

Testing the limits of lacustrine faecal lipid biomarkers as tools to document human and herbivore presence in Alaskan and Eastern Beringian landscapes

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ABSTRACT

Sedimentary archives of faecal steroid biomarkers offer insights into human and animal presence and environmental impacts in past landscapes. However, interpretations of faecal stanol records, used to suggest early human arrivals in regions such as northern Alaska, are constrained by limited modern reference data and uncertainties associated with mixed stanol sources in wetlands. Here, we assess the sensitivity and validity of lacustrine faecal steroid reconstructions of human and animal presence within the landscape by combining analyses of modern faecal samples and a sediment archive from Chisholm Lake in central Alaska, where relative historical and archaeological population changes are well documented.

Results from modern faecal samples reveal clear dietary signatures: herbivore steroid distributions are discrete from omnivores/carnivores, and multivariate analyses reveal differences between ruminant species and even herds, highlighting the influence of diet on steroid distributions. Comparisons of sedimentary 5 β -stanols reconstructions with independent records of human activity show that large-magnitude population changes are reliably resolved, whereas small changes often are not, likely due to the signal-to-noise characteristics of the depositional systems. Bile acid detection was intermittent in this sedimentary archive, which limits characterisation of faecal sources and requires further taphonomic examination in lake sediments.

Our findings caution against solely relying on 5 β -stanol ratios to identify human faecal input in sedimentary archives because of overlapping distributions between humans and other omnivores/carnivores (e.g., Canidae, such as wolves and dogs) and mixed sources of sterol production and transformation in wetlands. Robust faecal steroid reconstructions of population change require multivariate analytical approaches that are integrated with archaeological evidence and expanded modern reference faecal steroid datasets.

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1. Introduction

The timing of human arrival(s) in North America and their movement through the continent remains subject to extensive debate (Bennett et al., 2021; but see Meltzer, 2021; Potter et al., 2022; Rachal and Dello-Russo, 2025). There is broad consensus, however, that ancestral Native Americans expanded from northeast Asia to the Americas via Beringia, whether through the interior or along the Pacific Coast (Meltzer, 2021). Delineating ancient human occupations relies on archaeological evidence, and the earliest unequivocal archaeological evidence of humans in eastern Beringia (Alaska) dates to c. 14,200 cal yr BP (Reuther et al., 2023). Archaeological studies alongside genetic insights constrain the expansion of Native American ancestors into the Americas after c. 16,000 cal yr BP (Llamas et al., 2016; Moreno-Mayar et al. 2018a, 2018b; Sikora et al., 2019). Other sites with equivocal evidence support human settlement in North America prior to the Last Glacial Maximum (26,500–19,000 cal yr BP; Becerra-Valdivia and Higham, 2020), but they are subject to contextual and dating concerns (Fiedel, 2013; Haynes, 2015; Krasinski and Blong, 2020; Potter et al., 2022). Recent research based on a selection of faecal steroid and fire biomarkers point to an earlier arrival of humans in northern Alaska (Eastern Beringia) > 32,000 cal yr BP (Vachula et al., 2019, 2020), which is c. 15,000 years before most accepted archaeological models of Native American colonisation of the region and the Americas more broadly (e.g., Gusareva et al., 2025; Posth et al., 2018; Llamas et al., 2016). While palaeofaecal steroid biomarkers have been used as a potential proxy for human presence, their use is often complicated by several confounding factors such as incomplete reference databases (Gryczewska et al., 2025; Larson et al., 2022), overlapping faecal steroid distributions between organisms (Prost et al., 2017) and *in-situ* faecal stanol transformation through time (Gaskell and Eglinton, 1975); we have yet to fully evaluate these methodologies and limitations.

Lacustrine sediment archives provide an opportunity to develop independent continuous reconstructions of human and animal presence and activity (Dubois et al., 2018). Lakes incorporate biomolecular, geochemical and microscopic signals of environmental change as their sediments accumulate. These sedimentary archives have the potential to detect early human signals of landscape use and manipulation that may not be recorded in the archaeological record (Curtin et al., 2021). Faecal steroid biomarkers (stanols and bile acids) are biomolecules that are deposited in the landscape from faeces of higher vertebrates. Faecal steroid methodologies and applications were first developed for water quality studies (e.g., Kirchmer, 1971) and later in archaeological studies of discrete faecal remains such as coprolites and manuring deposits (Bethell et al., 1994; Bull et al., 1999, 2001; Evershed et al., 1997; Gill et al., 2009; Knights et al., 1983; Prost et al., 2017; Simpson et al., 1999; Sistiaga et al., 2014; van Geel et al., 2008). However, sedimentary-based faecal steroid reconstructions have since contributed a diversity of insight into human settlement and land use in the Late Quaternary (Argiriadis et al., 2018; Battistel et al., 2017; Birk et al., 2022; Brown et al., 2021, 2022; Curtin et al., 2021; D'Anjou et al., 2012; Li et al., 2024a, 2024b, 2026; Mackay et al., 2020; Wendt et al., 2024; White et al., 2018, 2019, 2020).

Lacustrine faecal steroid studies in North America have reconstructed the presence and local lake catchment use of both animal and human populations. In addition to the application of stanols to early human presence in eastern Beringia (Vachula et al., 2019, 2020), steroid reconstructions from the Yellowstone Northern Range inferred herbivore-landscape interactions over the last c. 2300 years (Wendt et al., 2024). These lacustrine stanol and bile acid reconstructions identified bison and/or elk as the dominant ungulates and linked their elevated presence in the local catchment to declines in forage taxa during the early to mid 20th century. Late Holocene lake sediment stanol records from Illinois have reconstructed population change associated with Cahokia, the largest prehistoric mound centre in North America (White et al., 2018, 2019). These stanol reconstructions show

minimum regional population levels at 1400 CE and comparisons with palaeoenvironmental data highlight the importance of climate change associated with the Medieval Climatic Anomaly to Little Ice Age transition to population and sociopolitical transformations at Cahokia (White et al., 2019).

The robustness of any sedimentary faecal steroid record is dependent on a studies' understanding of a diverse range of steroid sources and their movement and transformation within the landscape. Sterols are cell membrane components of living organisms that are deposited in lake sediments from aquatic organisms and by transportation of terrestrial organic matter from lake catchments (Fig. 1). Δ^5 -sterols are produced by eukaryotes including fungi, algae, vegetation and animals, and are transformed to 5α -stanols, predominantly *in-situ* by bacteria in soils and sediments (Schubert and Kaufmann, 1965), or to 5β -stanols (often termed 'faecal' or 'zoo' stanols), predominantly by bacteria within the digestive tracts of animals and excreted in faeces (Björkhem and Gustafsson, 1971), often prior to transportation into the lake (Fig. 1). The hydrophobic nature of stanols combined with their low lability compared with other compounds, ensures that they are preserved in lake sedimentary archives over millennia. However, 5α - and to a much lesser extent, 5β -stanols are also produced *in-situ* within sediments by post-depositional diagenesis of Δ^5 -sterols, altering their relative composition in sediments over time (Gaskell and Eglinton, 1975, 1976; Grimalt et al., 1990).

Bile acids, unlike stanols, are exclusively excreted in waste products of vertebrates. Primary bile acids, which exist in bile in the form of conjugates with glycine or taurine, are produced from cholesterol in the liver, whilst secondary bile acids are metabolised from primary bile acids in the intestine, and tertiary bile acids are transformed from secondary bile acids in the liver (Bull et al., 2002; Di Ciaula et al., 2017). Unconjugated bile acids are more recalcitrant than sterols and exhibit low hydrophilicity at low pH (Elhmmali et al., 1997; Bull et al., 2002). However, the solubility of bile acids increases with polarity, as dictated by their degree of hydroxylation and stereochemistry (cholic acid (CA) > hyodeoxycholic acid (HDCA) > chenodeoxycholic acid (CDCA) > ursodeoxycholic acid (UDCA) > deoxycholic acid (DCA) > lithocholic acid (LCA)), and with increasing pH, especially above pH 5–6.5 (Fini et al., 1985; Igimi and Carey, 1980; Roda and Fini, 1984). Conjugated bile acids are more soluble than free bile acids (Roda and Fini, 1984), which limits bile acid accumulation-potential within neutral-basic lake sediments and at higher temperatures (Fini et al., 1985; Igimi and Carey, 1980). Furthermore, rates of microbial degradation of bile acids in the environment are also optimised at neutral or slightly alkaline pH and differ between bile acids compounds based on their degree of hydroxylation and stereochemistry (tri-hydroxyl > di-hydroxyl > mono-hydroxyl; reviewed by Feller et al., 2021).

Distributions of faecal steroids are characteristic of different source organisms since they are determined by diet, digestive processes and gut microbiome (Bull et al., 2002, Fig. 1). Diagnostic faecal steroid ratios of modern reference materials (e.g., Bethell et al., 1994; Bull et al., 1999; Leeming et al., 1997; Prost et al., 2017) and applications of multivariate statistics (e.g., Derrien et al., 2012; Harraut et al., 2019; Wendt et al., 2024) are effective at distinguishing between herbivore and omnivore dung and can often identify to species level. However, identification of faecal sources is limited by overlapping faecal steroid distributions between organisms and a lack of modern reference values. 5β -stanol reconstructions from lake sediments have been used to infer relative changes in populations over time in areas with high land use intensity (Keenan et al., 2022; White et al., 2019), and while greater validation is required for populations of wild animals (Wendt et al., 2024), relationships between soil faecal stanol ratios and herbivore dung counts from wild herbivore enclosures demonstrate their potential as a proxy for palaeo-herbivore abundance (Karp et al., 2025).

Faecal steroid characterisation in lake sediments is challenging, owing to (i) mixed faecal inputs, (ii) sediment transport and storage and (iii) non-faecal sterol sources. Firstly, interpretations of diagnostic ratios

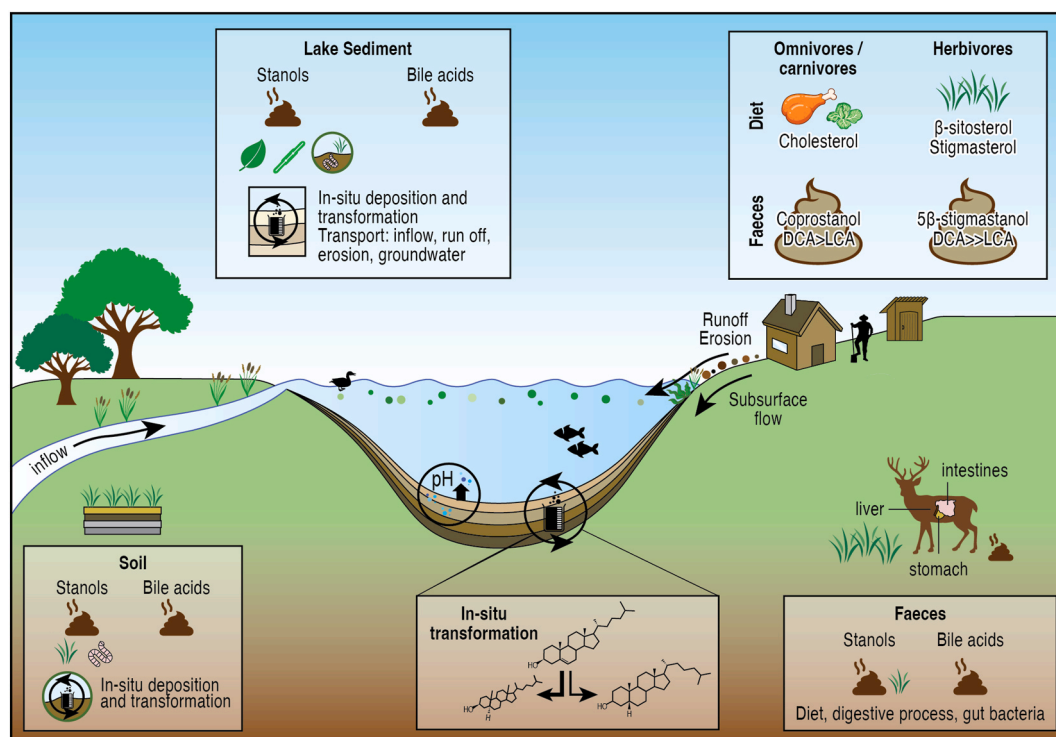


Fig. 1. Sources, transportation and transformation of faecal steroids in lake catchments. Steroid distributions of faeces differ based on diets, digestive systems and gut bacteria. Anaerobic bacteria in the rumen and intestine transform ingested sterols to stanols. Primary bile acids are produced from cholesterol in the liver and secondary (tertiary) bile acids are transformed from primary (secondary) bile acids in the intestine (liver). Stanols in the terrestrial environment (e.g., soils) originate from excrement, vegetation, other organisms and *in-situ* transformation from sterols. Lacustrine sedimentary stanols reflect catchment soil sources following sediment transport into the lake as well as sources from aquatic organisms and further anaerobic *in-situ* sterol transformation. Δ^5 -sterols are primarily transformed to 5 α -stanols or oxidised, but smaller proportions of Δ^5 -sterols are also transformed to 5 β -stanols *in-situ*. Bile acids are only produced via digestive processes and excreted into the environment. Bile acid preservation or retention may be favoured under slightly acidic conditions (pH 5–6.5).

and steroid distributions are complicated by the mix of faecal source organisms whose products accumulate over years to centuries and millennia in sediment archives. Steroid distributions are therefore better suited to identifying the dominant faecal source organism when inputs are mixed, rather than the full suite of contributing organisms (e.g., Derrien et al., 2012). Secondly, faecal signatures in soils can be temporarily stored in catchments prior to sediment transport and deposition in lakes, and therefore may not be contemporaneous with the horizon in which they are detected (Zolitschka et al., 2003; Fuchs et al., 2011). Transportation of 5 β -stanols is driven by erosion (Lloyd et al., 2016), which presents the possibility of oxidation prior to reaching the lake, and transportation rates will be sensitive to changes in precipitation and anthropogenic disturbances over time. Thirdly, diagnostic ratios from archaeological and modern water quality studies include compounds that are produced by non-faecal sources in wetland environments, including vegetation, microorganisms and chemical transformation of sterols in sediments (Fig. 1; Gaskell and Eglinton, 1975; Nishimura and Koyama, 1976; Soliman and Radwan, 1981; Taylor et al., 1981). Thus, stanol ratios are sensitive to changes in ecosystems and depositional environments that may occur independently from changes in faecal input. Threshold values of diagnostic sterol ratios attempt to account for this, but these are based on limited empirical data, not consistently applied, and vary between sites.

In this study we integrate faecal steroid analysis of modern dung samples and lake sediments from a small lake in central Alaska to evaluate the efficacy of lacustrine faecal biomarkers to reconstruct human and animal presence within the landscape. Chisholm Lake, located in the Tanana Valley of eastern Beringia, has independent historical records of Euro-American activity over the past c. 125 years and regional prehistoric human activity since c. 14,000 cal yr BP (Reuther et al., 2023). The prehistoric record of humans and large mammals in

the region is well documented from multiple archaeological sites (Section 2.1), which underpin our hypothesis that changes in faecal sources and relative intensities of use over time drive the lake sediment record of steroids.

Our objectives are to assess the sensitivity of faecal steroids to distinguish between different animals by expanding available faecal steroid dung reference material and to evaluate the sensitivity and validity of lake faecal steroid records by comparing dung and lake sediment steroid data with known or inferred population changes. We hypothesise that:

- H1.** Faecal sterols and bile acid distributions measured from modern faecal samples are effective at differentiating between animals with different diets.
- H2.** Lake-sediment faecal steroid records indicate the relative intensity of human and animal activity in the catchment.
- H3.** The diagnostic power of sedimentary 5 β -stanols to identify human presence depends on both the degree of overlap in 5 β -stanol distributions among contributing species and the relative contributions of human and non-human faecal sources to the sedimentary signal.

2. Materials and Methods

2.1. Study site

Chisholm Lake (WGS84 64.30229 N, –146.68623 W) is located in the Tanana Valley of interior Alaska, north of the Alaska Range at 240 m a.s.l (Fig. 2). The maximum lake depth is 11 m, the surface area is approximately 31 ha and the catchment area is approximately 780 ha. There is one inflow in the eastern corner of the lake, and one

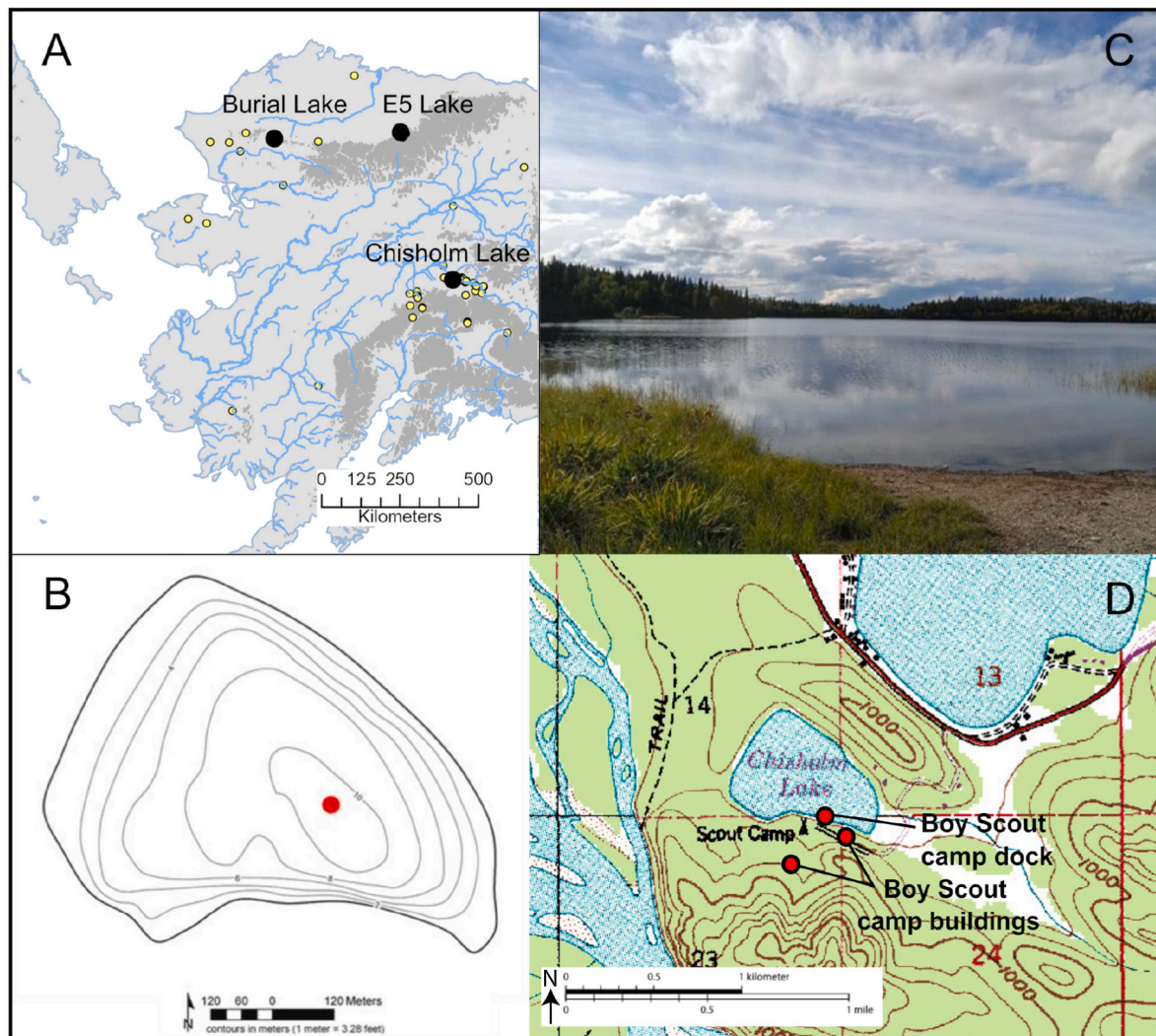


Fig. 2. A: Overview map; Alaskan lakes referred to in-text shown as black circles and archaeological sites >12,000 cal yr BP (Potter, 2025) shown as yellow circles. B: Chisholm Lake bathymetry and core location (red circle). C: View from southeast edge of Chisholm Lake. D: Chisholm Lake surroundings, showing Richardson Highway (red line), trail and Boy Scout camp dock and buildings (red circles).

intermittent outflow during high water periods on the southwestern shore that drains into a wetland adjacent to the Tanana River. The present forest is dominated by spruce (*Picea glauca* and *P. mariana*) with occasional larch (*Larix laricina*) and hardwood trees and shrubs such as birch (*Betula* spp.), alder (*Alnus viridis*) and willow (*Salix* spp.). Aquatic plants such as the yellow pond lily (*Nuphar polysepalum*), dwarf water lily (*Nymphaea tetragona*) and pondweed (*Potamogeton* spp.) grow densely in the shallow (1–3 m) margins of the lake. The surface water pH is 8.2, which is associated with increased bile acid solubility compared with acidic and neutral pH (Fini et al., 1985; Igimi and Carey, 1980; Roda and Fini, 1984).

Archaeological research has been conducted in the region for over 60 years. Numerous sites recorded within the vicinity of Chisholm Lake indicate that humans have been present in the area since c. 14,200 years ago, and faunal remains show a change in the dominant mammal species. While archaeological research at Chisholm Lake itself has been limited, extensive excavations at sites to the west and east inform us on the human history of the area. The Upward Sun River and McDonald Creek sites are located 10 km west of Chisholm Lake (Potter et al., 2011), while a series of key sites located within the Shaw Creek area lie approximately 29 km east of Chisholm lake, including Broken Mammoth, Mead, Swan Point and others (Holmes, 2001; Potter et al., 2014; Reuther, 2013; Reuther et al., 2023). Archaeological sites in the

region are situated in a variety of geological contexts including lakeside shorelines, dune fields, and terraces and hill tops overlooking wetlands and rivers. Many of these settings have deep (>1 m thick) loess (wind-blown silts) that display a series of stratified soils dating back to the late Pleistocene and relatively alkaline sediments that preserve organic materials, including faunal remains, that help us reconstruct changes within regional animal populations (Dilley, 1998; Reuther, 2013; Potter, 2008; Potter et al., 2014). Bison (*Bison priscus*) and elk (wapiti; *Cervus canadensis*) decreased from the middle Holocene, when caribou (*Rangifer tarandus*) and to a lesser extent moose (*Alces alces*) increased (Skarland and Giddings, 1948; Potter, 2008; Reuther et al., 2016). Over the last 1000 years, faunal components were dominated by caribou, followed by salmon, hare, moose, canid and freshwater fish, with sheep, beaver, bear and birds also present (Potter, 2008, 2025).

