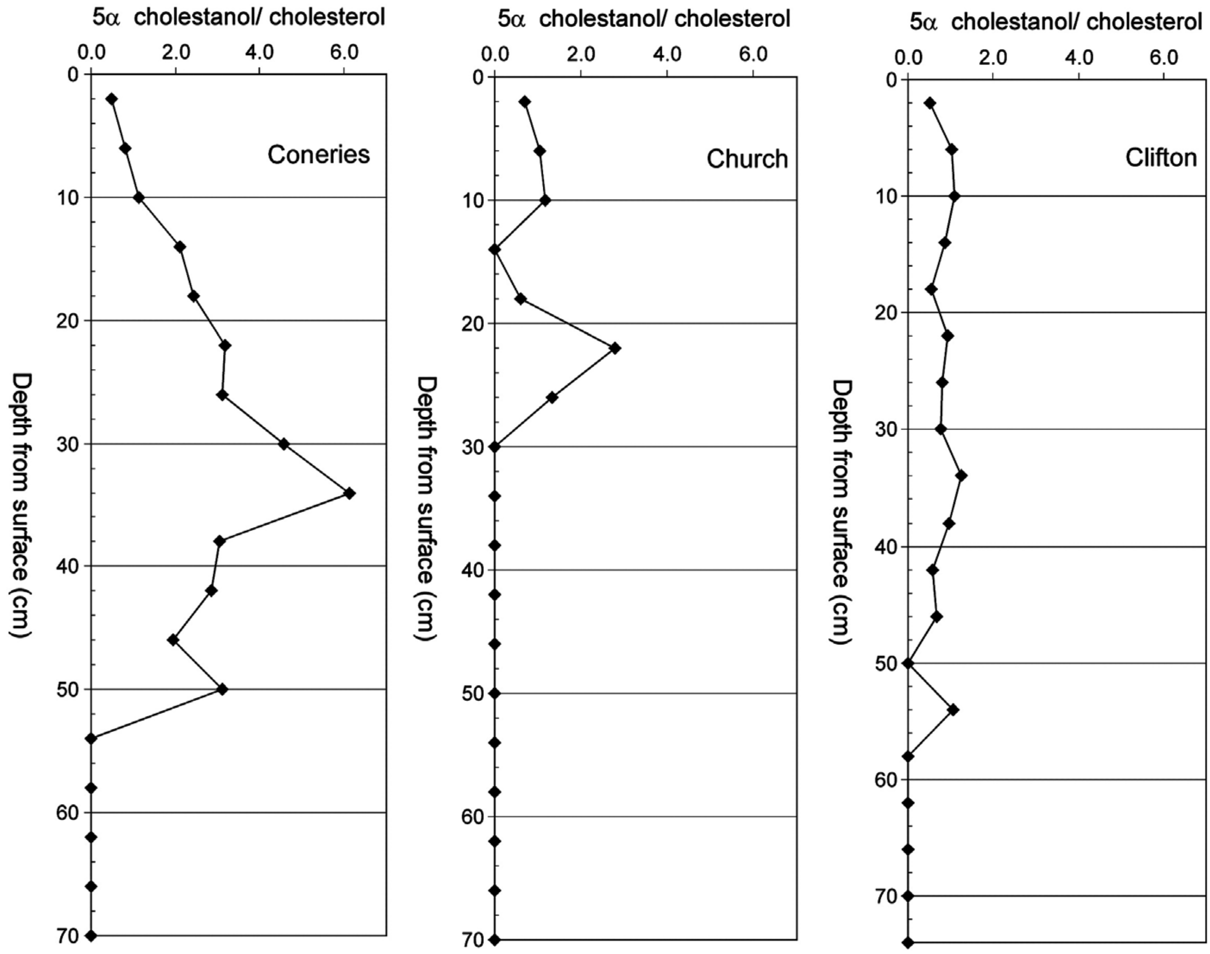


Fig 9. Variation in the ratio of 5α-cholestanol to cholesterol in sediment cores from Attenborough Ponds.