There is an undated prehistoric site on the ridge above Chisholm Lake and several around nearby Birch Lake (Skarland and Giddings, 1948). A group of Salcha Dene-Athabaskan peoples seasonally occupied Birch Lake in the late 1800s (Andrews, 1975). Historical records of Euro-American activity around Chisholm Lake show that the Fairbanks-Valdez trail was established in 1905 CE following the existing mail route after gold was discovered in the Tanana Valley in 1902 CE. The summer trail (the modern Richardson Highway) passes c. 700 m to the northeast of Chisholm Lake. A short-lived community (Washburn)

was founded in 1905 CE c. 1.5 km south of Chisholm and served as a winter stop until c. 1920 CE (Marsh, 2008). The post office closed in 1913 CE and the telegraph station moved in 1915 CE (Potter et al., 2007). A few cabins were first constructed on the shore of Chisholm Lake in the 1920s and development increased since the 1950s, resulting in c. 30 cabins near the lake shore today. A large Boy Scout camp on the southern shore of the lake was established in the early 1960s.

2.2. Sediment core retrieval and sampling

Sediment core 19Chis-C was obtained from the deepest region of the lake (WGS84, 64.30229 N, −146.68623 W) in September 2019 using a Bolivia corer. Cores were stored in polycarbonate tubes, wrapped in plastic film and stored in cold storage at 4°C. The core was split longitudinally and samples were extracted at 0.5 cm resolution for the top 4.5 cm of the core and then approximately 10 cm resolution for the remainder of the top 1 m. Sub-sampling was conducted at University Alaska Fairbanks using sterile tools. Care was taken to minimise exposure of samples to room temperature and light, and to ensure that the outer surfaces of the core were not used to avoid contamination from plastic and the coring procedure. Sub-samples were immediately freeze-dried in preparation for lipid analysis.

2.3. Modern reference material sampling

Fresh modern-dung samples were collected in spring-summer 2020 from four common large herbivore species found in Holocene archaeological sites in interior and northern Alaska: moose (*Alces alces*, $n = 6$), elk (wapiti; *Cervus canadensis*; $n = 6$), plains bison (*Bison bison bison*, $n = 6$) and caribou (*Rangifer tarandus*, $n = 6$) (Table 1). Wood bison (*Bison bison athabasca*; $n = 6$) dung collected in 2017 was analysed to examine differences between bison subspecies. Omnivore (sandhill crane (*Antigone canadensis*; $n = 6$), mallard (*Anas platyrhynchos*; $n = 6$), American black bear (*Ursus americanus*; $n = 1$, with 4 replicates)) and carnivore (wolf (*Canis lupus*; $n = 1$, with 4 replicates)) dung were collected and analysed in summer 2024. All faecal samples represent a single excretion from a single organism. The maximum feasible extents of the sampling areas were surveyed for samples to reduce the chance of repeat sampling of individuals and only fresh dung deposited within the last c. 48 hrs was sampled. Faecal samples were frozen at −18°C immediately after collection.

The origin of the dung deposits was identified prior to sampling into aluminium foil to avoid contamination from plastic or organics. The species of bear was identified by DNA analysis at the Canadian Centre for DNA Barcoding using standard protocols. The sample was freeze-dried, homogenised and mixed with ethanol. Following the removal of ethanol, 200–500 µL of lysis buffer with 0.5 mg mL^{−1} of proteinase K

Table 1
Modern dung sample collection details.

Species	Sample name	Latitude	Longitude	General area collected	Date collected
Moose (<i>Alces alces</i>)	MP-20-01	N64.79022	W148.05306	Fairbanks, Interior Alaska	01/05/2020
	MP-20-02	N64.27145	W146.11394	Delta Junction, Interior Alaska	06/06/2020
	MP-20-03	N64.27110	W146.11501	Delta Junction, Interior Alaska	06/06/2020
	MP-20-04	N64.27125	W146.11710	Delta Junction, Interior Alaska	01/06/2020
	MP-20-05	N64.27087	W146.11595	Delta Junction, Interior Alaska	01/06/2020
	MP-20-06	N64.27215	W146.11545	Delta Junction, Interior Alaska	01/06/2020
Plains bison (<i>Bison bison bison</i>)	BP-20-01	N64.033861	W145.160556	Delta Junction, Interior Alaska	03/07/2020
	BP-20-02	N64.033861	W145.16075		
	BP-20-03	N64.034111	W145.160917		
	BP-20-04	N64.034250	W145.160944		
	BP-20-05	N64.034306	W145.161222		
	BP-20-06	N64.034651	W145.160814		
Elk/wapiti (<i>Cervus canadensis</i>)	EP-20-01	N64.034306	W145.161306	Hollembaek Herd, Delta Junction, Interior Alaska	03/07/2020
	EP-20-02	N64.034444	W145.161528		
	EP-20-03	N64.034599	W145.161639		
	EP-20-04	N64.035278	W145.161500		
	EP-20-05	N64.036000	W145.161720		
	EP-20-06	N64.03577	W145.161793		
Caribou (<i>Rangifer tarandus</i>)	CP-20-01	N69.56979	W150.32225	North Slope, Northern Alaska	25/07/2020
	CP-20-02	N70.01305	W153.01225		27/07/2020
	CP-20-03	N70.07822	W152.99937		31/07/2020
	CP-20-04	N70.71356	W153.85884		01/08/2020
	CP-20-05	N70.69131	W152.85553		04/08/2020
	CP-20-06	N70.69096	W153.31960		04/08/2020
Black bear (<i>Ursus americanus</i>)	UP-24-01	N64.755378	W145.353458	Delta Junction, Interior Alaska	15/06/2021
Wolf (<i>Canis lupus</i>)	CLP-24-01	N64.035308	W145.165256	Delta Junction, Interior Alaska	01/06/2021
Wood Bison (<i>Bison bison athabasca</i>)	WBP-17-01	N64.04634	W145.18990	Hollembaek Herd, Delta Junction, Interior Alaska	26/06/2017
	WBP-17-02	N64.04643	W145.18837		
	WBP-17-03	N64.04647	W145.18620		
	WBP-17-04	N64.04637	W145.18420		
	WBP-17-05	N64.04644	W145.18311		
	WBP-17-06	N64.04636	W145.18053		
Crane (<i>Antigone canadensis</i>)	SCP-24-01	N64.864834	W147.73477	Fairbanks, Interior Alaska	22/08/2024
	SCP-24-02	N64.865862	W147.73545		
	SCP-24-03	N64.86357	W147.739658		
	SCP-24-04	N64.866074	W147.737127		
	SCP-24-05	N64.864833	W147.738649		
	SCP-24-06	N64.864098	W147.741141		
Mallard (<i>Anas platyrhynchos</i>)	APP-24-01	N64.841651	W147.777664	Fairbanks, Interior Alaska	22/08/2024
	APP-24-02	N64.841522	W147.778048		
	APP-24-03	N64.841367	W147.777577		
	APP-24-04	N64.841327	W147.776881		
	APP-24-05	N64.841181	W147.77655		
	APP-24-06	N64.841250	W147.775979		

(Promega) was added to the sample and DNA was extracted from 50 μL of lysate. Total genomic DNA was extracted using a validated spin column DNA extraction protocol and the extract was diluted to 1:10 and purified using clean-up columns. The target genetic marker COI (a DNA barcode region of the mitochondrial cytochrome c oxidase subunit I gene) was amplified using polymerase chain reaction (PCR) employing full length C_VF1Lft1/C_VR1Lrt1 and short length C_BloodmealF1_t1/ModMamRev_t1 primer cocktails (Ivanova et al., 2007). Cycle sequencing was performed using a standardized commercially available BigDye Terminator v3.1 kit. Sequencing reactions were analysed by high-voltage capillary electrophoresis on an automated ABI 3730xL DNA Analyzer. The DNA sequence recovered from the sample was compared against the Barcode of Life Data System (BOLD) accessible at <https://boldsystems.org/>. The match was determined since the percentage of nucleotide substitutions between the queried sequence and a reference sequence was less than 2% (1.1%). The identity of the closest matching reference sequence (*Ursus americanus*) was used to infer species identity of the DNA contributor in the sample.

2.4. Chronology

The age-depth model for core 19Chis-C is based on a combination of ^{210}Pb and five ^{14}C measurements, the latter of which were calibrated using IntCal20 (Reimer et al., 2020, Tables 2 and 3, respectively).

The ^{14}C plant samples were sieved and washed with distilled water prior to submission to the laboratory. The two pollen samples (which are rejected – see below), were processed using techniques modified from Brown et al. (1989) and Vandergoes and Prior (2003), which used weak KOH and HCl washes, as well as sieving (250, 125, and 90 μm) and multiple heavy liquid separations, starting at 2 σ and decreasing incrementally to 1.4 σ , and which yielded samples dominated by spruce pollen.

The age-depth model for the full 19Chis-C record was constructed with R packages *Rplum* (Aquino-López et al., 2018) and *Rbacon* (Blaauw and Christen, 2011) combining the ^{210}Pb and the ^{14}C ages. One ^{14}C date is from a nearby core from Chisholm Lake (17Chis-A; Clarke et al., 2024), correlated using a distinct silt band present in both cores. Due to a laboratory error (probable ^{14}C -contaminated heavy liquids), the two pollen dates from 19Chis-C are too old and are not included in the age model. Reported ages from this model represent rounded median cal yr BCE/CE.

A second shorter age-depth model for the historical period (1895 to 1998 CE) was constructed with the R package *Rplum* (Aquino-López et al., 2018), using only the ^{210}Pb ages to remove the influence of ^{14}C -based accumulation rates on the modelled ages and therefore provide a more accurate estimate of the age and sedimentation rate at the core top. Reported ages represent rounded median yr CE.

2.5. Faecal steroid analysis

Sediment and dung samples were freeze-dried and homogenised using a mortar and pestle prior to lipid biomarker analysis. Total lipids were extracted from approximately 1 to 3 g of sediment and 0.1 to 0.2 g

of modern dung samples. All samples were spiked with internal standards (preg-5-en- β -ol and hyocholic acid; 50 μL , 0.1 mg mL^{-1} solution) at the start of the sample processing and carried through all stages of analysis to normalise concentrations for recovery and instrumental variability. Lipids were microwave-extracted using an Ethos EX microwave-assisted extraction system [10 min ramp to 70°C (1000 W), 10 min hold at 70°C (1000 W) and 20 min cool down] using 10 mL DCM: CH_3OH , 2:1 (v/v), saponified using 5M NaOH in CH_3OH . Following acidification to pH 3–4 and liquid-liquid extraction using CHCl_3 , ‘neutral’ and ‘acid’ fractions were separated using aminopropyl solid phase extraction columns (Elhmmali et al., 1997). Silica gel column chromatography isolated a ‘sterol’ fraction (Bull et al., 2001) and, following methylation using $\text{BF}_3\text{-CH}_3\text{OH}$ (Shillito et al., 2020), a ‘bile acid’ fraction (Elhmmali et al., 1997). The sterol and bile acid fractions were trimethylsilylated using HMDS:TMCS:pyridine (3:1:9) at 70°C for 1 h and 12 h, respectively.

Derivatised sterol and bile acid fractions were dissolved in hexane and ethyl acetate respectively, prior to analysis by gas chromatography-flame ionisation (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Sterol and bile acid fractions were screened using an HP 5890 Series II gas chromatograph. Trimethylsilylated sterols and methylated and trimethylsilylated bile acids were introduced (1.0 μL) via a splitless injector (temperature 300°C, purge time 0.75, purge flow 15.0). The analytical column was a 50 m $\times$ 0.32 mm fused silica capillary column coated with a 100% dimethylpolysiloxane nonpolar stationary phase (HP-1, 0.17 μm ; Agilent). For the sterol analyses, the GC temperature program was set to hold at 50°C for 2 min, followed by a gradient increase to 200°C at 10°C min^{-1} , and then to 300°C at 3°C min^{-1} , before a final isothermal at 300°C for 20 min. For the analyses of bile acids, the GC temperature program was set to hold at 40°C for 1 min, followed by a gradient increase to 230°C at 20°C min^{-1} , and then to 300°C at 2°C min^{-1} , before a final isothermal at 300°C for 20 min. Helium was used as the carrier gas set to constant flow of 2.0 mL min^{-1} , and the flame ionisation detector (FID) used to monitor column effluent was kept at a constant temperature of 300°C. Data were acquired using DataApex Clarity (version 2.6.2.226).

GC-MS analyses were performed using a ThermoScientific Trace 1300 gas chromatograph coupled to an ISQ single quadrupole mass spectrometer. Samples were introduced via a PTV injector set to splitless mode, with a splitless time of 2 min, onto a 50 m $\times$ 0.32 mm (Agilent) fused silica capillary column coated with a 100% dimethylpolysiloxane HP 1 non-polar stationary phase (0.17 μm ; Agilent). The GC temperature programmes employed were the same as for the corresponding GC analyses. Helium was used as the carrier gas, set to a constant flow of 2 mL min^{-1} . The MS was operated in electron ionisation (EI) mode operating at 70 eV, with a GC transfer line temperature of 300°C and a source temperature of 300°C. The emission current was set to 150 μA and the MS was set to acquire in the range of m/z 50–650 at 0.2 scans s^{-1} in full scan mode. The data acquisition and processing were carried out using XCalibur software, version 3.0. GC-MS peaks were identified using extracted ion chromatograms (Supplementary, Appendix A1), characteristic fragment ions (Supplementary, Appendix A2) and comparison with reference mass spectra (NIST08; Prost et al., 2017 and a laboratory reference library) and retention behaviour comparisons with example chromatograms (Prost et al., 2017 and laboratory reference library). External sterol standards were analysed alongside samples to account for variation in ionisation. Analytes were quantified based on internal standards, therefore concentrations are considered semi-quantitative. Full details of selected analytes and their nomenclature are outlined in the supplementary data (Appendix A2).

2.6. Statistical analyses of faecal steroids distributions

Concentrations of target dung steroids are reported in $\mu\text{g g}^{-1}$ or mg g^{-1} (dry weight) and lake sediment concentrations are reported as mass accumulation rates ($\text{ng cm}^{-2} \text{yr}^{-1}$). Values are reported to two

Table 2

^{210}Pb ages based on analyses from Flett Research, using a constant rate of supply (CRS). CRS-derived ages are calculated for samples with activity levels that exceed sediment-supported ^{210}Pb activity (measurements in blue in Fig. 3B).

Core name and sediment depth (cm)	Years before core collection year (2019)	Years CE
19Chis-C (0 - 0.5 cm)	21.57	1998.23
19Chis-C (0.5 - 1 cm)	41.00	1978.80
19Chis-C (1 - 1.5 cm)	63.76	1956.04
19Chis-C (1.5 - 2 cm)	81.11	1938.69
19Chis-C (2 - 2.5 cm)	103.64	1916.16
19Chis-C (2.5 - 3 cm)	127.79	1892.01

Table 3

¹⁴C dates from 19Chis-C and one date from an adjacent core. Depth represents the depth below sediment-water interface (mid-point) at 19Chis-C core site. N/A = not available.

Lab number	Sample ID	Material	Depth (cm)	δ ¹³ C (‰)	¹⁴ C age, years BP ± 1 σ	Year CE calibrated median	Year CE calibrated ± 2 σ	Citation
UGAMS-58995	19Chis-C_19-21	4 Birch seeds	20.00	−23.60	430 ± 55	1475	1407–1633	This study
UGAMS-51576p ^a	19Chis-C_37-38	Pollen	37.50	−28.78	1680 ± 20	385	262–418	This study
UGAMS-51576s	19Chis-C_37-38	1.5 birch seeds	37.50	−23.44	1230 ± 45	798	669–892	This study
Poz-124407	17ChisA-D1-19-21	Plant fragments	55.00	N/A	1575 ± 30	489	423–560	Clarke et al. (2024)
UGAMS-58993	19Chis-C_76-77.5_nuphar	Water lily seed	76.75	−26.84	2160 ± 25	−199	−353–−60	This study
UGAMS-58994	19Chis-C_76-77.5_herb	Herbaceous fragments	76.75	−29.62	2310 ± 50	−374	−537–−203	This study
UGAMS-51577 ^a	19Chis-C_77.5-78.5	Pollen	78.00	−27.99	3030 ± 20	−1282	−1386–−1217	This study

^a Dates are too old, due to a laboratory error at UAF. They are not included in the age model.

significant figures. The relative amounts of (i) 5β-stanols and (ii) bile acids in each sample were calculated to account for variability in concentrations between samples. The presence and source of faecal matter was identified using published ratios and diagnostic threshold values (full details of ratios, threshold values and references are provided in Table 4). Stanol ratios 1 (C₂₇-stanol faecal presence index) and 2 (C₂₉-stanol faecal presence index) were used to identify the presence of faecal matter input (ratio references and threshold values for all equations below are provided in Table 4):

$$\text{Stanol ratio 1 (C}_{27}\text{-stanols)} = \frac{(\text{coprostanol} + \text{epicoprostanol})}{(\text{coprostanol} + \text{epicoprostanol} + 5\alpha\text{-cholestanol})} \quad (1)$$

$$\text{Stanol ratio 2 (C}_{29}\text{-stanols)} = \frac{(\text{5}\beta\text{-stigmastanol} + \text{epi-5}\beta\text{-stigmastanol})}{\text{5}\beta\text{-stigmastanol} + \text{epi-5}\beta\text{-stigmastanol} + 5\alpha\text{-stigmastanol}} \quad (2)$$

Several ratios have been developed to distinguish between faecal matter from different source organisms (Table 4). Stanol ratios that consider 5β-stanol epimers and bile acid ratios were used to distinguish between faecal matter source organisms:

$$\text{Stanol ratio 3 (herbivore index)} = \frac{\text{epi-5}\beta\text{-stigmastanol}}{\text{5}\beta\text{-stigmastanol}} + \frac{\text{epicoprostanol}}{\text{coprostanol}} \quad (3)$$

$$\text{Stanol ratio 4 (dietary index)} = \frac{(\text{coprostanol} + \text{epicoprostanol})}{(\text{5}\beta\text{-stigmastanol} + \text{5}\beta\text{-epi-stigmastanol})} \quad (4)$$

$$\text{Bile acid ratio 1 (dietary index)} = \frac{\text{deoxycholic acid (DCA)}}{\text{lithocholic acid (LCA)}} \quad (5)$$

$$\text{Bile acid ratio 2 (dietary index)} = \frac{\text{deoxycholic acid (DCA)}}{\text{chenodeoxycholic acid (CDCA)}} \quad (6)$$

The application of multiple ratios has the potential to refine dietary separation in modern dung (H1) and track changes in faecal source contributions in palaeoenvironmental reconstructions (H2 and H3): Stanol ratio 3 differentiates among different herbivores, whilst Stanol ratio 4 differentiates between omnivores and herbivores (Table 4). The bile acid ratios differentiate ruminant dung from other herbivore and

omnivore sources (Bile acid ratio 1), and human faeces from herbivore dung (Bile acid ratio 2) (Prost et al., 2017, Table 4).

The distributions of the relative steroid abundances (% of total weight of (i) sterols + stanols and (ii) bile acids) and untransformed diagnostic ratio values of the modern faecal samples are displayed using boxplots. Ordination analyses of the dung data followed Harrauld et al. (2019) using *FactoMineR* R package (Le et al., 2008) in RStudio (Posit Team, 2025). Principal Component Analysis (PCA) was performed using arcsine transformed relative abundances of stanols and bile acids. PCA analyses were conducted on (i) 5β-stanols, (ii) bile acids, (iii) all sterols and bile acids (to assess the contributions of Δ⁵-stanols and 5α-stanols on faecal source identification and the influence of faecally-derived Δ⁵-stanols and 5α-stanols on lake sediments) and (iv) 5β-stanols and bile acids combined (to assess interacting influences of

faecally-derived stanols and bile acids in source identifications). Secondary 5β-stanol data from Harrauld et al. (2019) and 5β-stanol and bile acid data from Wendt et al. (2024) are included in PCAs to expand the modern reference material available for faecal source identification. These studies were selected due to their overlapping species of interest, which facilitates assessment of the extent to which steroid distributions from different populations of the same species differ. Compound assignments for 5β-stigmastanol and 5β-epi-stigmastanol in the dataset from Harrauld et al. (2019) were based on their published mass spectra rather than the names provided (i.e. here we present 24-Ethylcoprostanol data as 5β-stigmastanol and 24-Ethylepicoprostanol data as 5β-epi-stigmastanol). Lake samples were added as supplementary individuals to the dung PCAs.

Changes in the trends of lacustrine 5β-stanol accumulation rates, ratios and PC1 values over time were assessed using piecewise linear regression in the *strucchange* R package (Zeileis et al., 2002). A minimum segment size constraint of 0.2 was selected to ensure model stability after testing the impact of different values. The optimal number of breakpoints was determined using the Bayesian Information Criterion (BIC), with lower BIC values indicating a better model fit.

Ratios of 5α-stanols:Δ⁵-sterols, 5β-stanols:5α-stanols, 5β-stanols:Δ⁵-stanols and epi-5β-stanols:5β-stanols were used to assess environmental transformation pathways and sources of sterols downcore and relationships were quantified with generalised additive models (GAMs).

Table 4

Faecal steroid groups and indicator ratios. Threshold values from stanol ratios 1–3 and 5 and bile acid ratios 1–2 are reported from [Prost et al. \(2017\)](#), based on primary and secondary reference faecal samples primarily from Western Europe as well as Australia and USA. Sterol references include: sheep $n = 8$, horse $n = 39$, donkey $n = 6$, cow $n = 47$, chicken $n = 23$, pig $n = 48$, human $n = 83$. Bile acids: horse $n = 26$, cow $n = 25$, chicken $n = 17$, pig $n = 26$, human $n = 70$. Threshold values serve as a guide only since steroid distributions vary based on diet and digestive processes ([see Introduction](#)) and therefore will differ between locations and populations.

Steroid Group	Category/Indicator	Target compounds	Source organisms and processes
Sterol	Δ^5 -sterols	cholesterol, β -sitosterol, campesterol, stigmasterol	Produced by eukaryotes. β -sitosterol, campesterol and stigmasterol predominantly from vegetation and cholesterol in variable amounts from nearly all eukaryotic cells including fungi, vegetation (including algae) and animal tissue (including vertebrates and protozoa). Δ^5 -sterols are also present in faeces.
Sterol	5 α -stanols	5 α -cholestanol, 5 α -stigmasterol, 5 α -campestanol	Predominantly derived from microbial hydrogenation of Δ^5 -sterols in the environment. Small amounts of 5 α -stanols are present in fresh plant and animal tissues as well as faeces.
Sterol	5 β -(faecal) stanols	coprostanol, epicoprostanol, 5 β -campestanol, 5 β -epi-campestanol, 5 β -stigmasterol, 5 β -epi-stigmasterol	Predominantly derived during the digestive process (small amounts transformed in the anaerobic environments) and are the dominant compounds in faeces.
Bile acid	Primary bile acids	cholic acid (CA), chenodeoxycholic acid (CDCA)	Synthesised from cholesterol in the liver.
Bile acid	Secondary bile acids	lithocholic acid (LCA), deoxycholic acid (DCA), hydoxycholic acid (HDCA), ursodeoxycholic acid (UDCA)	Primary bile acids are concentrated (10x) in the gall bladder before being transformed to secondary bile acids in the intestine.
Stanol ratio 1 (C ₂₇ -stanol faecal index)	Presence of faecal matter inferred from the ratio of faecal to environmentally derived cholesterol reduction products (C ₂₇ -stanols)	(coprostanol + epicoprostanol)/(coprostanol + epicoprostanol + 5 α -cholestanol)	>0.7 confirms presence of faecal matter and >0.3 is thought to indicate possible presence of faeces, however more empirical testing is needed and exact values are context specific (Bull et al., 1999 ; Grimalt et al., 1990 ; Prost et al., 2017). Applied here to address H1 : faecal steroid source differentiation and H2/H3 : faecal deposition intensity and identification in past landscapes.
Stanol ratio 2 (C ₂₉ -stanol faecal index)	Presence of faecal matter inferred from the ratio of faecal to environmentally derived β -sitosterol reduction products (C ₂₉ -stanols)	(5 β -stigmasterol + epi-5 β -stigmasterol)/(5 β -stigmasterol + epi-5 β -stigmasterol + 5 α -stigmasterol)	>0.7 confirms presence of faecal matter and >0.3 is thought to indicate possible presence of faeces, however more empirical testing is needed and exact values are context specific (Bull et al., 1999 ; Prost et al., 2017). Applied here to address H1 : faecal steroid source differentiation and H2/H3 : faecal deposition intensity and identification in past landscapes.
Stanol ratio 3 (Herbivore differentiation index)	Source identification between herbivores	(epi-5 β -stigmasterol/5 β -stigmasterol) + (epicoprostanol/coprostanol)	<0.8: not horse. Absence of CDCA: cattle/sheep; CDCA present = goat/goose Horse: >1.2 (Prost et al., 2017). Applied here to test the ability of this ratio to distinguish between a more diverse range of herbivore (H1 , H3).
Stanol ratio 4 (Dietary differentiation index with epimers)	Source identification	(coprostanol + epi-coprostanol)/(5 β -stigmasterol + 5 β -epi-stigmasterol)	Omnivore >1 (Shillito et al., 2011), expanding the ratio from Evershed and Bethell (1996) by including epimers. Applied in this study to distinguish between omnivores and herbivores (H1 , H3).
Dietary differentiation index (R')	Source identification	(coprostanol + epi-coprostanol)/(coprostanol + epi-coprostanol + 5 β -stigmasterol + 5 β -epi-stigmasterol)	To distinguish between omnivores and herbivores (Li et al., 2024a) updated Bull et al. (2002) ratio to include epimers). Will reflect the same trends as Stanol ratio 4 above; therefore, it has not been applied in this study.
Dietary differentiation index	Source identification	coprostanol/(coprostanol + 5 β -stigmasterol) * 100	Herbivore: <29% Pig: 29–65% (supported by HDCA presence) Human: >65% (Leeming et al., 1997), with thresholds updated by Prost et al. (2017) . This ratio is similar to Stanol ratio 4 but does not account for 5 β -epimer transformation. It has therefore not been applied in this study.
Bile acid ratio 1 (Dietary differentiation index)	Source identification	DCA/LCA	Values used to distinguish between dietary types and digestive systems. E.g., ruminant species have higher values than other domesticated species and humans: Geese, pig: 0.01–0.4 Human, horse, donkey: 1.1–4.5 Cattle, sheep, goat: 5–48 (Prost et al., 2017) Applied here to address H1 : faecal steroid source differentiation and H3 : faecal deposition intensity and identification in past landscapes.
Bile acid ratio 2 (Dietary differentiation index)	Source identification	DCA/CDCA	Values used to distinguish between dietary types and digestive systems. Humans are expected to have high values compared with other omnivores/herbivores (other species are order of magnitude lower than humans) e.g.: (continued on next page)

Table 4 (continued)

Steroid Group	Category/Indicator	Target compounds	Source organisms and processes
			Horse: 0.8–1.1 Geese: 0.03–0.09 Goat: 19–53 Human: 80–171 (Prost et al., 2017). Applied here to address H1: Faecal steroid source differentiation and H2/H3: Faecal deposition intensity and identification in past landscapes. First proposed by Evershed and Bethell (1996) to distinguish between omnivore (>1.5) and herbivore. Lower thresholds values were applied by Vachula et al. (2019) to identify human input in northern Alaska: Human >0.18, Mammoth <0.18. This ratio does not account for 5 β -epimer transformation; it has not been applied to characterise the modern faecal material in this study.
Stanol dietary index	Source identification: omnivore versus herbivore faeces	coprostanol/5 β -stigmastanol	

GAMs were also used to quantify relationships between sediment core age and both individual compounds and aggregate sterol groups (Δ^5 -sterols, 5 α -stanols, 5 β -stanols) to assess sterol source and diagenesis. GAMs were run using the *mgcv* R package (Wood, 2011; Wood et al., 2017) and statistical significance was assessed at the 95% confidence interval ($p < 0.05$).

Estimates of faecal production rates for humans and animal species identified from archaeological faunal assemblages in the Tanana Valley (Potter, 2008) are derived from published data (Supplementary, Appendix A3) and are reported to two significant figures. Mean excretion rates of C₂₇- and C₂₉-5 β -stanols are estimated using faecal production rates and faecal 5 β -stanol data from modern reference material (this study; Gryczewska et al., 2025; Harrault et al., 2019; Leeming et al., 1996; Li et al., 2024a; Wendt et al., 2024).

3. Results

3.1. Chronology

The 77 cm 19Chis-C core represents approximately 2200 years of sedimentation, with the base of the core dating to 165 BCE (Fig. 3A). Sedimentation rates range from 20 years cm⁻¹ to 55 years cm⁻¹, with a mean sedimentation rate of 28.4 ± 5.2 years cm⁻¹. Bayesian modelling of the ²¹⁰Pb measurements report a date of 1895 CE at 3 cm and produce sedimentation rates between 39 and 44 years cm⁻¹ in the top 3 cm of sediments (Fig. 3B). The combined ¹⁴C and ²¹⁰Pb Bayesian age-depth model, which dates 3 cm to 1879 CE, has slightly lower sedimentation rates in the top 3 cm of the record, ranging from 43 to 55 years cm⁻¹ (Fig. 3A).

3.2. Dung steroids

Avian (mallard and crane) guano contains the highest average total sterol concentrations (19 to 29 mg g⁻¹), which are an order of magnitude higher than bear (6 mg g⁻¹), wolf (2 mg g⁻¹) and ruminant faeces (1 to 4 mg g⁻¹) (Table 5). The most prevalent sterols are β -sitosterol in avian (67 to 71% of total sterols), bear (74%) and wood bison (40%) faeces, cholesterol in wolf scat (74%), 5 β -stigmastanol in plains bison (27%) and 5 α -stigmastanol in caribou (34%) and elk dung (24%) (Fig. 4). Ruminant dung contains more 5 α -stanols than the other faeces and the relative abundances are similar between ruminant species with the exceptions of lower 5 α -cholestanol in moose dung and lower 5 α -campestanol in elk dung. 5 β -stanols are not detected in bear or mallard

faeces and very low concentrations of coprostanol are detected in some crane faeces (0.8 μ g g⁻¹, <1%). Coprostanol is the most abundant 5 β -stanol in wolf scat (3%), although plains bison, caribou and elk dung contain higher relative abundances (4 to 7%). Elevated 5 β -stigmastanol and epi-5 β -stigmastanol are characteristic of the ruminant dung, together comprising 14 to 66% of their total sterols, but are absent in bear and bird faeces and negligible in wolf scat. The two bison subspecies have overlapping distributions of 5 α -stanols but distinct relative abundances of Δ^5 -sterols and 5 β -stanols.

Bears excrete up to two orders of magnitude more primary bile acids (4 mg g⁻¹) than birds (0.3 to 0.8 mg g⁻¹) and herbivores (0.06 to 0.1 mg g⁻¹) (Table 5). Secondary bile acids vary within and between faeces of all dietary groups, but are highest in bear faeces (0.9 mg/g). Bear and bird faeces are dominated by primary bile acids (64 to 81% of total bile acids), most of which is CDCA (Fig. 5). Ruminant dung contains more of the secondary bile acid DCA than the other species (52 to 86%). LCA is variable within and between dietary types, with wolf faeces containing the highest relative proportion (73%), followed by ruminant faeces (7 to 44%). With the exception of some mallard faeces, LCA and DCA are present in all dung analysed here. UDCA is detected only in the bear and bird faeces. CA is detected in all analysed faeces, except crane guano.

Existing sterol identification and characterisation ratios (Table 4) cannot be applied to the bear, mallard or crane samples analysed in this study because the required compounds were not detected. Stanol ratios 1 and 2 (C₂₇- and C₂₉-stanol faecal presence indices, respectively) exceed 0.3 in wolf and ruminant samples and therefore are within the range of possible faecal matter (Fig. 6). Only wolf and moose dung consistently have C₂₇-stanol ratio 1 values > 0.7 to confirm faecal input, whereas wood bison samples do not exceed this threshold. Stanol ratio 2 (C₂₉-stanol based) are more variable than stanol ratio 1 (C₂₇-stanol based): most exceed 0.3, but many remain below the 0.7 threshold derived from other modern dung samples (Prost et al., 2017), preventing definitive faecal identification. Stanol ratio 3 (herbivore differentiation index) exhibits overlapping distributions for most ruminants but separates moose dung from both bison species. Dung from all ruminant species except from moose fall within the range of horse manure as characterised by Prost et al. (2017), whereas moose values exceed the 1.2 horse threshold. Stanol ratio 4 (dietary index) is highest in wolf scat samples, which is consistent with the omnivore range of >1 (Shillito et al., 2011). All ruminant samples are lower than the omnivore range and moose faeces have lower values than the other ruminants (<0.06).

Bile acid ratio 1 values for ruminants span above and below the

threshold to distinguish between human/horses and cattle/sheep/goat. Mallard bile acid ratio 1 values align with those of geese, but crane values are higher (reaching 0.3), bear and wolf also exhibit low values (maximum values of 0.3 and 0.6, respectively). Ruminant bile acid ratio 2 values are variable, but they are greater than the published range of horse, whilst bear, mallard and crane are lower.

Multivariate analyses demonstrate that 5β -stanols provide the clearest species-specific separation of steroid compositions (PC1 and PC2 = 87% of total variance; (Fig. 7A)), compared with bile acids or combined sterols and bile acids (Fig. 7B–D). Wolf (carnivore) scat is isolated based on elevated coprostanol and ruminants are characterised by elevated 5β -stigmastanol. Within the ruminants, there is a distinct clustering between moose, wood bison, plains bison and elk, but caribou have a wide and overlapping distribution. The first two principal components of the bile acid PCA account for 80% of total variance (Fig. 7B), with PC1 facilitating the isolation of bear and avian faeces (elevated UDCA and CDCA) from ruminant and carnivores (elevated DCA and LCA). Whilst the bile acid PC2 identifies differences between bear and avian faeces based on UDCA and CDCA, respectively, the differences among the ruminants are less pronounced than the 5β -stanol PCA.

When all sterol and bile acid distributions are considered together, the first two principal components account for 72% of the total variance (Fig. 7C) and PC axes 1 and 2 remain effective at distinguishing between ruminants and the carnivores and omnivores. Wolf, bear, mallard and crane are characterised by elevated concentrations of Δ^5 -sterols and ruminants by elevated levels of DCA and 5β -stanols (Fig. 7C). Carnivore (wolf) scat is separated based on elevated cholesterol and campesterol. The combination of sterols, stanols and bile acids achieve greater separation of wood bison and moose dung than 5β -stanols or bile acids

alone, driven by differences in both distributions of secondary bile acids and 5α -stanol compositions. Differentiation among the ruminant species is improved when only 5β -stanols and bile acids are considered (PC1+PC2 = 74%) (Fig. 7D).

The first two principal components of 5β -stanol data, from this study and published faecal reference samples from more species and different populations (Harrault et al., 2019; Wendt et al., 2024), account for 81% of the total variance, with coprostanol isolating human, dog and wolf scat from herbivore dung along PC1 (Fig. 7E). PC2 distinguished lemming faeces from the ruminant dung based on elevated 5β -stigmastanol. The amount of total PCA variance accounted for reduces when both 5β -stanols and bile acids are considered (PC1+PC2 = 53%; note reduced number of species included owing to data availability; Fig. 7F).

3.3. Lake sedimentary steroids

Δ^5 -sterols are the most abundant group of sterols in the lake sediment record, accounting for $790 \pm 270 \text{ ng cm}^{-2} \text{ yr}^{-1}$, whilst 5α -stanols and 5β -stanols account for 200 ± 100 and $140 \pm 130 \text{ ng cm}^{-2} \text{ yr}^{-1}$, respectively (Fig. 8, Supplementary, Appendices A4–A5). Accumulation rates of Δ^5 -sterols, 5α -stanols and 5β -stanols are high from c. 1900 CE to the present day, with all groups exceeding millennial base levels from c. 1940 CE onwards (Fig. 8, Supplementary, Appendix A5). C_{29} - 5β -stanols (5β -stigmastanol and epi- 5β -stigmastanol) are the dominant 5β -stanols within the lake record, increasing from 88 to $290 \text{ ng cm}^{-2} \text{ yr}^{-1}$ over the last century, whilst C_{27} - 5β -stanols (coprostanol and epicoprostanol) increased from 11 to $88 \text{ ng cm}^{-2} \text{ yr}^{-1}$ over the same period. The accumulation rate of all groups of sterols doubled from 1980 to 1998 cal yr CE (1 to 0.5 cm), which is the most abrupt and pronounced change in the

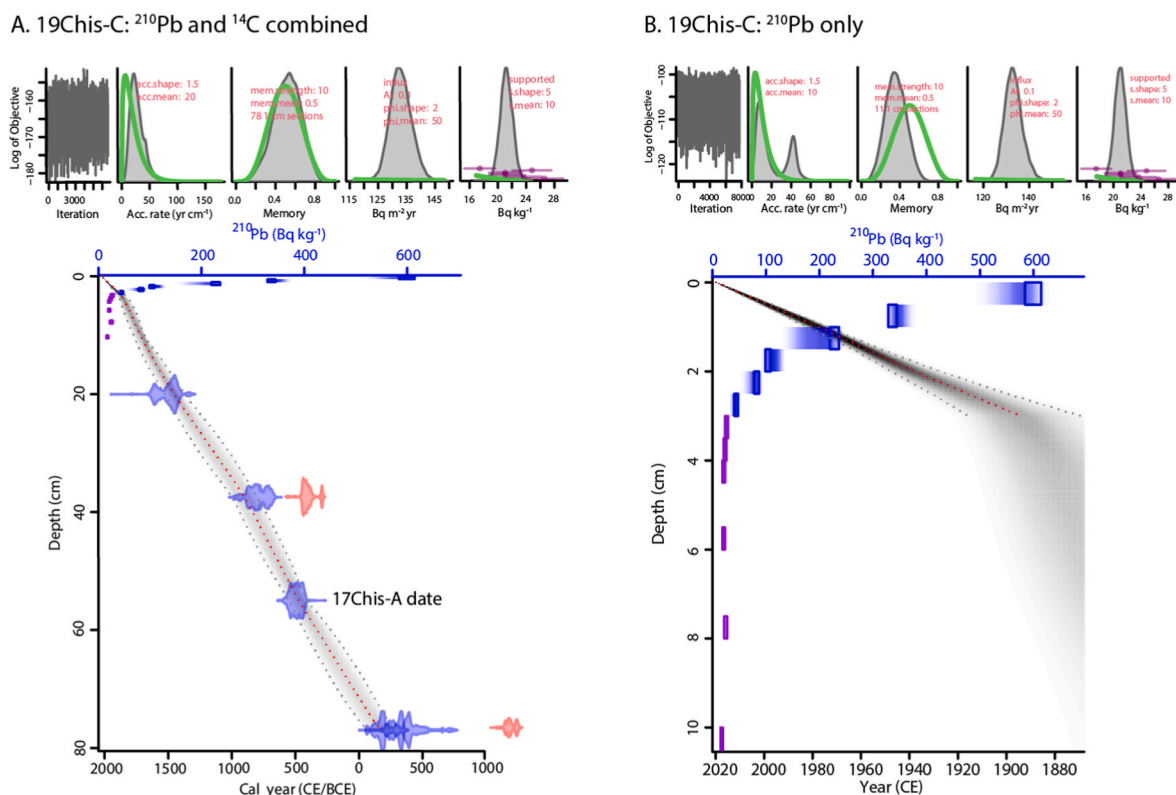


Fig. 3. Bayesian-age model for core 19Chis-C. A: Combined ^{210}Pb (PLUM modelled) and ^{14}C age-depth model. Blue markers represent accepted chronological constraints (dark blue markers between 0 and 3 cm = ^{210}Pb ; light blue markers between 20 and 80 cm = ^{14}C). The rejected pollen dates are in red and are not included in the age model (see Materials and Methods). B: PLUM ^{210}Pb -only model for the historical period (1892–2019 CE) of 19Chis-C. Blue boxes represent measured ^{210}Pb activities ($\pm 1 \sigma$) and blue shading represents the Bayesian modelled activity. Purple boxes represent the sediment-supported ^{210}Pb activity levels. Only measurements in blue (those exceeding sediment-supported ^{210}Pb) are used in the Constant Rate of Supply (CRS) age model (Table 2). Grey shading in A and B is the 2σ range of the age-depth model and the red dashed line marks the mean age at each depth. The panels at the top of A and B show prior (green) and posterior (grey) distributions for model iterations for the accumulation rate, memory, supply of ^{210}Pb and supported ^{210}Pb .

Table 5

Steroid composition of dung samples (average values and standard deviations, reported to 2 significant figures).

	Steroid content ($\mu\text{g g}^{-1}$ dry matter)								
	Wolf	Bear	Crane	Mallard	Wood bison	Plains bison	Caribou	Elk	Moose
Samples analysed	1 (4 reps)	1 (4 reps)	6	6	6	6	6	6	6
Δ^5 -Sterols									
Cholesterol	1500 $\pm$ 1900	1500 $\pm$ 500	2200 $\pm$ 840	3600 $\pm$ 2400	730 $\pm$ 410	110 $\pm$ 70	95 $\pm$ 51	170 $\pm$ 330	32 $\pm$ 24
β -Sitosterol	290 $\pm$ 89	4700 $\pm$ 1500	13000 $\pm$ 6100	21000 $\pm$ 15000	1600 $\pm$ 770	140 $\pm$ 69	300 $\pm$ 180	360 $\pm$ 500	170 $\pm$ 130
Stigmasterol	28 $\pm$ 6	49 $\pm$ 6	2500 $\pm$ 1400	2500 $\pm$ 1700	43 $\pm$ 32	11 $\pm$ 11	16 $\pm$ 10	37 $\pm$ 58	2 $\pm$ 3
Campesterol	120 $\pm$ 49	81 $\pm$ 20	1600 $\pm$ 700	2200 $\pm$ 1800	150 $\pm$ 77	28 $\pm$ 20	15 $\pm$ 8	26 $\pm$ 39	2 $\pm$ 2
5 α -Stanols									
5 α -Cholesterol	7 $\pm$ 7	3 $\pm$ 1	13 $\pm$ 4	14 $\pm$ 9	77 $\pm$ 46	39 $\pm$ 30	27 $\pm$ 7	80 $\pm$ 130	4 $\pm$ 2
5 α -Stigmastanol	7 $\pm$ 2	22 $\pm$ 7	54 $\pm$ 24	60 $\pm$ 31	640 $\pm$ 320	320 $\pm$ 150	490 $\pm$ 500	720 $\pm$ 880	170 $\pm$ 76
5 α -Campestanol	2 $\pm$ 1	0 $\pm$ 1	7 $\pm$ 4	9 $\pm$ 6	63 $\pm$ 43	29 $\pm$ 17	20 $\pm$ 9	24 $\pm$ 46	12 $\pm$ 10
5 β -Stanols									
Coprostanol	62 $\pm$ 89	0 $\pm$ 0	1 $\pm$ 2	0 $\pm$ 0	50 $\pm$ 33	110 $\pm$ 69	60 $\pm$ 15	190 $\pm$ 360	23 $\pm$ 12
5 β -Stigmastanol	9 $\pm$ 7	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	390 $\pm$ 300	430 $\pm$ 130	270 $\pm$ 110	600 $\pm$ 11000	420 $\pm$ 220
5 β -Campestanol	3 $\pm$ 4	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	19 $\pm$ 23	70 $\pm$ 41	30 $\pm$ 16	77 $\pm$ 140	6 $\pm$ 3
Epi-5 β -stanols									
Epicoprostanol	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	12 $\pm$ 7	19 $\pm$ 16	17 $\pm$ 17	48 $\pm$ 76	9 $\pm$ 3
Epi-5 β -stigmastanol	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	160 $\pm$ 150	270 $\pm$ 95	99 $\pm$ 43	540 $\pm$ 690	410 $\pm$ 180
Epi-5 β -campestanol	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	16 $\pm$ 8	8 $\pm$ 7	70 $\pm$ 130	4 $\pm$ 3
Primary bile acids									
Cholic Acid	6 $\pm$ 5	170 $\pm$ 180	0 $\pm$ 0	1 $\pm$ 3	3 $\pm$ 4	7 $\pm$ 6	49 $\pm$ 92	11 $\pm$ 13	46 $\pm$ 93
Chenodeoxycholic Acid	9 $\pm$ 9	3500 $\pm$ 2900	820 $\pm$ 600	280 $\pm$ 110	3 $\pm$ 6	2 $\pm$ 3	72 $\pm$ 140	7 $\pm$ 6	0 $\pm$ 0
Secondary bile acids									
Lithocholic Acid	120 $\pm$ 95	6 $\pm$ 7	40 $\pm$ 47	130 $\pm$ 290	63 $\pm$ 110	82 $\pm$ 71	190 $\pm$ 220	35 $\pm$ 60	50 $\pm$ 68
Deoxycholic Acid	30 $\pm$ 30	3 $\pm$ 2	10 $\pm$ 8	2 $\pm$ 3	74 $\pm$ 81	520 $\pm$ 470	400 $\pm$ 330	58 $\pm$ 39	590 $\pm$ 1000
Ursodeoxycholic Acid	0 $\pm$ 0	880 $\pm$ 690	200 $\pm$ 110	28 $\pm$ 46	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Average sum of identified compounds									
Sterols	2000	6400	19000	29000	3900	1600	1400	2900	1300
Bile acids	160	4600	1100	430	140	610	710	110	690