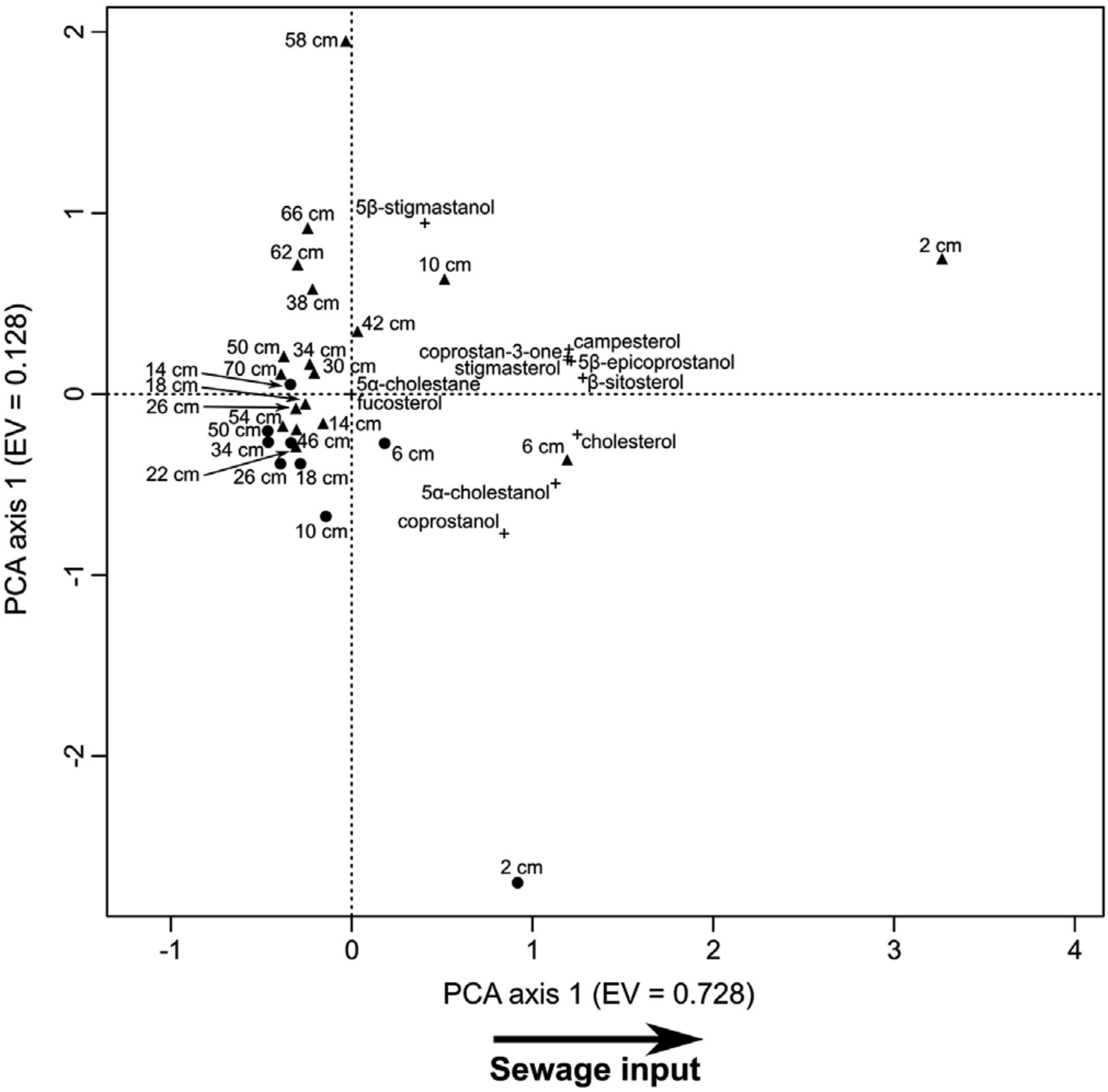


Fig 10. PCA axis one and two biplot for sterol sewage marker compounds Coneries Pond core.