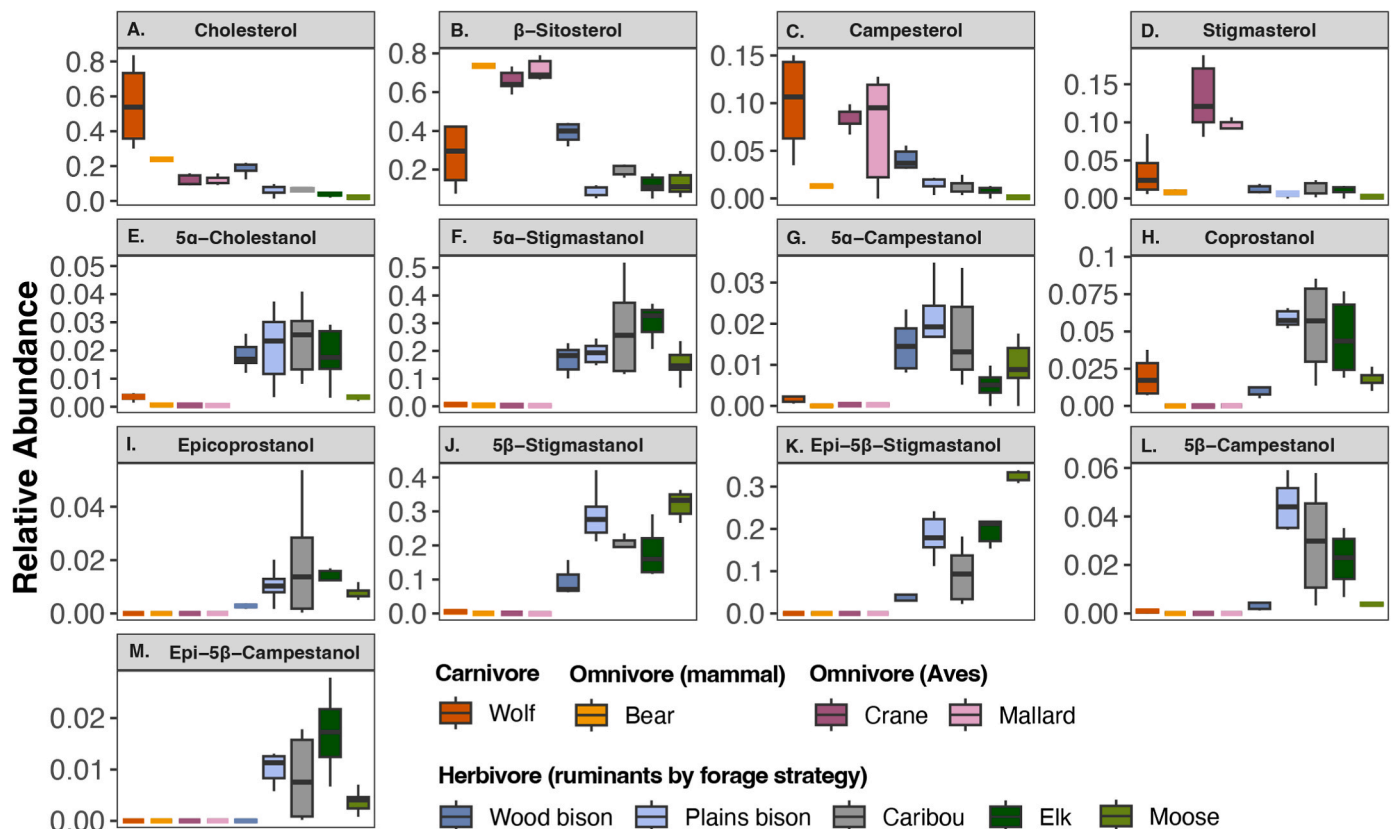


Fig. 4. Box plots of sterol relative abundances (% of total sterols) from faecal reference material (note differences in scale for legibility). A-D: Δ^5 -sterols, E-G: 5 α -stanols, H-M: 5 β -stanols and their epimers. Colours represent differences in dietary types: light red = carnivore, orange = omnivore (mammal), purple = omnivore (Aves), blue = herbivore (grazers), grey = herbivore (intermediate feeders), green = herbivore (browsers).

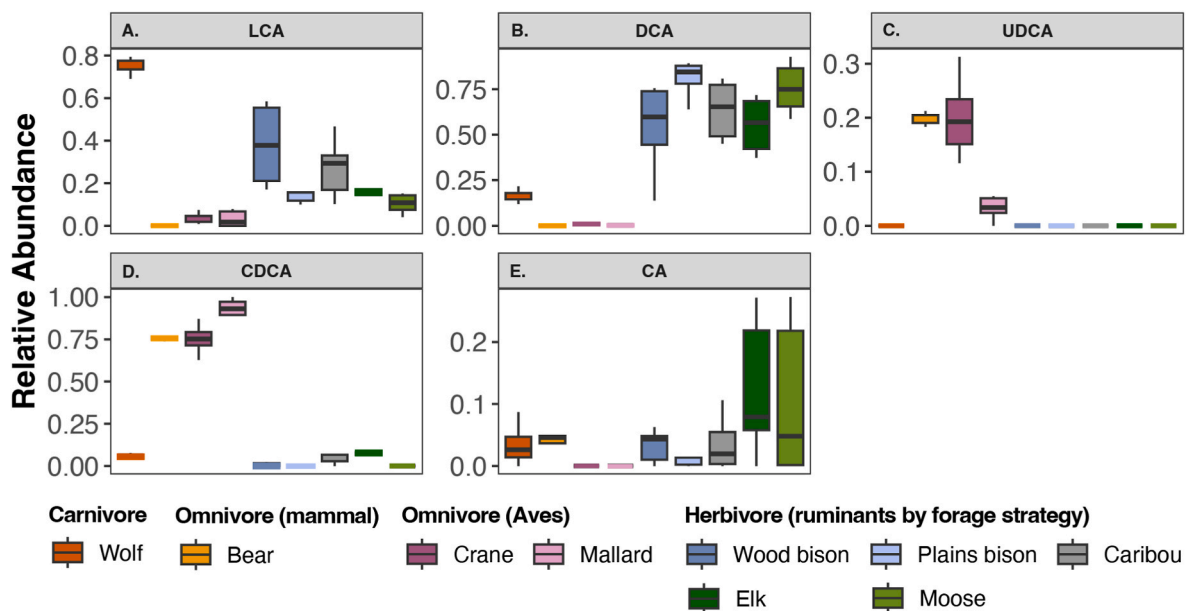


Fig. 5. Box plots of bile acid relative abundances (% of total bile acids) by species (note differences in scale for legibility). A-C: secondary bile acids, lithocholic acid (LCA), deoxycholic acid (DCA), ursodeoxycholic acid (UDCA). D-E: primary bile acids, chenodeoxycholic acid (CDCA), cholic acid (CA). Colours represent differences in dietary types: light red = carnivore, orange = omnivore (mammal), purple = omnivore (Aves), blue = herbivore (grazers), grey = herbivore (intermediate feeders), green = herbivore (browsers).

2000-year record. There are no statistically significant relationships between the age or depth of the sediment and the accumulation rate of Δ^5 -sterols nor 5 β -stanols, but 5 α -stanols increase non-linearly over time (edf = 2.17, $p < 0.05$; Fig. 8). The elevated biomarker accumulation rates in the surface sediment sample (1998 CE) have a strong influence on the fitted GAM (Supplementary, Appendix A6). Following removal of this outlying surface sediment sample (2 σ ; Supplementary, Appendix A7), statistically significant relationships are detected between 5 α -cholestanol and coprostanol (edf = 2.65, $p < 0.05$); cholesterol and coprostanol (edf = 1; $p < 0.05$); β -sitosterol and 5 α -stigmastanol (edf = 2.46, $p < 0.05$) and 5 β -stigmastanol and 5 β -epi-stigmastanol (edf = 1.83, $p < 0.05$). Ratios of 5 α -cholestanol:cholesterol (edf = 2.21, $p < 0.05$) and 5 α -stigmastanol: β -sitosterol (edf = 2.46, $p < 0.05$) increase non-linearly towards the present day (Supplementary, Appendix A8).

Despite detection of 5 β -stanols in all lake sediments analysed, bile acids are only intermittently detected in 25% of the samples analysed and all occur within the last 600 years (Fig. 8, Supplementary, Appendix A5). LCA is at least an order of magnitude more abundant than the other detected compounds and the largest amount of LCA was detected in 1420 CE (290 ng cm⁻² yr⁻¹). There is, however, no accompanying prominent increase in 5 β -stanols at this time. LCA, DCA, HDCA and UDCA were detected in 1860 (75 ng cm⁻² yr⁻¹) and LCA, HDCA and UDCA were detected in 1980 (33 ng cm⁻² yr⁻¹) prior to an isolated presence of LCA in 1998 (Fig. 8, Supplementary, Appendices A4-A5). There is no significant relationship between bile acid presence/abundance and age of the sediments.

C₂₇-stanol ratio 1, which identifies the presence of faecal matter based on cholesterol reduction products, supports possible faecal input in the upper surface sample (values > 0.3 in 1998 cal yr CE; Fig. 8F, Supplementary, Appendix A4), whilst C₂₉-stanol ratio 2, which identifies the presence of faecal matter based on the phytosterol β -sitosterol reduction products, indicates the possible presence of faecal matter (values > 0.3) in all samples. Stanol ratio 3 and 4 support a dominant herbivore faecal source and the source organisms change over time: moose dung thresholds of stanol ratio 4 (<0.07; Fig. 6E) are intermittently present but decreasing towards the present day whilst other

browsers dominate samples analysed at the start and end of the record (Stanol ratio 3 < 2.6). The majority of stanol ratio 3 values are higher than 2.75, however, these values are not well represented by the reference material analysed within this study (overlaps with only 1 elk sample), nor those analysed by Prost et al. (2017) who reported human and pig faeces as ranging between 0.01 and 0.13 and the highest herbivore dung values being horse manure (2.08 to 2.71).

Multivariate analyses of steroid compounds in Chisholm Lake sediments provide further insights into sources of faecal input and temporal changes in faecal input are identified using breakpoint analysis. PC1 and PC2 in the PCA with all sterols included account for 71% of the total variance (Fig. 9A) and three breakpoints, were identified, indicating shifts in the rate of change of the PC1 score derived from all sterols, occurring prior to 810 cal yr CE, 1740 cal yr CE and 1958 CE (Fig. 9C, Supplementary, Appendix A9). When the PCA is restricted to only 5 β -stanols, PC1 and PC2 account for 85% of the total variance (Fig. 9B) and a single breakpoint in 5 β -stanols PC1 scores is identified prior to 1958 CE (Fig. 9D, Supplementary, Appendix A9). Passive PCA plotting of the Chisholm Lake 5 β -stanols and bile acids distributions reveals no overlap with the modern faecal reference material from this study or published data (Harrault et al., 2019; Wendt et al., 2024, Fig. 9E-F).

4. Discussion

4.1. Steroid-based characterisation of modern faecal deposits

4.1.1. Evaluating steroid approaches for detecting the presence of faeces

Stanol ratios 1 (C₂₇-stanol faecal index; Equation (1)) and 2 (C₂₉-stanol faecal index; Equation (2)), which distinguish between environmentally derived 5 α -stanols (predominately non-faecal) and 5 β -stanols from faecal sources, are effective at identifying the faecal origin of the North American carnivore and herbivore faecal deposits in this study when the lower threshold values (0.3) are applied. Ratio values less than the upper threshold (0.7) are required to consistently capture the herbivore dung deposits in this study, as was the case for dung identification from European domesticates (Prost et al., 2017) and herbivores in the Tibetan Plateau (Li et al., 2024a). The lower threshold of 0.3 was first

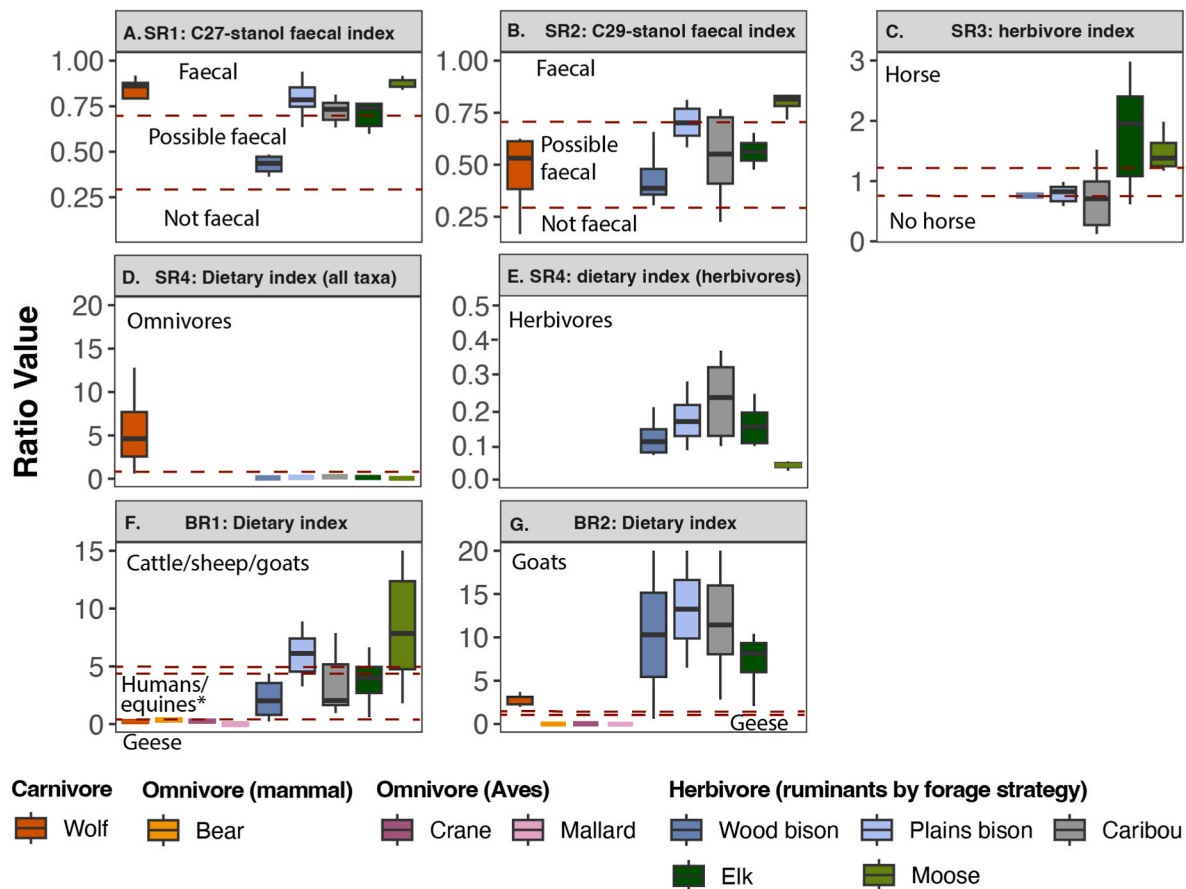


Fig. 6. Box plots of stanol and bile acid ratios (equations and references outlined in Table 4). A-B: Stanol ratios 1 and 2 (SR1 and SR2), which identify the presence of faeces (<0.3 not faecal, 0.3 to 0.7 possible faecal, >0.7 faecal). C: Stanol ratio 3 (SR3), which was designed to discriminate among herbivores (<0.8 no horses, cattle/sheep or goats/geese >1.2 horse). D-E: Stanol ratio 4 (SR4), which identifies the presence of omnivores (>1), D presents all dietary types and E is scaled to show differences in herbivores. F: Bile acid ratio 1 (BR1) which was designed to discriminate between geese (0.01 to 0.4), humans and equines (*specifically horses and donkeys) (1.1 to 4.5) and cattle/sheep/goats (5 to 48). G: Bile acid ratio 2 (BR2), which was designed to discriminate between geese (0.03 to 0.09), horses (0.8 to 1.1), goats (19 to 53) and humans (80 to 171). Colours represent differences in dietary types. Herbivore forage strategies: grazers (blue) intermediate feeders (grey) browsers (green).

identified as a boundary separating sediments in remote areas from those polluted by sewage effluent (Grimalt et al., 1990) and is applied to identify 'possible faeces' in European-focused studies of discrete deposits of domesticated mammal faeces (Bull et al., 1999; Prost et al., 2017). Whilst the 0.3 threshold captures all faecal input in the C₂₇-stanols ratio 1 and is supported by a review of published data by Li et al. (2024a), the C₂₉-stanol ratio 2 was less consistent: two samples in this study did not meet the stanol ratio 2 threshold value of 0.3 due to relatively high proportions of 5 α -stigmastanol. This is supported by stanol data from soils samples from wild herbivores enclosures in Kenya (Kemp et al., 2022) and ruminant herbivore dung from species such as giraffe, topi, wildbeast, buffalo and domesticated cattle collected in South Africa (Karp et al., 2025) but differs from assessments of European domesticates (Prost et al., 2017). These findings emphasise that the current 0.7 threshold for confirmation of faecal input is too high for the identification of wild herbivore faeces. Exact threshold values for capturing faecal input from all sources are likely to be context specific; however, the lower 0.3 value is more appropriate in this study. Furthermore, these findings highlight the importance of using multiple approaches for robust identification of faecal sources and that it is critical to expand modern faecal steroid databases with reference data (Gryczewska et al., 2025; Larson et al., 2022).

Stanol ratios to detect the presence of faeces are not applicable for all species due to low or absent 5 β -stanol excretion as detected in omnivore faeces at the time of the sample collection in this study (bear faeces were collected in spring and mallards and cranes in summer; Table 1; Fig. 4). Another study of faecal steroid compositions of modern faeces from eastern Europe reported low to absent 5 β -stanol concentration in fresh samples of other omnivores (brown bear, *Ursus arctos* and badgers, *Meles meles*) as well as carnivores (polecats, *Mustela putorius* and pine martins, *Martes martes*) and insectivores (bats, *Myotis myotis* and *Rhinolophus hipposideros*) (Gryczewska et al., 2025). Omnivores and carnivores have larger intra-species dietary variation than herbivores and all wild animals exhibit seasonal and geographical changes in diets, which have the potential to alter 5 β -stanol production and excretion. Indeed, the impact of seasonal dietary differences on faecal steroid distributions has yet to be comprehensively tested. Guano samples from other bird species contain minimal amounts or an absence of 5 β -stanols (Davies et al., 2022; Florini et al., 2020; Gryczewska et al., 2025; Leeming et al., 1996, 1997; Martin et al., 1973), although chickens show a different pattern, producing around 15 to 20 mg of coprostanol and c. 35 mg of cholesterol per individual per day (Sugano, 1967). The stanol ratios are also unlikely to robustly detect fish faeces, following the identification of 5 α -stanols dominance over 5 β -stanols in discrete fish excrement

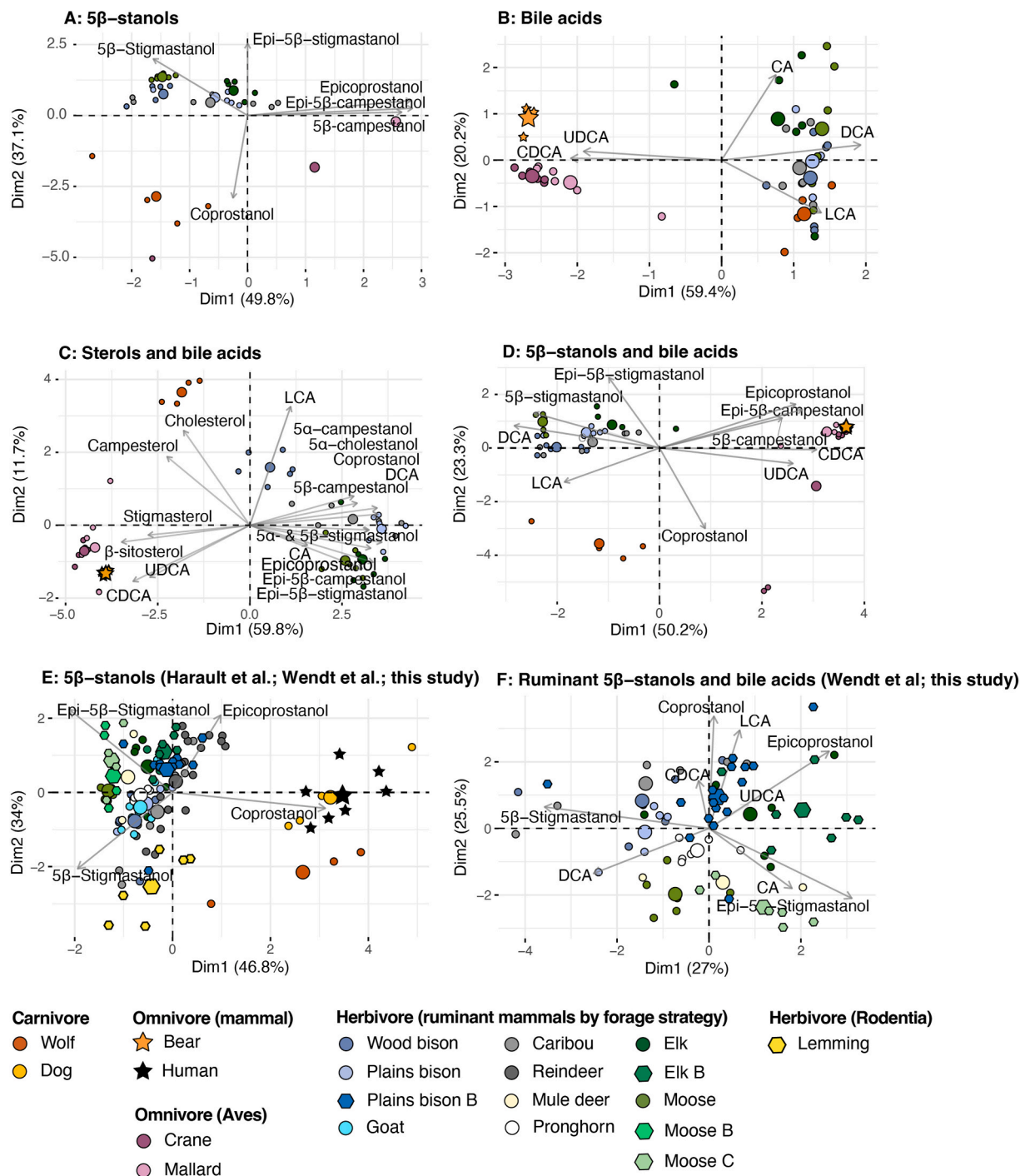


Fig. 7. PC1 and PC2 scores from PCA analysis of dung steroids. A-D: dung analysed in this study. A: 5 β -stanols only; B: bile acids only; C: all sterols and bile acids (position of compound labels adjusted for readability and therefore represent relative positions); D: 5 β -stanols and bile acids. E: PCA of 5 β -stanols (this study; Harrault et al. (2019); Wendt et al. (2024)). F: PCA of 5 β -stanols and bile acids of herbivores (this study and Wendt et al. (2024)). Symbol size within each plot reflects the quality of representation (cosine squared) of each point on the first two PCA axes, with larger symbols better represented by the two PCA axes than smaller ones. Colours and symbols represent differences in dietary types. Herbivore forage strategies: blue = ruminant grazers, grey and white = ruminant intermediate feeders, green = ruminant browsers and yellow circles = Rodentia.

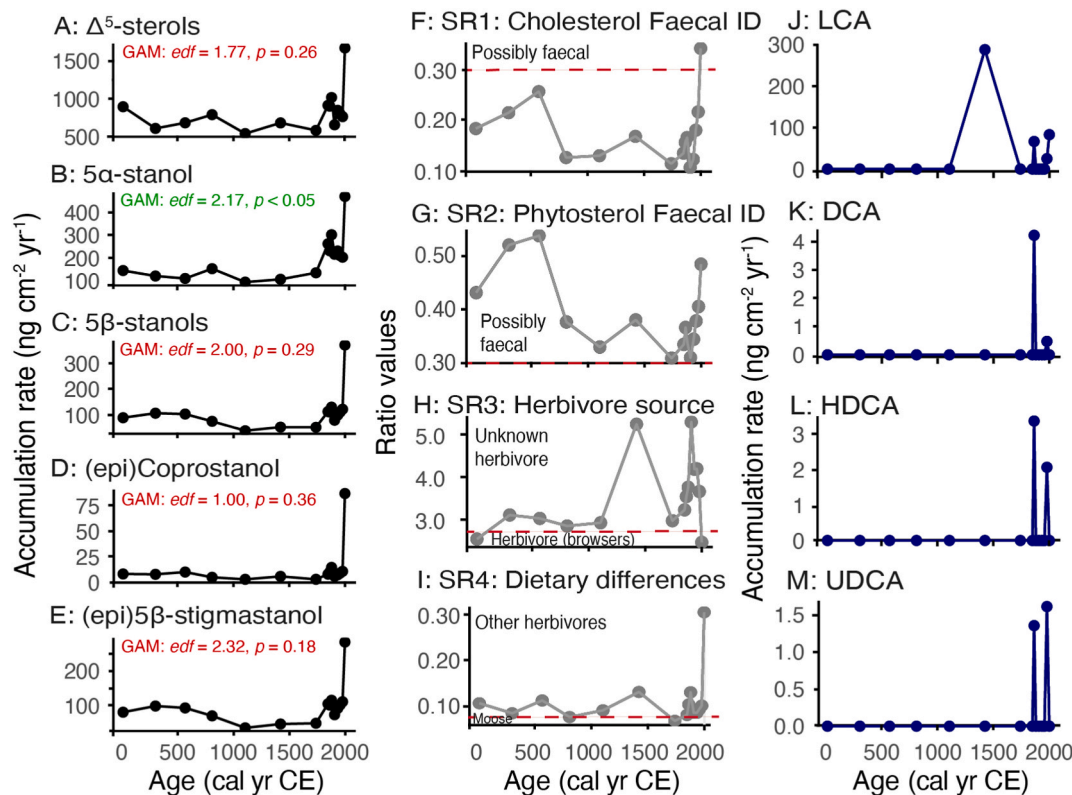


Fig. 8. Chisholm Lake steroid compositions and ratios over the last 2000 years. A-C: accumulation rates of different groups of sterols: A: Δ^5 -sterols (cholesterol + β -sitosterol + stigmasterol), B: 5 α -stanols (5 α -cholestanol + 5 α -stigmastanol) and C: 5 β -stanols (coprostanol + epicoprostanol + 5 β -stigmastanol + epi-5 β -stigmastanol); D: accumulation rates of coprostanol + epicoprostanol and E: 5 β -stanols (coprostanol + epi-5 β -stigmastanol), F-I: stanol ratios to identify faecal input and sources (equations and threshold values in Table 4). F-G: C₂₇-stanol and C₂₉-stanol faecal input indices respectively, where 0.3 to 0.7 are considered as a possible faecal input, >0.7 = confirmed faecal input. H: Stanol ratio 3, herbivore differentiation index, where <1.2 = grazers and intermediate herbivore feeders, 1.2 to 2.6 = herbivore browsers, >2.75 = no robust modern reference match, some overlap with elk; I: Stanol ratio 4, dietary differentiation index, where >1 = omnivore faeces (Shillito et al., 2011) and <0.07 = moose faeces (this study; Fig. 6E); J-M: accumulation rates of individual bile acids. Generalised Additive Model (GAM) results for sterol groups show the effective degrees of freedom (edf) and p-values (A-E). p-values in green (red) are significant (not significant) at a 95% confidence level. Steroid concentrations are provided in the supplementary data (Appendix A5) for comparative purposes.

massaged from live salmon (McGhie et al., 2000). Bile acids are therefore required to detect faecal input from species with absent or low-level 5 β -stanols.

4.1.2. Evaluating steroid approaches for source identification of discrete faecal deposits

Ratios of steroid compounds exploit differences in steroid distributions to identify faecal sources, in addition to faecal presence (Section 4.1.1). Stanol ratios are most effective at discriminating between discrete faecal sources with different diets: stanol ratios evaluating the relative inputs of animal derived (C₂₇) versus plant derived (C₂₉) 5 β -stanols (Equations (3) and (4)) clearly discriminate between discrete faecal deposits of carnivore (wolf) and herbivores (ruminants) (H1; Fig. 6). These ratios also distinguish between North American ruminants, especially moose owing to their relative higher abundance of plant derived 5 β -stanols driving lower ratio values (stanol ratio 4 < 0.07). Stanol ratio 3 could not distinguish all ruminant stanol distributions, however a boundary value of 1.1 separated plains and wood bison (<1.1) from moose (>1.1).

A key limitation of stanol ratios is their inability to identify avian faecal deposits because of a lack of 5 β -stanols present in their faeces, however, these species can be separated by bile acid ratios. Both bile acid ratios 1 (DCA relative to LCA; Equation (5)) and 2 (DCA relative to CDCA; Equation (6)) were effective at distinguishing the ruminants (>0.6) from carnivores and omnivores (<0.6; H1). Bile acid ratio 2 separates wolf faeces (2 to 4) from the omnivores (<0.02) and herbivores (>4) but cannot characterise moose faeces owing to the absence of

CDCA. There is no single steroid ratio that can be used to universally characterise sources of discrete faecal remains (Fig. 6). While the most effective approach varies based on the species of interest, combining stanol and bile acid ratios offers the best discrimination between dietary groups and, in some cases, between species within the same dietary group (Fig. 6).

Indicator compounds have been effectively used alongside steroid ratios to discriminate between faecal sources of European domesticates (Prost et al., 2017). This approach enhances ruminant identification of North American species, for example, in addition to high bile acid ratio 1 (>5) and low stanol ratio 4 (<0.07), moose can be characterised by an absence of CDCA. The presence of indicator compounds alone, however, would lead to incorrect faecal identifications. For example, UDCA is often primarily associated with bears, but similar proportions are deposited in crane guano and it is present in mallard guano (Fig. 5; Table 5). Additionally, UDCA was only detected in one out of three brown bear (*Ursus arctos*) fresh faecal matter analysed from eastern Europe (Gryczewska et al., 2025).

Dimensionality reducing statistical techniques such as PCA are increasingly applied to identify faecal steroid sources (e.g., Harrault et al., 2019; Wendt et al., 2024), and they have proved effective in discriminating between species in this study: the primary differences are reflected in dietary types, although different species occupy more subtle separate ordination spaces, which facilitates separation within ruminant and bird species. PCA can also support the identification of faecal input based on matches of samples with reference material; however, incomplete modern faecal reference databases prevent confirmation of

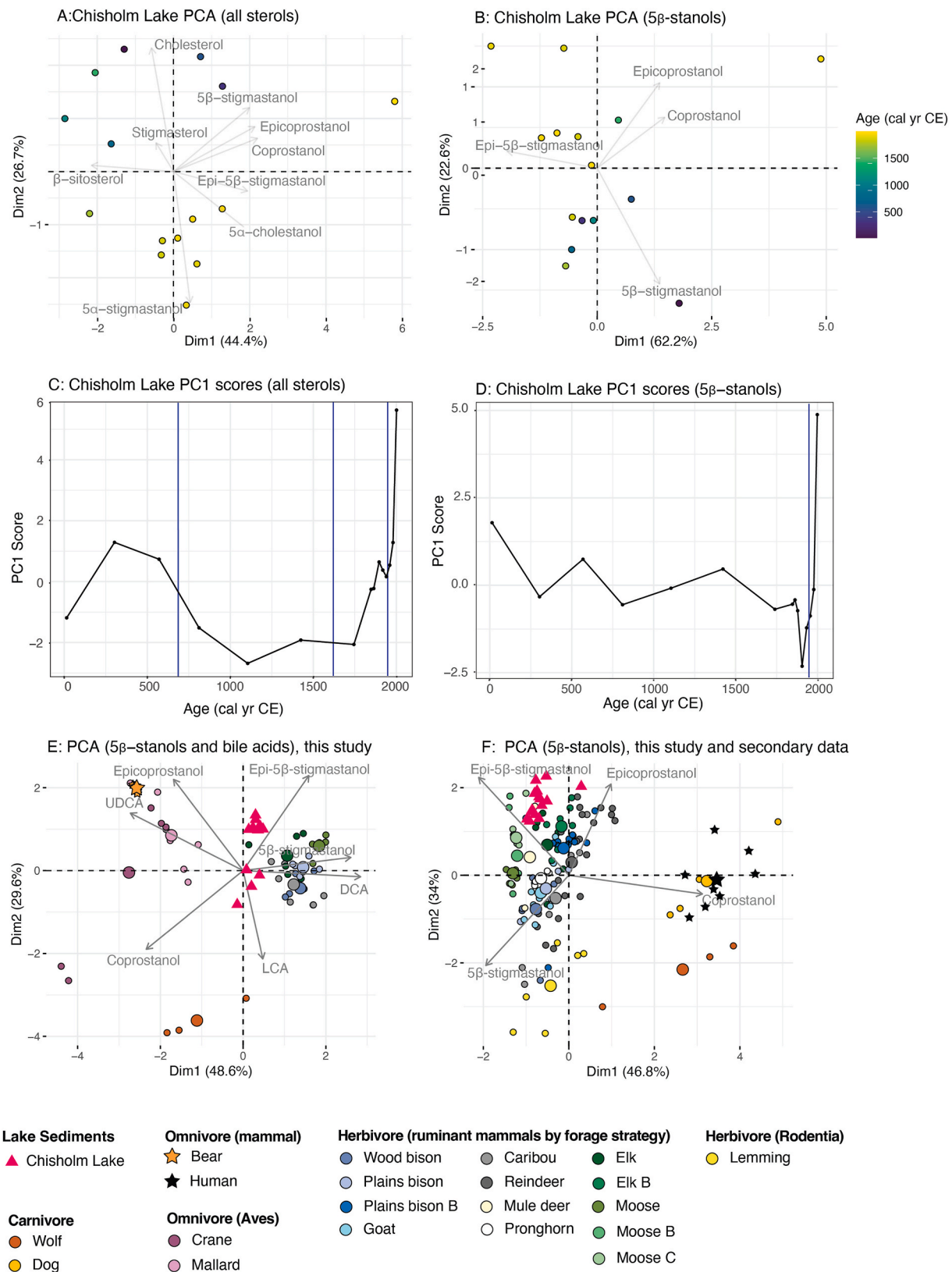


Fig. 9. Multivariate analysis of steroid distributions of Chisholm Lake sediments. A-B: PC1 and PC2 scores from PCA analysis of all sterols and 5 β -sterols only, respectively, with colours of sample point indicating changes with age. C-D: PC1 scores for each PCA model against sediment core age. Blue horizontal lines indicate breakpoints detected (minimum segment length = 3 data points; Supplementary, [Appendix A9](#)). E-F: PC1 and PC2 scores from PCA analysis of dung steroids with lake sediments plotted passively. E: dung analysed in this study (only compounds detected in the lake sediments are included). F: PCA of 5 β -sterols based on this study, [Harrault et al. \(2019\)](#) and [Wendt et al. \(2024\)](#). Symbol size reflects the quality of representation (cosine squared) of each point on the first two PCA axes, with larger symbols better represented by the two PCA axes than smaller ones. Colours and symbols represent differences in dietary types. Herbivore forage strategies: blue circles = ruminant grazers, grey and white circles = ruminant intermediate feeders, green = ruminant browsers and yellow circles = Rodentia.

an absence of faecal input. PCA analysis for source identification has focused on 5 β -stanols only (Harrault et al., 2019; Wendt et al., 2024), and this approach was effective at discriminating between different species in this study. However, consideration of PCAs of (i) all sterols and (ii) 5 β -stanols and bile acids is needed to assess the presence of birds due to their lack of 5 β -stanols. Whilst 5 β -stanols were also not detected in black bear (*Ursus americanus*) faeces analysed as part of this study, the presence of 5 β -stanols varies between *Ursidae* species and individuals; *Ursus arctos* faeces sampled from Central Europe contained 0.1 to 67% of 5 β -stanols as a proportion of total sterols and stanols analysed (n = 3; Gryczewska et al., 2025).

4.1.3. Examining the influence of diet on steroid source identification of discrete faecal deposits

The most pronounced differences in faecal steroid distributions are driven by differences in dietary types (Bull et al., 2002; Prost et al., 2017): the carnivore, omnivore and herbivore faeces analysed in this study have different predominant bile acids associated with their dietary types (LCA, CDCA and DCA, respectively), which differs from other carnivore and omnivore species that are predominated by DCA (e.g., Gryczewska et al., 2025; H1). The predominant herbivore bile acid (DCA) and sterols (5 α -stigmastanol, 5 β -stigmastanol and epi-5 β -stigmastanol) detected for moose, elk, caribou and bison are consistent with a plant-based diet and those detected in faeces of European domesticates (Prost et al., 2017) and herd herbivores from Montana, USA (Wendt et al., 2024). The predominant compounds in crane and mallard guano (CDCA and β -sitosterol) align with other bird species, such as geese (Prost et al., 2017), ducks, swans and chickens (Leeming et al., 1996); however, there are some differences between bird species, since cholesterol is the dominant sterol in seagull guano (Leeming et al., 1996) and chicken guano contains coprostanol (Sugano, 1967).

Faecal steroids reflect more nuanced differences in diets and gut microbes that exist within dietary types, as demonstrated by the consistent differences detected between ruminant herbivore species. Moose, which are obligate browsers adapted to eat aquatic and woody plants with minimal grass, differ from the other ruminants based on higher relative abundances of 5 β -stigmastanol and epi-5 β -stigmastanol and low LCA (Figs. 4 and 5; Fig. 7). Wood bison, which are grazers but also incorporate more woody browsing than plains bison, show distinct differences in stanol ratios, reflecting low abundances of coprostanol, epicoprostanol, 5 β -stigmastanol and epi-5 β -stigmastanol and cluster more closely to moose in the 5 β -stanols multivariate analyses than the other ruminants, including the other sub-species of bison (plains bison). Concentrations of cholesterol and β -sitosterol in wood bison faecal samples are twice as high as average concentrations of other herbivores in this study and those analysed by Li et al. (2024a) and Gryczewska et al. (2025). Elevated Δ^5 -sterols in faeces suggest reduced microbial hydrogenation of sterols during digestion and/or the dietary differences. Further research of herds in different locations and with different dietary compositions is required to assess the consistency of Δ^5 -sterols in wood bison faeces. Caribou and elk eat a wide range of plants, compared with more specialised ruminants, which is reflected in the wider steroid and bile acid distributions and 5 β -stanol and bile acid multivariate analyses. Faecal steroids are therefore most effective at identifying species that are more specialist feeders, owing to reduced variability in their faecal steroid distributions, and when dietary differences are most pronounced (H1).

Faecal steroids can characterise within-species dietary differences,

reflecting the availability of food: comparisons of faecal steroid profiles of populations of ruminants (plains bison, elk and moose) show that whilst different herds occupy similar species-specific ordination space, herd-specific characteristics are evident (Fig. 7E–F). Within-species dietary variation is also evident in omnivores, e.g., studies of dog faeces have reported both 5 β -stanol absence (Leeming et al., 1994) and presence (Harrault et al., 2019; Leeming et al., 1996; Shah et al., 2007). Some studies of pig species report approximately twice as much 5 β -stigmastanol compared with coprostanol (Leeming et al., 1996; Prost et al., 2017), whilst others reported only slightly higher 5 β -stigmastanol (Derrien et al., 2011), and others reported more coprostanol than 5 β -stigmastanol (Bull et al., 2002). Differences in carnivores also exist, with wolf faeces from the Tibetan Plateau (Li et al., 2024a) characterised by lower C₂₇-stanols ratio 1 values and stanol ratio 4 values than this study. Considering spatial and temporal changes in diet, informed by known climatic, vegetation and cultural changes, is therefore important when identifying faecal steroid sources in both modern and palaeo-environmental records.

Dietary diversity and variability assist with distinguishing between and within species, however, they also have the potential to contribute to overlapping sterol distributions between species, reducing confidence in faecal source identification. This is reflected in the wide ranging 5 β -stanols ordination space that is occupied by caribou/reindeer, which are more generalist feeders than other ruminants (Fig. 7). Humans also have potential for large dietary variation both in terms of the range of potential food ingested and dietary preferences of individuals. In addition, the efficiency of cholesterol-coprostanol conversion in modern human populations is highly variable depending on cholesterol-reducing bacteria (Midtvedt and Midtvedt, 1993; Midtvedt et al., 1990); most have efficient conversion, but some have low to no conversion (Juste and Gérard, 2021; Wilkins and Hackman, 1974). Human faeces are predominated by coprostanol (Leeming et al., 1996; Prost et al., 2017) and DCA (Prost et al., 2017), which differs from the species analysed in this study, however individual humans have a range of sterol distributions (Harrault et al., 2019; Leeming et al., 1996). Indeed, human 5 β -stanol distributions can be similar to those of dogs due to potential shared food sources, and in some instances, they are indistinguishable in multivariate analyses (Fig. 7E).

The ratio of coprostanol to 5 β -stigmastanol, which is similar to Stanol ratio 4 in this study but does not account for 5 β -epimer transformation, has been used to identify human faecal input in lake sediments when values exceed 0.18 (Vachula et al., 2019, 2020). This value is lower than the 0.25 ratio value applied in the seminal application of faecal sterol analysis in lake sediments to reconstruct mid-late Holocene human and herbivore presence by D'Anjou et al. (2012) on the basis of a review that reported ratio values of c. 5.5 in human faeces and 0.25 in ruminant dung (Bull et al., 2002; *sensu* Evershed and Bethell, 1996). Coprostanol to 5 β -stigmastanol ratio values of both 0.18 and 0.25 are an order of magnitude lower than modern reference material of human, dog and wolf faeces, which have overlapping ratio distributions (1 to 64; Fig. 10) and align more closely with the original ratio threshold value (<1.5) proposed by Evershed and Bethell (1996) and those recommended in the review by Bull et al. (2002) (>1 for humans, canines and porcines). The need for a higher threshold value for the coprostanol:5 β -stigmastanol ratio to identify human faecal matter is further supported by the median modern herbivore dung value of 0.18 (Fig. 10). Indeed, the majority of sterol data from North American lake records fall within the herbivore ratio range (Fig. 10). This example highlights the

difficulties of unambiguous identification of human presence when using only 5 β -stanol ratios and the need for more empirical testing of modern faecal ratio values to account for population differences.

4.2. Sensitivity of faecal steroid reconstructions from lake sedimentary archives

4.2.1. Steroid-based faecal characterisation at Chisholm Lake

Steroid characterisation of faecal input into Chisholm Lake is more complex than the modern discrete faecal samples (Section 4.1), because of mixing of organic matter and faecal inputs and changes in erosion rates through time, influencing the transport of faecal matter (Lloyd et al., 2016). The chronology for the Chisholm Lake sediment record core does not contain any age reversals or hiatuses and has a steady accumulation rate throughout the sequence suggesting there has been no redeposition of older catchment material. As a result, the steroid biomarkers are considered contemporaneous with their deposition horizon.

5 β -stanols are present throughout the last 2000-years, and the stanol ratios (Fig. 8F–I) indicate they originate primarily from a herbivore faecal source, which is supported by multivariate statistical analyses that show that the lake sediments are more similar to ruminant dung than omnivore/carnivore faeces (Fig. 9E–F). The presence of human faecal input is, however, more intermittent and uncertain: the C₂₇-stanol index (stanol ratio 1) only exceeds the threshold for confirmed faecal input in the uppermost sample (0.5 cm, 1998 CE). There is no evidence of a dominant human faecal input based on the multivariate statistics (Fig. 9F) nor of a dominant omnivore input in stanol ratio 4 (Fig. 8I).

Despite the persistence of herbivore dung input identified by the sterols in Chisholm Lake sediments, bile acids (predominately LCA) were only present in 4 of the samples analysed. Bile acid concentrations were between 0.5 and 10 times lower than 5 β -stanols in the majority of fresh

faeces analysed in this study. However, bile acid presence does not align with elevated input of 5 β -stanols, which would be expected if the absence of persistent bile acids was a result of below-detectable concentrations. In addition, DCA is absent in half of the samples containing LCA, which is unexpected given the sterol-based detection of a herbivore faecal source and the ubiquitous presence of both LCA and DCA in faeces of large terrestrial mammals (e.g., this study; Prost et al., 2017; Wendt et al., 2024; Zocatelli et al., 2017). Pigs are the only species currently known to have high LCA and order-of-magnitude lower DCA (Prost et al., 2017), however they are absent from Chisholm Lake historical and local archaeological records. Wolf scat has three times more LCA than DCA so could be contributing to the Chisholm Lake record; however, dung production rates of wolves are much lower than those of ruminants (Fig. 11, Supplementary, Appendix A3). Concentrations of DCA in other lake sedimentary archives with mixed faecal sources are within GC-MS limits of detection (e.g., Birk et al., 2022; Brown et al., 2022, 2021; Wendt et al., 2024; Zocatelli et al., 2017). The consistency of positive faecal input identification across different stanol ratios and multivariate statistical analyses, and the independently recorded human and animal activity in the catchment and wider region, supports a persistent historical faecal source. Therefore, bile acids are most likely not consistently incorporated, preserved or detected in the Chisholm Lake sediments.