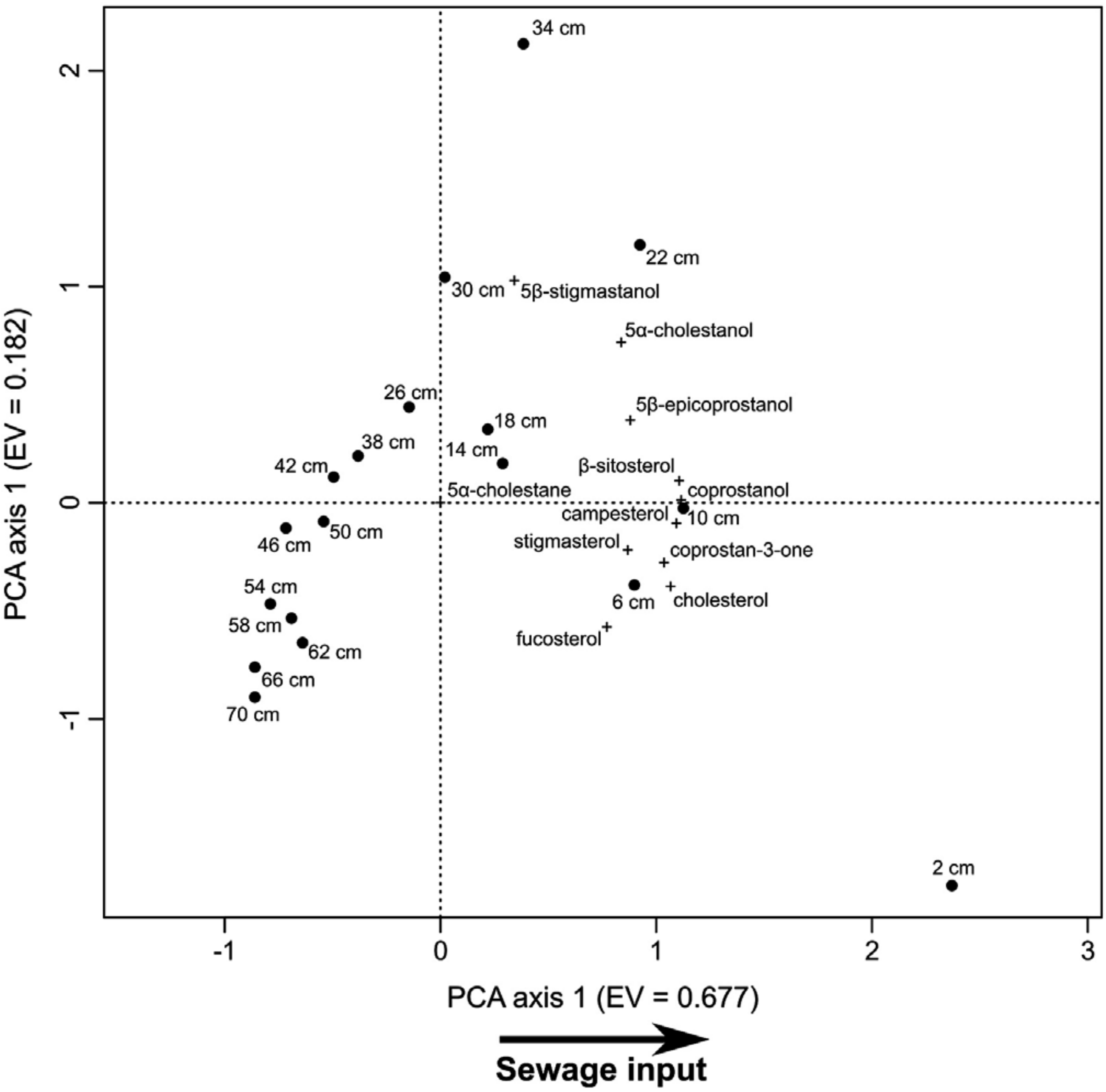
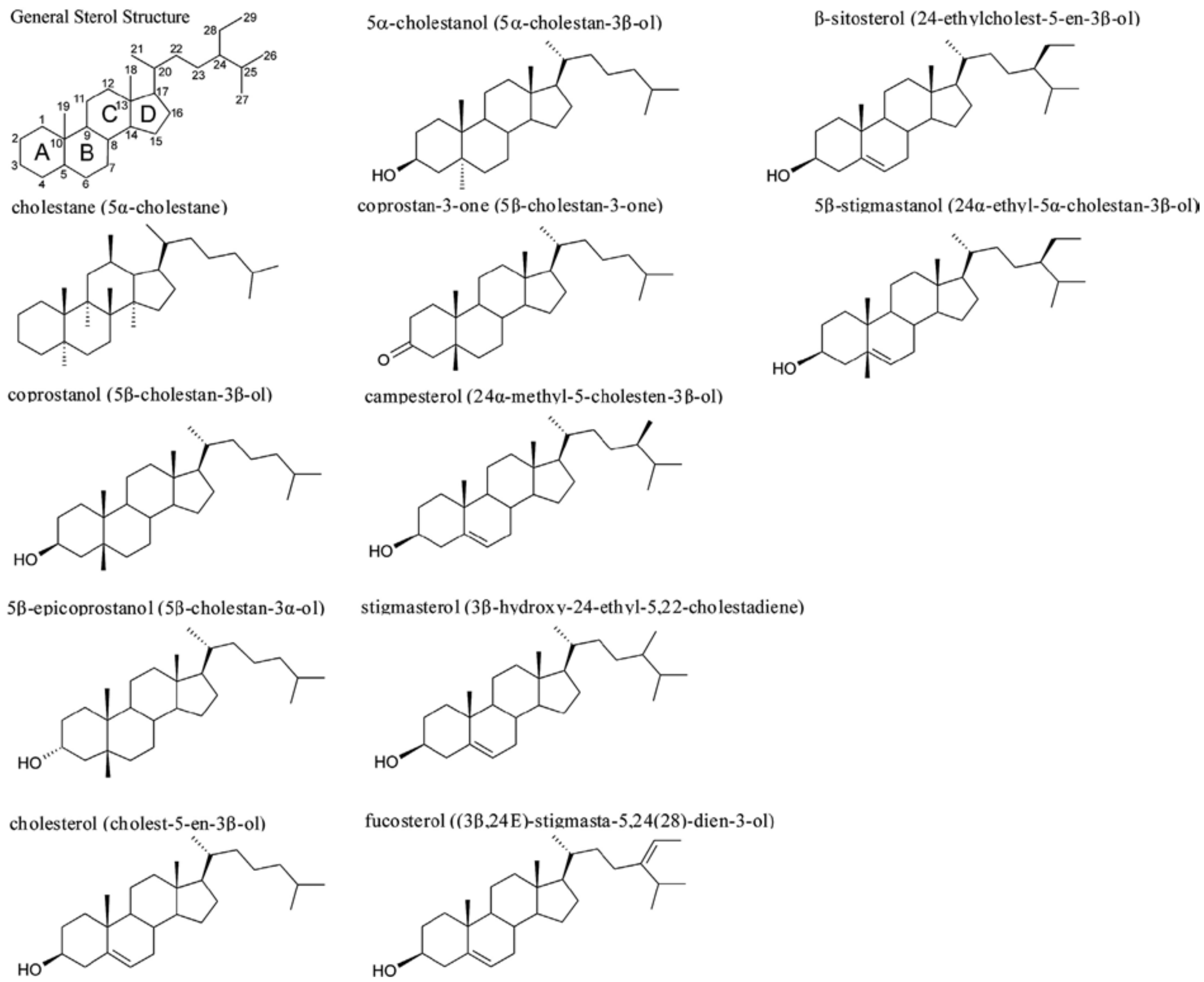


Fig 11. PCA axis one and two combined biplot for Clifton and Church Ponds with the removal of the ordination outliner 62 cm at Church.



Appendix 1. The structure of sterol and stanol sewage markers analysed in this study.