The modern pH of surface water in Chisholm Lake is 8.2 and, whilst experimental studies of lacustrine bile acid hydrological mobility and sedimentary preservation are lacking, the solubility of bile acids increases above pH 5 to 6.5 (Fini et al., 1985; Igimi and Carey, 1980; Roda and Fini, 1984). At a pH of c. 8 in Chisholm Lake, the majority of the bile acid compounds will exist as water-soluble anions, particularly those in a conjugated form, and therefore less likely to partition onto settling particles and will be bioavailable for microbial mineralisation, ultimately restricting sedimentary accumulation of bile acids to compounds

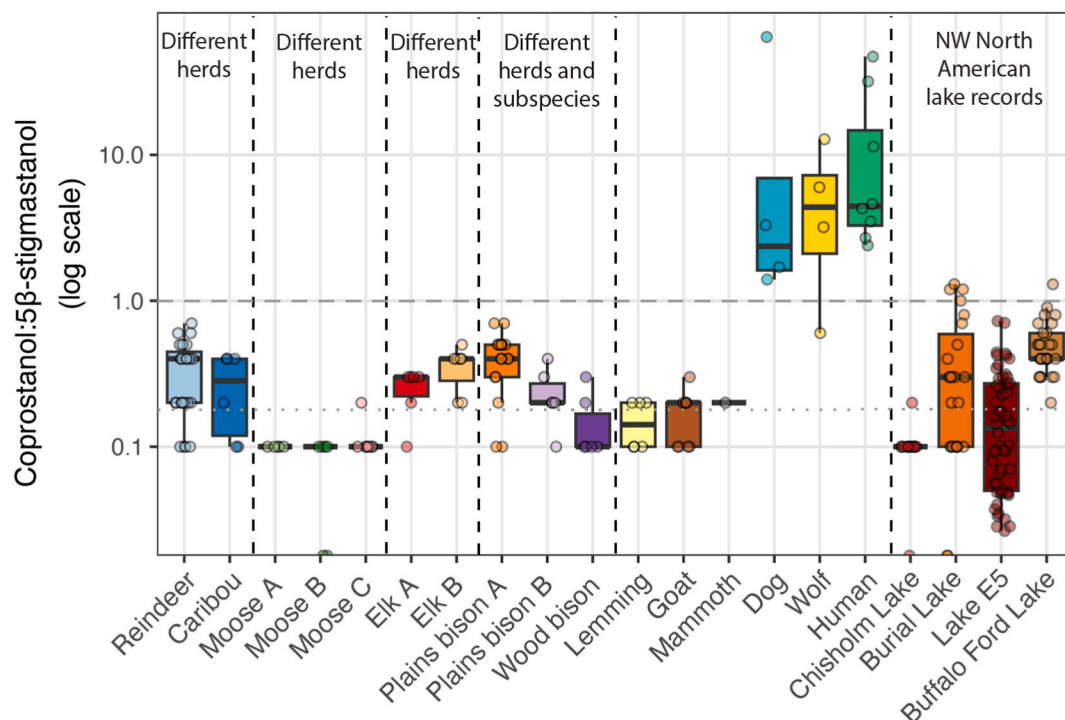


Fig. 10. Boxplot of coprostanol:5 β -stigmastanol (log scale) ratios. Ratio values of different herds (labelled A to C) and subspecies are highlighted. Modern faecal reference data are from this study, Harrault et al. (2019) and Wendt et al. (2024). Human, wolf and dog values are indiscriminate and are higher than all herbivores and North America lake sediment records presented here. Chisholm Lake: this study; Burial Lake: Vachula et al. (2020); Lake E5 (Vachula et al., 2019); Buffalo Ford: Wendt et al. (2024). Grey dashed line represents the threshold used to identify omnivore faecal input (>1 including epimers when available; Shillito et al., 2011) and grey dotted line represents the threshold applied to identify human input in northern Alaska (>0.18; Vachula et al., 2019, 2020).

that are occluded with organic debris. Further support for a pH-influence on bile acid preservation within Chisholm Lake sediments is that the most dominant bile acid in the record is the mono-hydroxylated LCA, which has a greater relative preservation potential and resistance to microbial decay than the other bile acids (Feller et al., 2021; Fini et al., 1985; Igimi and Carey, 1980; Roda and Fini, 1984).

Discrepancies between LCA and DCA have been detected in other lake records, for example DCA is absent from 41% of samples analysed (all of which contained LCA) from a site dominated by ruminant faecal input in Yellowstone National Park (Wendt et al., 2024), although other di-hydroxy bile acids (HDCA and UDCA) were more consistently detected alongside LCA in this record. Other records contain samples with no detectable bile acids despite persistent 5β -stanol and mammal sedaDNA records to support the presence of mammals (Brown et al., 2022). Bile acid preservation appears compromised at $\text{pH} > 8$ and therefore can vary between sites and over time within the sedimentary archives. Optimum preservation of bile acids is likely at slightly acidic conditions (pH 5 to 6.5; Feller et al., 2021; Fini et al., 1985; Igimi and Carey, 1980; Roda and Fini, 1984), but this requires systematic testing.

4.2.2. Assessing faecal versus environmental sources of 5β -stanols in Chisholm Lake sediments

Ratios of 5β - and 5α -stanols are important for characterising environmental transformation of sterols that occur under anoxic conditions

(Gaskell and Eglinton, 1975; Nishimura, 1982; Nishimura and Koyama, 1977; Taylor et al., 1981). However, ratio threshold values are not universal, since they depend on the sterol composition of faecal input and rates of post-depositional transformation (Chan et al., 1998; Pratt et al., 2007). These ratios were designed in studies of discrete and concentrated faecal input, such as sewage effluent and archaeological studies of manuring, and are not optimised for application in lacustrine environments, which contain organisms that biosynthesise 5α -stanols such as algae (Grimalt et al., 1990; Nishimura and Koyama, 1977), zooplankton (Nishimura and Koyama, 1977), aquatic plants (Nishimura and Koyama, 1977) and fish (Lee et al., 2025; McGhie et al., 2000). The possible faecal input threshold (0.3) for stanol ratio 1 and 2 could therefore be lower in lakes to account for the additional 5α -stanol sources. Furthermore, lacustrine stanol ratio values are likely to be site-specific, which may limit the utility of widely applicable threshold value definitions.

Diagenetic pathways, such as the reduction of Δ^5 -sterols to 5α -stanols and 5β -stanols and the epimerisation of 5β -stanols to 5β -epi-stanols, can be evaluated using relationships between ratios of compounds and sediment core age. This approach can provide insight into long-term contributions from chemical transformation and degradation reactions. There is no evidence of a dominant long-term (e.g., millennial-scale) diagenesis influence on aggregate Δ^5 -sterol and 5β -stanol flux (Fig. 8) nor transformation associated with the production of 5β -

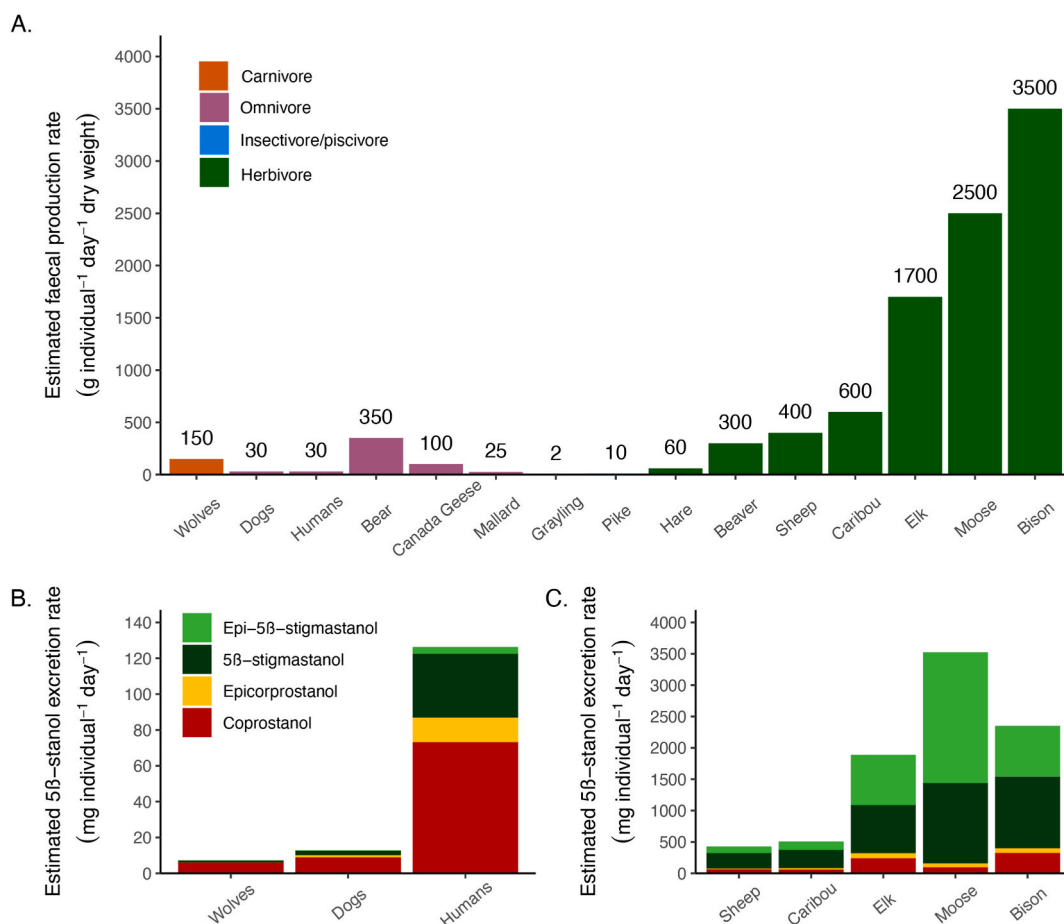


Fig. 11. Estimated faecal production and 5β -stanol excretion rates of species present in Tanana Valley based on assemblages from Potter (2008). Differences in magnitude are of most relevance to this study. A: Faecal production: rates vary based on many factors such as diet, hydration, activity level, age of individual and hibernation patterns; therefore, these rates are example estimates. Total faecal production rates are reported to two significant figures. References for the estimates are provided in the supplementary data (Appendix A3). B-C: Estimated mean excretion rate of C_{27} - and C_{29} - 5β -stanols based on estimated faecal production rates and species with available modern faecal 5β -stanol distribution data from this study; Gryczewska et al. (2025); Harrault et al. (2019); Leeming et al. (1996); Li et al. (2024a); Wendt et al. (2024). B: carnivores and omnivores; C: herbivores. Note different scales in B. and C.; herbivores have an order of magnitude higher estimated 5β -stanol excretion rates (C) compared with omnivores/carnivores (B).

stigmasterol in Chisholm Lake based on precursor (β -sitosterol) and degradation (5 β -stigmasterol) compounds in the C₂₉sterol transformation pathway (Supplementary, Appendix A7G). There is a significant relationship between coprostanol and cholesterol (Supplementary, Appendix A7C), but this likely reflects a common source rather than *in-situ* transformation since there are no statistical relationships between age of sediment and 5 β -stanols and Δ^5 -sterols fluxes nor downcore ratios (Fig. 8, Supplementary, Appendix A8). The absence of a significant relationship between the primary diagenetic pathway of cholesterol to 5 α -cholesterol (Supplementary, Appendix A7A) and the source-influenced significant relationship between 5 α -cholesterol and coprostanol (Supplementary, Appendix A7B) provide additional support for a source-derived cholesterol:coprostanol relationship. Further evidence to reject *in-situ* transformation as a major source of 5 β -stanol production in Chisholm Lake is provided by the 5 α -stanol: Δ^5 -sterol and 5 β -stanol: Δ^5 -sterol ratio values, all of which are less than the 0.5 threshold indicative of diagenetic processes (Wakeham and Canuel, 2006). There is a significant relationship between epi-5 β -stigmasterol and 5 β -stigmasterol (Supplementary, Appendix A7), but relative proportions of 5 β -stigmasterol increase towards the surface (Supplementary, Appendix A8), which does not support a long-term diagenetic signature.

There are significant non-linear relationships between sediment age and aggregate 5 α -stanol flux and downcore records of 5 α -stanols: Δ^5 -sterols ratios, which increase from c.1850 CE (4 cm sediment depth) to towards the present day (Fig. 8, Supplementary, Appendix A8). The timing and sediment depth of the increase in 5 α -stanol correspond with (i) the start of seasonal occupation of nearby Birch Lake and (ii) sediment depth changes in redox conditions that influence compound degradation, transformation and resilience in this section of microbially active sediments (Nishimura and Koyama, 1977; Wakeham, 1989; Bogus et al., 2012). Elevated human activity alters the composition of lacustrine sterols by increasing terrestrial and nutrient inputs, which increase the supply of labile organic matter and microbial respiration. Changes in terrestrial inputs have the potential to increase aquatic productivity of 5 α -stanol-producing organisms such as phytoplankton (Nishimura, 1977; Nishimura and Koyama, 1977; Robinson et al., 1987), including diatoms (Nishimura and Koyama, 1976). The majority of 5 α -stanols and Δ^5 -sterols in the top centimetre of lake sediment deposition originate directly from organisms rather than being influenced by degradation and transformation processes (Nishimura and Koyama, 1976). The top sample depth of the Chisholm Lake core has the maximum values of 5 α -stanol: Δ^5 -sterol ratios and fluxes, which supports increased aquatic productivity as a driver of 5 α -stanols: Δ^5 -sterols ratios in the historic period. Disentangling the influence of changing sterol sources and influence of redox conditions on degradation beyond the top centimetre of core depth is however not possible here with the data available. Additional analyses to reconstruct redox conditions (e.g., XRF-derived Fe/Mn ratios) and aquatic productivity (e.g., diatom or pigment analysis) would facilitate discrimination between the connected processes of primary productivity, microbial respiration, microbial hydrogenation of Δ^5 -sterols.

Contextualising natural background sterol distributions is another approach to identify external faecal inputs in sediment archives (Birk et al., 2011; Harrault et al., 2019; Mackay et al., 2020), since it characterises low-level 5 β -stanols production from environmental transformation as well as *in-situ* faecal 5 β -stanol deposition from organisms living in the lake and the catchment soils (e.g., fish; Lee et al., 2025;

McGhie et al., 2000). However, it is challenging to obtain 'undisturbed' base-level sediments that can act as a meaningful control in many modern environmental settings (Bull et al., 1999) and using lake catchment soils for contextualising a lake is not ideal because of the differences in *in-situ* sterol production sources. In modern aquatic sediments, threshold concentrations of >100 ng g⁻¹ of coprostanol plus epicoprostanol have been proposed to represent faecal contamination, and concentrations >2000 ng g⁻¹ proposed as significant faecal contamination (Li et al., 2024a). Combined concentrations of coprostanol and epicoprostanol in Chisholm Lake exceed the significantly faecally contaminated threshold (ranging from c. 7600 to 98,000 ng g⁻¹) throughout the last 2000 years. This threshold is a useful guide, but exact thresholds may vary across wider geographical regions and time periods, and concentrations are influenced by changes in sedimentary accumulation rates. Statistical approaches such as breakpoint analysis help identify changes in faecal inputs from background levels in sedimentary faecal steroid reconstructions. This approach is useful in sites such as Chisholm Lake, when the length of the faecal steroid record is (or may be) shorter than the known presence of humans and animals in the region. A common breakpoint is detected in the Chisholm Lake PC axis 1 scores of 5 β -stanols, accumulation rates of coprostanol plus epicoprostanol, accumulation rates of 5 β -stigmasterol plus epi-5 β -stigmasterol and in ratios of coprostanol plus epicoprostanol:5 β -stigmasterol plus epi-5 β -stigmasterol prior to 1958 CE (Fig. 9C–D, Supplementary, Appendix A9), which supports a change in the amount and delivery of 5 β -stanols at this time.

4.2.3. Comparisons of Chisholm Lake faecal steroid reconstruction with historical and archaeological context

The following interpretations of faecal input at Chisholm Lake are based primarily on the sterol composition since the preservation and therefore integrity of the bile acids in this sediment archive is uncertain (Section 4.2.1). 5 β -stanol records are interpreted as faecal sources since environmental transformation pathways are not a dominant source of 5 β -stanols in this record (Section 4.2.2). Relative proportions of 5 β -stanols and 5 β -stanols to 5 α -cholestanol only meet threshold values to support omnivore faecal input in one sample analysed (dated to 1998 CE), however, the absence of optimised lacustrine ratio threshold values currently prohibits robust interpretations using these threshold values (Section 4.2.2). Fluxes of steroids account for changes in sedimentation rate (and therefore levels of input); however, they are dependent on robust chronological control. Whilst the chronology of core 19Chis-C is robust (Section 3.1), comparisons of both ratios and fluxes are useful to account for changes in sedimentation.

Coprostanol and epicoprostanol, the predominant 5 β -stanols in human/omnivore faeces, are present throughout the last 2000 years in the Chisholm Lake sedimentary archive, as expected based on evidence of human presence from nearby sites from throughout the Holocene (Potter, 2025). The period between 810 CE and 1740 CE exhibits low coprostanol and epicoprostanol accumulation rates and stanol ratio 1 values (Fig. 8), which supports a reduced human/omnivore presence, however, this does not align with modelled population declines based on regional archaeological components (Tremayne and Brown, 2017). Coprostanol and epicoprostanol accumulation rates increase from 1850 CE to 1900 CE, when they exceed base level values for the last 2000 years, which corresponds with approximate timings of seasonal occupation of Birch Lake by Salcha Dene-Athabaskan peoples (late 1800s;

Andrews, 1975; Supplementary, Appendix A4). The increased Euro-American activity around Chisholm Lake, which took place from 1902 CE (Supplementary, Appendix A4), does not register a beyond-base-level coprostanol plus epicoprostanol accumulation rate increase until 1998 CE, when stanol ratio 1 values exceed earlier base level values and the threshold to indicate possible faecal input. Breakpoint analysis of coprostanol and epicoprostanol accumulation rate record does, however, separate the record into two segments, with segment 1 spanning from 15 cal yr CE to 1940 CE and segment 2 spanning from 1958 to 1998 CE (Supplementary, Appendix A9). The breakpoint corresponds with elevated human presence at Chisholm Lake as documented by the increased construction and use of cabins (1950s) and the establishment of the boy scout camp (early 1960s; Supplementary, A4). This change is also detected by the breakpoints in the multivariate analysis (PC1 scores) of all sterols and 5 β -stanols (Fig. 9C–D). The coprostanol and epicoprostanol accumulation rate and stanol ratio 1 values therefore identify some key moments of change in the occupation of the Chisholm Lake region (Fig. 8D and F). However, historical periods of increased occupation intensity are only distinguishable from periods of lower intensity presence c. 2000 years ago when using multivariate and segmented regression analysis (H2; Fig. 9C–D, Supplementary, Appendix A9).

Comparisons of the ratio of stanol ratio 1 as described in (Grimalt et al., 1990; coprostanol/(coprostanol+5 α -cholestanol)) and architectural-based prehistoric population histories at Cahokia, Illinois, have demonstrated the ability of coprostanol to track human population change (White et al., 2018), but this approach did not correlate with prehistoric populations or proximity to population centres in the Yucatán Peninsula and northern Guatemala (Keenan et al., 2022). Including epicoprostanol in stanol ratio 1 at Chisholm Lake does not significantly alter the trends or relative changes over time (Supplementary, Appendix A10) but does account for diagenetic epimerisation of coprostanol that occurred over time. The direction of change in Chisholm Lake stanol ratio 1 tracks the approximate timings of historically documented increases in population densities (Supplementary, Appendix A4), but the magnitude of change does not consistently capture the elevated population density in the historical period compared with the archaeological period (Fig. 8). The findings from Chisholm Lake therefore add further evidence that use of the coprostanol:5 α -cholestanol ratio is not a universally robust quantitative indicator of human population change in all environmental settings.

The faecal sterol input at Chisholm Lake is dominated by 5 β -stigmastanol and epi-5 β -stigmastanol from herbivore dung (Figs. 8 and 10, Supplementary, Appendix A4). Whilst 5 β -stigmastanol and epi-5 β -stigmastanol accumulation rates are often an order of magnitude higher than coprostanol and epicoprostanol, they reveal similar broad patterns of faecal input intensity: accumulation rates are lower between 1105 CE and 1740 CE, and elevated from 1850 CE (denoted by a breakpoint separating the segment prior to 1740 CE and post 1850 CE; Supplementary, Appendix A9). A second breakpoint detected a period of increasing 5 β -stigmastanol and epi-5 β -stigmastanol accumulation rates from 1958 CE that peaked in 1998 CE. There is no distinguishable difference in accumulation rates of herbivore dung input between the majority of the historical period (with the exception of 1998 CE) and the start of the record c. 2000 years ago (Fig. 8E).

The dominance of herbivore faecal input into Chisholm Lake is perhaps unsurprising: faecal steroids are most effective at identifying the dominant faecal source (Derrien et al., 2012) and throughout the

majority of the last 2000-years, ruminant biomass was larger than human biomass in the Chisholm Lake catchment. For example, population densities for regional Dene hunter-gatherers average from 0.035 persons km⁻² for hunters and 0.13 persons km⁻² for fishers (Keeley, 1988), whilst Fortymile and Nelchina caribou densities combined were estimated as 1.2 individuals km⁻² in 2000, when herd sizes were lower than those in the early 1900s (Healy, 2001) and moose densities in the interior of Alaska were 2.4 individuals km⁻². Comparisons of estimated average rates of daily faecal production per individual from published literature (Supplementary, Appendix A3) demonstrate that humans produce 1 to 2 orders of magnitude less than large herbivores (Fig. 11A), and the human coprostanol excretion rate is one order of magnitude less than the coprostanol excretion rates of elk and bison (Fig. 11B–C). These estimated 5 β -stanols rates illustrate the elevated human population size required to achieve the same faecal input as other large herbivores and caution against solely relying on changes in concentrations of coprostanol and epicoprostanol to identify human input. In addition, herbivore defecation occurs on the ground surface and is indiscriminate in terms of deposition location, unlike humans who traditionally bury their excrement, avoid locations proximal to water supplies and/or use manure to fertilise field systems. Whilst humans produce an order of magnitude more 5 β -stanols than canids, discriminating between the 5 β -stanols faecal input of these species using 5 β -stanol ratios is not robust (Fig. 10). The example from Chisholm Lake therefore highlights the difficulties of extracting a human faecal steroid signal in sites with low intensity human activity and other dominant mammalian sources of faecal input (H3).

Ruminant species have distinct faecal steroid signatures (Section 4.1); however, identifying specific source organisms is more challenging in lake sediments due to the potential for mixed faecal sources and more *in-situ* sterol producers. PCA analysis of all 5 β -stanols and bile acids indicate that Chisholm Lake sediment steroids are most similar to those of caribou and elk (Fig. 9E). The Chisholm Lake 5 β -stanols have most similarities with elk and moose dung (Fig. 9F) and the intermittent presence of moose is supported by stanol ratio 4 (Fig. 8I). Elk is the closest match to Chisholm Lake stanol ratio 3, however the values extend beyond the currently defined elk range and may therefore represent faecal input that has yet to be characterised. Indeed, the dominance of elk is at odds with the regional late Holocene archaeological records, which are dominated by caribou-rich components (Potter, 2008) and historical records (Healy, 2001).

Our source identifications are limited by the lack of bile acids detected. UDCA was detected in 1850 CE and 1980 CE; UDCA is associated with bear and avian dung (this study) and pigs (Prost et al., 2017) but absent in ruminant and human faeces. No pig remains have been detected in archaeological sites, but there are archaeological components with bear and bird remains (Potter, 2008). HDCA is also detected at these points in time, but this has only been detected in pigs and rats, with trace amounts in human urine.

4.3. Lacustrine faecal steroids as tools to detect human arrival and movement in North America

Using our modern reference faecal steroids and examining the Chisholm Lake record supports the potential of faecal steroids to provide insights into past human and animal populations, but it also highlights that, for robust interpretations of sedimentary archives, it is essential to assess environmental and/or *in-situ* 5 β -stanols and undertake a

comprehensive characterisation of steroid signatures of all faecal sources. Detecting low-level human faecal input is challenging when accompanied by more predominant faecal input from other animals such as large or domesticated herbivores. Moose and elk migrated into eastern Beringia at the same time as humans, c. 16,000–14,000 cal yr BP, according to evidence in the archaeological and genetic records (Guthrie, 2006; Meiri et al., 2014; Wooller et al., 2018), but many animals, such as mammoths, horses and bison, pre-dated human arrival in this region (Mann et al., 2013). The necessity to disentangle the mammal faecal sources is less critical when human and animal arrival in the prehistoric record is synchronous, such as the arrival of humans and domesticated pigs on islands in Oceania and Wallacea (Hofman and Rick, 2018; Stanton et al., 2026; Sear et al., 2020), compared with settings with high densities of wild animals that predate human presence. When wild mammals are present before humans, a high degree of confidence in the interpretation of a human-specific faecal steroid source is required to confirm human presence. This should be achieved using a combination of approaches such as statistical analysis of both sterols and bile acids (if preserved) using ratios and multivariate analyses, comparisons of faecal steroids with mammalian sedaDNA to assist with faecal source comparisons, comparisons with other proxies of human activity such as fire and agricultural activity and/or integration of faecal steroids within an archaeological context.

5 β -stanol records from two lakes (Burial and E5) in Northern Alaska (Vachula et al., 2019, 2020) have been interpreted as showing earlier evidence of human arrival. This interpretation is inconsistent with many models of human colonisation in eastern Beringia and North America after 16,000 cal yr BP, which are based on archaeological evidence (Goebel et al., 2008; Potter et al., 2018; Waters, 2019) and genetic studies of humans (Grugni et al., 2019; Moreno-Mayar et al., 2018a, Scenario 1; Pinotti et al., 2019; Potter et al., 2018; Wei et al., 2018) and dogs (Leathlobhair et al., 2018; Perri et al., 2021). These models are consistent with the earliest unequivocal archaeological evidence of humans in Eastern Beringia, i.e. unambiguous human-made artefacts in clear dating contexts such as hearth features, which all post-date 16,000 cal yr BP (Potter et al., 2017; Reuther et al., 2023). Whole-genome evidence is compatible with longer-term population structure; Native American ancestors (FAM) diverged from Ancient East Asian populations c. 25,900 cal yr BP and subsequently admixed with Ancient North Eurasians, most closely represented by Mal'ta and Afonova Gora individuals from southern Siberia (Moreno-Mayar et al., 2018a; Sikora et al., 2019; Zeng et al., 2025). The subsequent “Beringian” Standstill or Incubation model (Tamm et al., 2007) proposes a period of isolation of FAM from East Asian populations following their formation and prior to expansion c. 16,000–15,000 cal yr BP (Llamas et al., 2016; Wei et al., 2018). Interpreting the >32,000 cal yr BP lacustrine 5 β -stanol records (Vachula et al., 2019, 2020) as evidence of human presence in eastern Beringia would require the presence of a distinct population separate from the currently recognised Native American ancestral population. However, there is currently no independent archaeological or genetic evidence to support this. Furthermore, the interpretation of these 5 β -stanol signals as evidence of human presence does not account for potential faecal contributions from omnivores/carnivores or wide-ranging herbivore ratio values (Fig. 10).

The interpretation that the 5 β -stanol records from Burial and E5 Lakes are indicative of human presence is primarily based on the ratio of coprostanol:5 β -stigmastanol and the application of a low threshold for human faecal detection (>0.18; Vachula et al., 2019, 2020). This ratio threshold was selected based on ratio values from a mammoth dung sample (van Geel et al., 2008) and the rationale that animal biomass of

northern Alaska during the Glacial was predominated by mammoth (49%), along with two other key herbivores: horse (23%) and bison (23%) (Mann et al., 2013). Interpretations of this ratio value are uncertain: firstly, following correction for Glacial animal biomass in northern Alaska as estimated by Mann et al. (2013), the coprostanol:5 β -stigmastanol ratio of dung of modern horse (c. 0.25; Gill et al., 2010; Leeming et al., 1996; Prost et al., 2017) and bison (0.34; this study and Wendt et al., 2024) dung and the single mammoth faecal sample (c. 0.15; van Geel et al., 2008) produces a higher weighted average ratio value of 0.22 ± 0.13 . The possible ratio range for Glacial herbivores can therefore be twice as high as the applied ratio threshold. Secondly, 5 β -stanol data from Pleistocene fauna are lacking, aside from analysis of the single mammoth dung sample reported above, which prohibits insight into 5 β -stanol variability among mammoths. The dietary composition and therefore stanol ratio values of Pleistocene horse and bison could have differed from those of modern individuals. Thirdly, many species have different ranges and population concentrations based on habitat and heterogeneous spatial behaviours (Mann et al., 2013, 2019), which contribute to unequal use of landscapes, and therefore spatial patterns of faecal deposition. Examination of spatial variability in Pleistocene faunal communities is therefore required to improve estimates of the relative abundance of animal biomass, and their potential faecal contributions, in lake catchments during the Pleistocene. Fourthly, this study demonstrates that ratio values above 0.18 can be achieved through the input of ruminant dung based on 5 β -stanol compositions from modern faecal samples (Fig. 10). Indeed, plains bison, caribou and elk have values that exceed 0.18, and caribou are known to migrate across Alaska seasonally in large numbers (Nicholson et al., 2016). Finally, the applied threshold value of 0.18 is an order of magnitude lower than values for human faeces (Bull et al., 2002; Prost et al., 2017) and the threshold originally proposed for this ratio (Evershed and Bethell, 1996; Bull et al., 2002).

Further examination of the coprostanol:5 β -stigmastanol ratio demonstrates that whilst human faeces are associated with higher values than ruminants, human coprostanol:5 β -stigmastanol ratio values are not consistently distinct from values of omnivores and carnivores such as dogs and wolves (Fig. 10). Dog and human faeces can be differentiated based on examination of their full steroid profile, with canine faeces containing elevated CDCA and a dominance of cholesterol compared with coprostanol; however, these compounds were not reported in previous studies of lacustrine faecal steroids in Alaska. Dogs may have accompanied human dispersal into Beringia (Perri et al., 2021) and the similarities in faecal steroids between humans and dogs may even be greater in archaeological record since the reliance of dogs on humans for food would have led to greater homogeneity of dietary intakes compared with the present day (Lanoë et al., 2024). Wolves (independent of human presence) were a major component of the glacial steppe-tundra (Bocherens, 2015; Fox-Dobbs et al., 2008) and have persisted to the present day in northern Alaska (Lanoë et al., 2024). Lions, bears and wolves accounted for c. 2% biomass on the North Slope between 43.5 and 10 cal ka BP (Mann et al., 2013), but there are no Pleistocene 5 β -stanol reference data to characterise their potential contribution to the coprostanol:5 β -stigmastanol ratio. Other carnivores in this region include foxes, wolverines and other mustelids (Youngman, 1993). Coyotes moved into subarctic North America in the late 19th and early 20th centuries with the expansion of Euro-American settler populations associated with gold rushes and fur trade (Hody and Kays, 2018). The sterol composition of carnivore faeces is generally characterised by a predominance of cholesterol and relatively smaller proportions of 5 β -stanols (e.g., Kemp et al., 2022). However, the potential for

overlapping coprostanol:5 β -stigmasterol ratio values between human and other carnivore and omnivore faecal sources (Fig. 10) and their co-existence in the palaeorecord highlights that this ratio cannot provide unambiguous evidence of human presence in Pleistocene Beringia.

In addition to the mismatch in timings of northern Alaskan 5 β -stanol arrival dates and migration pathway chronologies, there is no support for the 5 β -stanol timings in existing regional archaeological records: surveys of E5 lake shores did not detect evidence for any Holocene human occupation at the lake (Goebel et al., 2022). Decades of archaeological research in adjacent areas to Lake E5 have produced a robust record of human occupation dating back to 12,600 cal yr BP (Reanier, 1995; Holloway et al., 2018), showing population increases around 4000 cal yr BP associated with the Arctic Small Tool tradition migration and around 2000 cal yr BP associated with later Paleo Eskimo groups (Tremayne and Brown, 2017, Fig. 12). However, 5 β -stanol concentrations and ratios do not consistently track changes in the presence/absence and amplitude of human population density in the region based on taphonomically-corrected regional archaeological components

(Fig. 12).

5. Conclusions

Faecal steroid analysis of modern faecal samples is effective at distinguishing species that have different dietary types, with the primary differences evident between herbivores and omnivores/carnivores. We demonstrate the ability of multivariate analyses and compound ratios of faecal steroids to distinguish between ruminant species, and moose (*Alces alces*) dung has the most distinct ruminant sterol profile in this study. We highlight that modern faecal samples, especially those from wild herbivores, have lower sterol ratio values than upper thresholds (0.7) currently applied to confirm the detection of faecal input but the majority are higher than the lower threshold (0.3) currently used to identify possible faecal input. Further research is therefore required to refine sterol faecal identification ratios and thresholds for different taxa in different environments, and assess the extent to which they are context specific. Faecal steroid PCA identified differences between

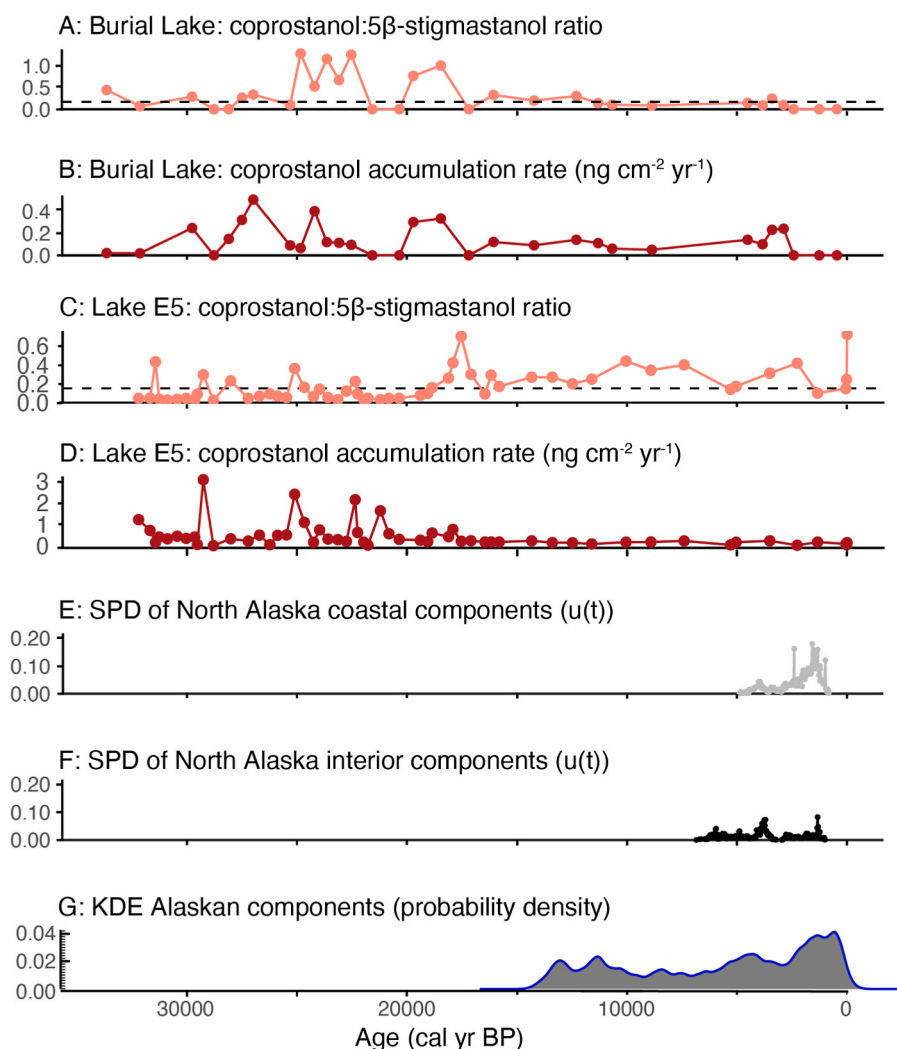


Fig. 12. Comparison of Northern Alaskan 5 β -stanol records with regional archaeological components. A-B: Burial Lake coprostanol:stigmasterol ratio and coprostanol accumulation rate (Vachula et al., 2020, data from NOAA Palaeoclimatology); C-D: Lake E5 coprostanol:stigmasterol ratio and coprostanol accumulation rate (Vachula et al., 2019; data from NOAA Palaeoclimatology). E-F: summed probability distributions (SPD) for coastal and interior Alaskan locations (Tremayne and Brown, 2017; data not available online, digitized using WebPlotDigitizer); G: Kernel density estimates (KDE) for Alaska (Potter, 2025). Horizontal black dashed lines in A and C indicate 0.18 ratio threshold used by Vachula et al. (2019, 2020) to identify the presence of humans, but herbivores extend beyond this threshold and dogs and wolves overlap with humans (Fig. 10).

different herds of the same species, highlighting the strong secondary influence of diet and its impact on gut microbiomes on the distribution of steroid compounds in faecal samples. Faecal steroid distributions of many species, especially carnivores and omnivores, have yet to be characterised, and impact of seasonal and geographical dietary differences on the range of species-specific characteristic faecal steroid distributions has yet to be experimentally tested. A community-wide standardised reference database of faecal steroid distributions would improve faecal source identifications and assess the influence of changing diets through time and space, both of which are critical for robust application in paleoenvironmental records.

Examination of the Chisholm Lake record highlights several challenges in obtaining definitive evidence of human presence from steroids in lake-sediment archives. Firstly, human coprostanol:5 β -stigmastanol ratio values overlap with other omnivores/carnivores, such as *Canidae*, and therefore wild populations of species such as wolves must be considered as possible faecal sources. This is especially important for interpreting human arrival and movement in areas such as Alaska, where wolves and other carnivores pre-date humans and could therefore be responsible for early faecal deposition in lake environments. Secondly, the dominant faecal source organism in the landscape is most reliably detected in lacustrine records of faecal steroids; detection is dictated not only by species biomass but also by faecal steroid production volumes. Since herbivores produce order(s) of magnitude more faeces than omnivores and carnivores, they have high potential to dominate faecal input in palaeoenvironmental records, especially in settings with low intensity or transient human populations. The combination of mammal sedaDNA and faecal steroid analysis of lake sediments respectively enables identification of the presence of non-dominant taxa and changes in the relative abundance of faecal input. Thirdly, the presence of 5 β -stanols in isolation is not unequivocal evidence of faecal input owing to the mixed sources of *in-situ* sterol production and environmental transformation in lakes. Consideration of base-level 5 β -stanols and ratios of sterol reduction are an effective approach to characterising the relative contribution of non-faecal or *in-situ* sources from external faecal input. Indeed, this approach demonstrated that environmental transformation was not a dominant source of 5 β -stanols in Chisholm Lake. Interpretations of faecal sources were limited by intermittent bile acid preservation in the sedimentary archive, which highlights the need for further research into the influence of pH, temperature, redox and sedimentary composition on the preservation of bile acids in lacustrine settings. 5 β -stanols were able to detect some noticeable points of change in the historical occupation of Chisholm Lake, such as the increased development since the 1950s, but they were not sensitive to small-scale changes in populations. Furthermore, despite historical records of increased human activity since the 1900s, only one sample analysed from 1998 CE contained significantly different sterol composition and accumulation rates within the context of the last 2000 years. The sensitivity of 5 β -stanols is therefore best suited to capture large magnitude changes in occupation histories.

Our findings demonstrate the utility of faecal steroids to provide insight into human and animal presence in palaeolimnological and archaeological contexts but also question the reliability of faecal steroids to detect transient/low human populations. This highlights the importance of integrating faecal steroid records with archaeological and

historical archives. More calibration studies of lake-sediment faecal steroid records with well constrained modern population archives and historical records and experimental testing of impacts of taphonomy within lakes and lake sediments are needed to improve the reliability of lacustrine faecal steroid reconstructions.

Author contributions

Helen Mackay: Conceptualisation, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review and Editing; Visualisation; Helen Whelton: Methodology; Formal Analysis, Investigation, Resources, Writing - Review and Editing; Joshua Reuther: Conceptualisation, Resources, Writing - Review and Editing; Ben Potter: Conceptualisation, Resources, Writing - Review and Editing; Mary Edwards: Conceptualisation, Writing - Review and Editing, Supervision; Andrew Henderson: Writing - Review and Editing, Supervision; Ian Bull: Resources, Writing - Review and Editing; Elinor Baldwin: Formal analysis; Writing - Review and Editing; Charlotte Clarke: Writing - Review and Editing; Roseanna Mayfield: Formal analysis, Visualisation, Writing - Review and Editing; Kimberley Davies: Writing - Review and Editing; Maarten van Hardenbroek: Writing - Review and Editing; Nancy Bigelow: Conceptualisation, Methodology, Resources, Writing - Review and Editing, Visualisation, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

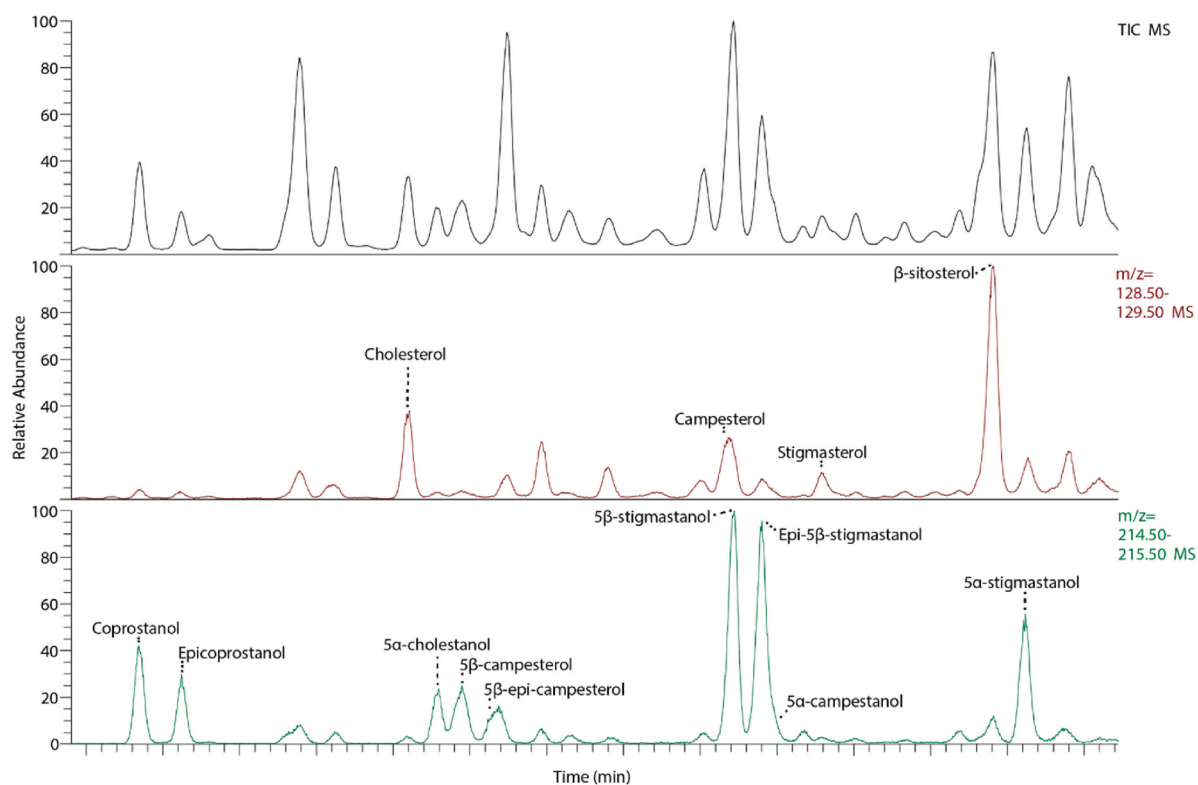
The authors declare that they have no known competing financial interests, personal relationships or professional roles that could have appeared to influence the work reported in this paper.

Acknowledgements

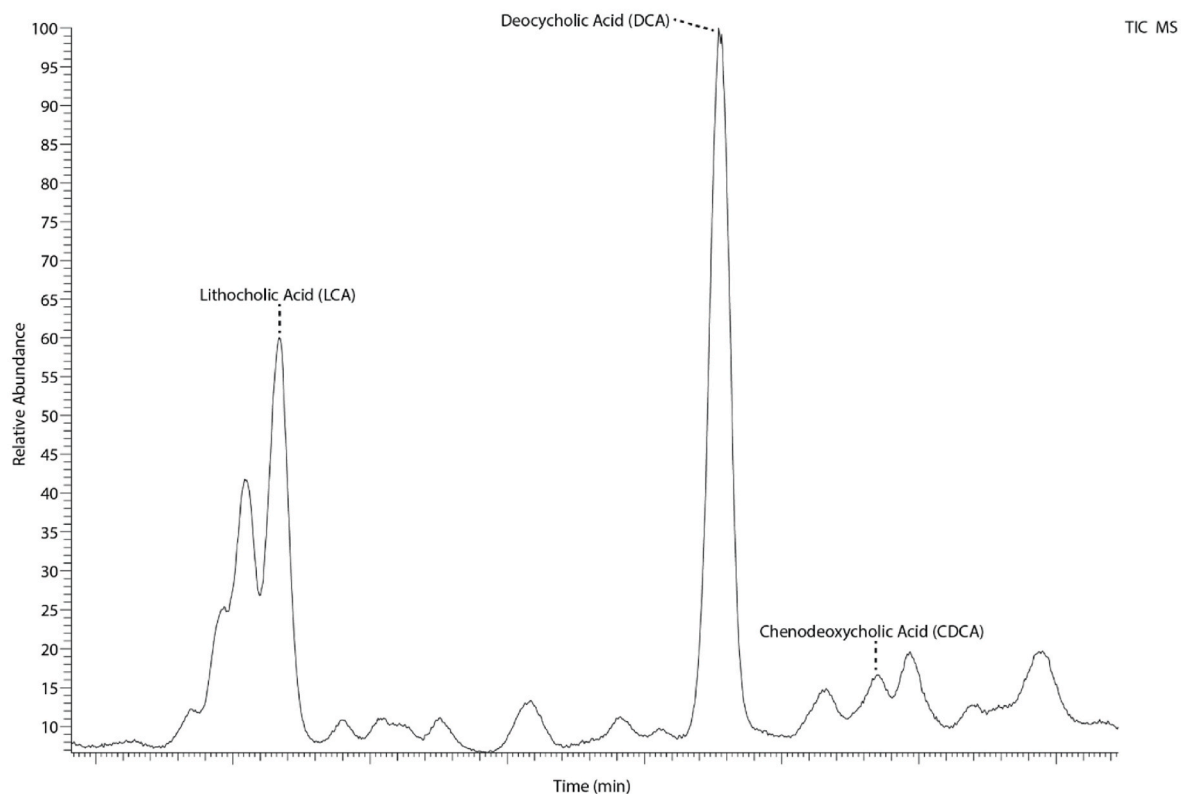
We thank Charles Holmes, Barbara Crass, Joseph Keeney, Thomas Allen and Gerard Smith for help collecting dung samples; the Hollembaek family for allowing us to collect wood bison and elk samples from their Alaska Interior Game Ranch; Kyndall Hildebrandt-Powers for support in sampling cores at the University of Alaska Museum of the North; Mallory Gulbranson, Genomic Resources Manager, and the UAMN Genomics Lab for faecal DNA analyses; Amanda Hayton and Martin West for analytical advice; Steve Juggins for support with R, Dan Law for assistance with Fig. 11 and Chris Orton for the production of Fig. 1 and the Graphical Abstract. We also thank Richard Vachula and two anonymous reviewers for their time and valuable comments and suggestions.

Appendices.

A.



B.



Appendix A1. Example sterol (A) and bile acid (B) chromatograms from a modern caribou dung sample.

Appendix A2

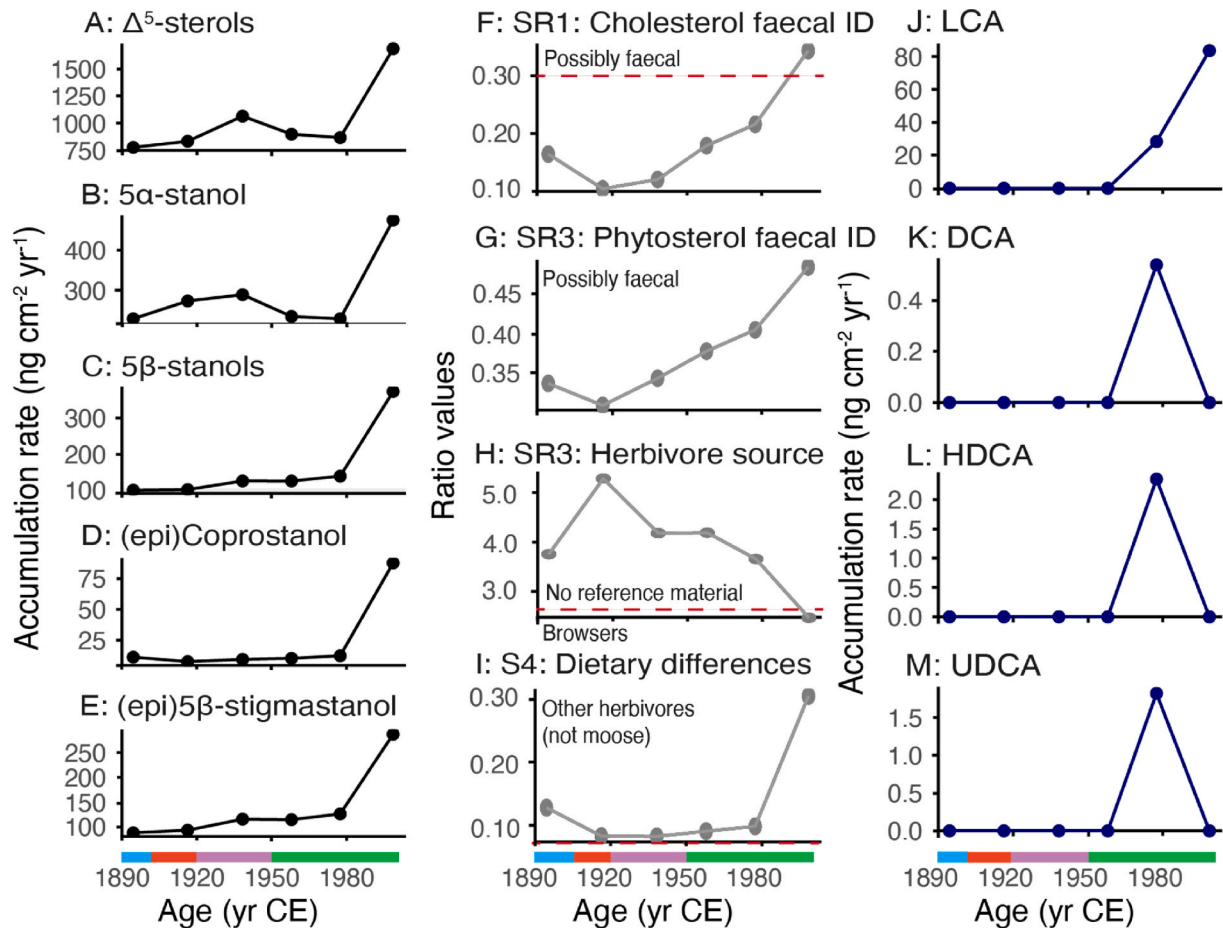
Table of selected analytes and their nomenclature. Fragmentation ions are based on electron ionisation (EI) as trimethylsilyl (TMS) ethers (sterols/stanols) and methylated (Me)-TMS (bile acids) and listed in mass order, with base peak in bold. $M+^{\cdot}$ is indicated.

Compound Class	Trivial Name	IUPAC nomenclature	Abbreviation	Precursor compound	CAS number	Fragment ions (m/z)
Δ^5 -sterol	cholesterol	cholest-5-en-3 β -ol			CAS 57-88-5	129 , 329, 368, 458 ($M+^{\cdot}$)
Δ^5 -sterol	β -sitosterol	stigmast-5-en-3 β -ol			CAS 83-46-5	129 , 357, 396, 486 ($M+^{\cdot}$)
Δ^5 -sterol	campesterol	campest-5-en-3 β -ol			CAS 474-62-4	129 , 343, 382, 472 ($M+^{\cdot}$)
Δ^5 -sterol	stigmasterol	stigmasta-5,22-dien-3 β -ol			CAS 83-48-7	83 , 129, 255, 394, 484 ($M+^{\cdot}$)
5 α -stanol	5 α -cholestanol	5 α -cholestan-3 β -ol		cholesterol	CAS 80-97-7	215 , 355, 445, 460 ($M+^{\cdot}$)
5 α -stanol	5 α -stigmastanol	5 α -stigmastan-3 β -ol		β -sitosterol	CAS 19466-47-8	215 , 383, 473, 488 ($M+^{\cdot}$)
5 α -stanol	5 α -campestanol	5 α -campestan-3 β -ol		campesterol	CAS-474-60-2	215 , 370, 384, 474 ($M+^{\cdot}$)
5 β -stanol	coprostanol	5 β -cholestan-3 β -ol		cholesterol	CAS 360-68-9	215 , 257, 355, 370 , 460 ($M+^{\cdot}$)
5 β -stanol	epicoprostanol	5 β -cholestan-3 α -ol		cholesterol	CAS 516-92-7	215 , 257, 355, 370, 460 ($M+^{\cdot}$)
5 β -stanol	5 β -stigmastanol	24-ethyl 5 β -cholestan-3 β -ol		β -sitosterol	CAS 4736-91-8	215 , 257, 383, 398 , 473 ($M+^{\cdot}$)
5 β -stanol	5 β -epi-stigmastanol	24-ethyl 5 β -cholestan-3 α -ol		β -sitosterol	CAS 5060-24-2	215 , 257, 383, 398, 473 ($M+^{\cdot}$)
5 β -stanol	5 β -campestanol	24 α -methyl-5 β -cholestan-3 β -ol		campesterol		215 , 257, 369, 384 , 474 ($M+^{\cdot}$)
5 β -stanol	5 β -epi-campestanol	24 α -methyl-5 β -cholestan-3 α -ol		campesterol		215 , 257, 369, 384, 474 ($M+^{\cdot}$)
Primary bile acid	cholic acid	(3 α ,7 α ,12 α)-trihydroxy-5 β -cholanoic acid	CA		CAS 81-25-4	253 , 343, 628, 468, 638 ($M+^{\cdot}$)
Primary bile acid	chenodeoxycholic acid	(3 α ,7 α ,8 ξ)-3,7-dihydroxy-5 β -cholanoic acid	CDCA		CAS 474-25-9	255 , 355, 370 , 460, 550 ($M+^{\cdot}$)
Secondary bile acid	deoxycholic acid	(3 α ,5 β ,12 α)-3,12-dihydroxy-5 β -cholanoic acid	DCA	cholic acid	CAS 83-44-3	208 , 255 , 245, 370, 550 ($M+^{\cdot}$)
Secondary bile acid	hyodeoxycholic acid	(3 α ,6 α)-3,6-dihydroxy-5 β -cholanoic acid	HDCA	lithocholic acid	CAS 83-49-8	255 , 355, 370, 460 , 550 ($M+^{\cdot}$)
Secondary bile acid	lithocholic acid	(3 α ,5 β)-3-hydroxy-5 β -cholanoic acid	LCA	chenodeoxycholic acid	CAS 434-13-9	215 , 257, 357, 372, 462 ($M+^{\cdot}$)
Secondary bile acid	ursodeoxycholic acid	(3 α ,7 β)-3,7-dihydroxy-5 β -cholanoic acid	UDCA	chenodeoxycholic acid	CAS 128-13-2	255 , 355, 370 , 460, 550 ($M+^{\cdot}$)

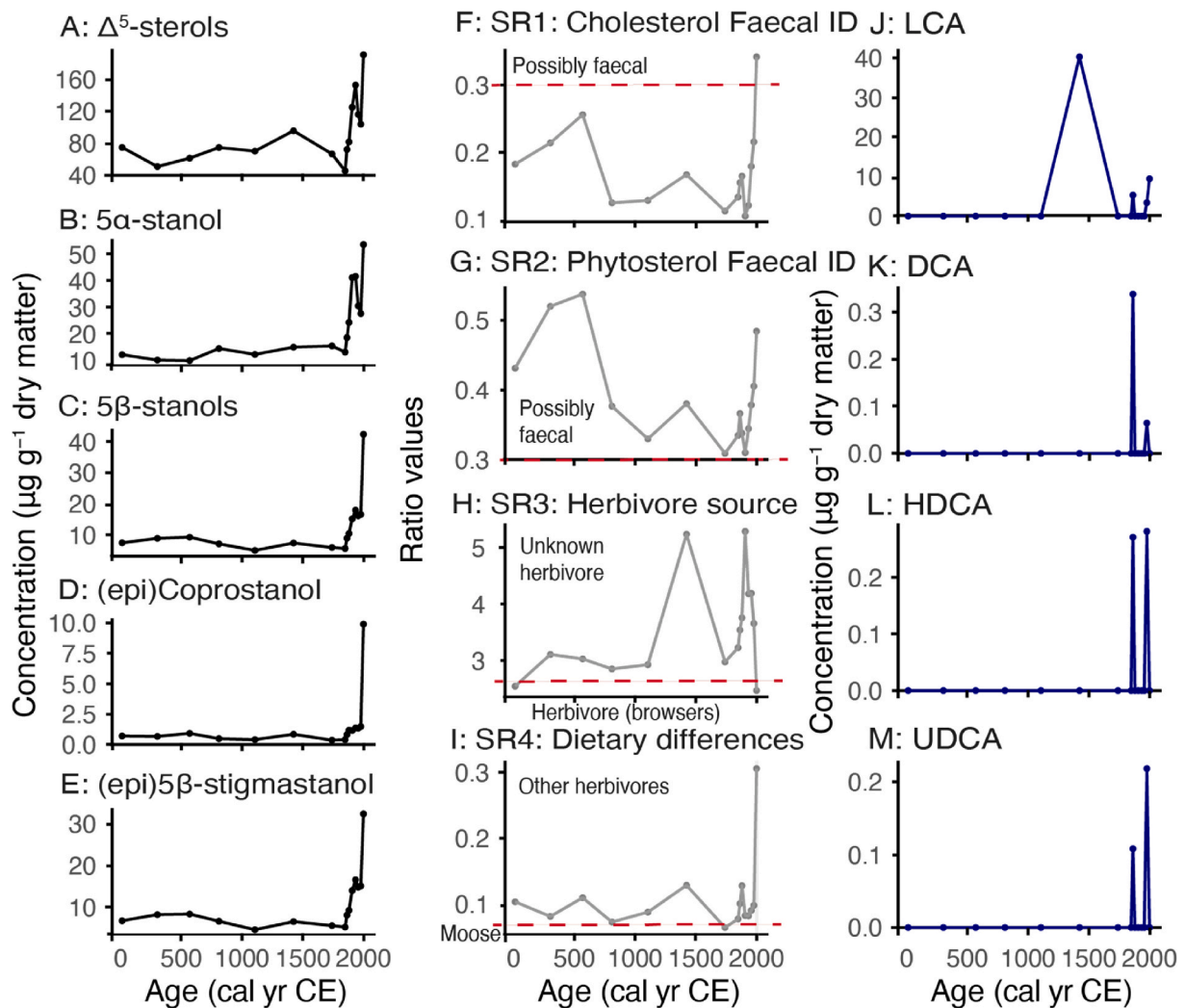
Appendix A3

Estimated faecal production rate of species in Tanana Valley based on faunal assemblages from [Potter \(2008\)](#) to accompany [Fig. 11](#). Faecal production rates vary on many factors such as diet, hydration, activity level, age of individual and hibernation patterns; therefore, these rates are example estimates. Differences in magnitude are of most relevance to this study.

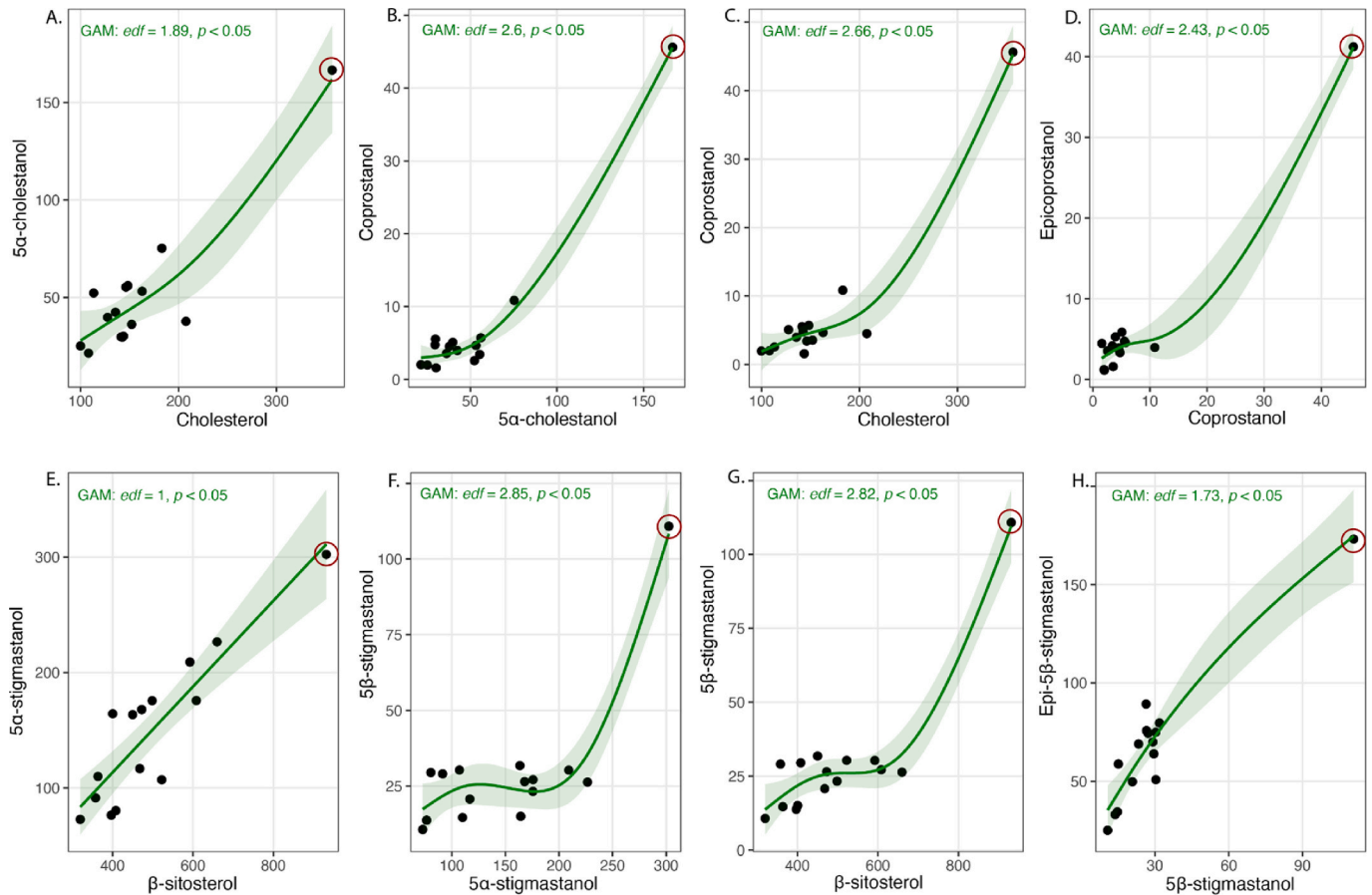
Species	Diet	Estimated faecal production rates (g individual ⁻¹ per day ⁻¹ , dry wt)	Basis for estimate and example reference
Humans	Omnivore	30	Faecal samples (Rose et al., 2015)
Canids	Omnivore	30 (dogs)	Dietary intake and digestibility (Do et al., 2021)
	Carnivore	150 (wolves)	Peterson and Ciucci (2003)
Bear	Omnivore	350	Dietary intake and digestibility (Pritchard and Robbins, 1990)
Birds	Omnivore	100 (Canada Geese)	Faecal counts and dropping rates (Kear, 1963)
		25 (Mallard)	
Fish	Piscivore/ insectivore	10 (Pike)	Bioenergetic modelling (Hanson et al., 1997 ; Hartman and Jensen, 2017 ; Harvey, 2011)
		2 (Grayling)	
Hare	Herbivore	60	Faecal counts (Hodges, 1999)
Beaver	Herbivore	300	Dietary intake and digestibility (Belovsky, 1984)
Sheep	Herbivore	400	Faecal counts (Welch, 1982)
Caribou	Herbivore	600	Dietary intake and digestibility (Boertje, 1990)
Elk	Herbivore	1700	Dietary intake and digestibility (Jiang and Hudson, 1992)
Moose	Herbivore	2500	Dietary intake and digestibility (Renecker and Hudson, 1986)
Bison	Herbivore	3500	Dietary intake and digestibility (Galbraith et al., 1998)



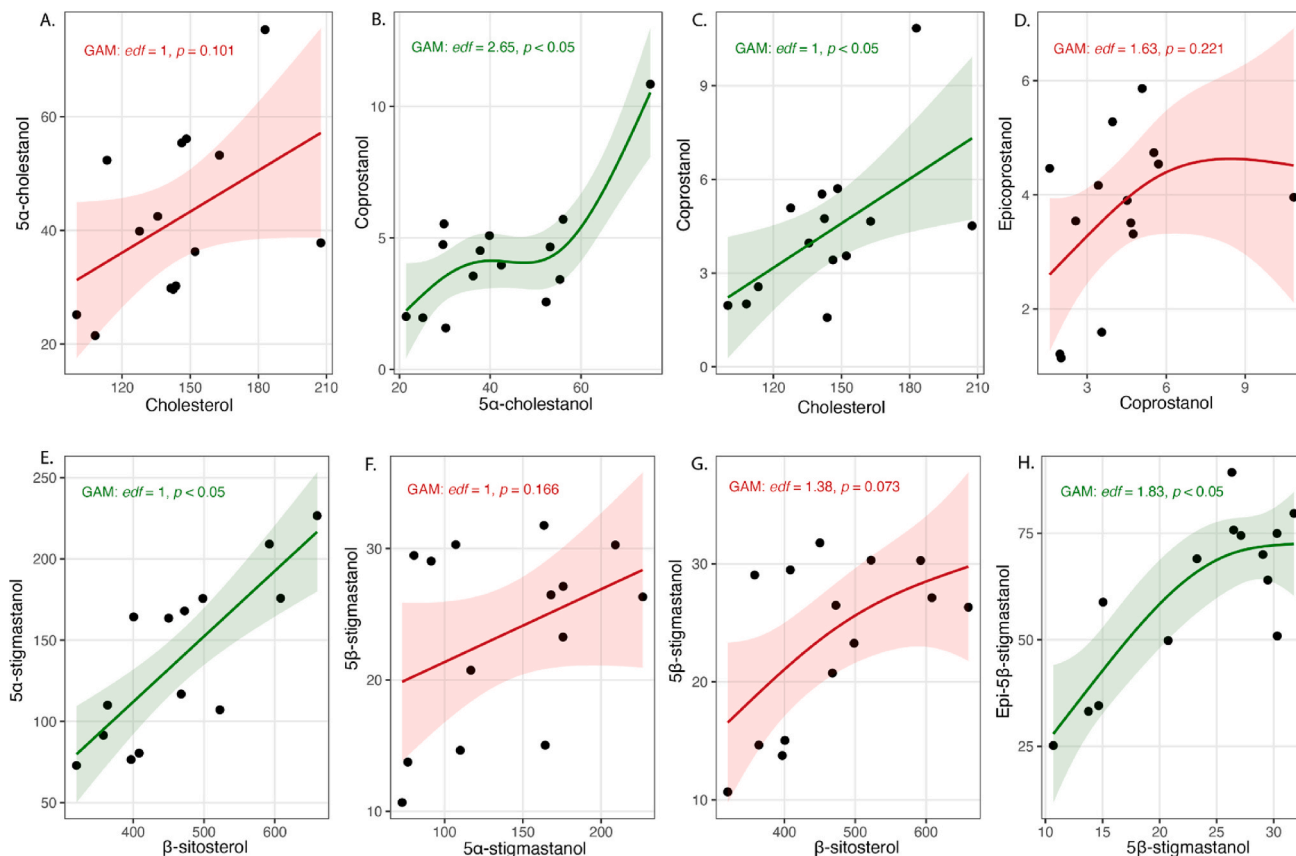
Appendix A4. Chisholm Lake steroid compositions and ratios over the last 125 years. A-C: accumulation rates of different groups of sterols: A: Δ^5 -sterols (cholesterol + β -sitosterol + stigmastanol), B: 5α -stanols (5α -cholestanol + 5α -stigmastanol) and C: 5β -stanols (coprostanol + epicoprostanol + 5β -stigmastanol and epi- 5β -stigmastanol); D: accumulation rates of coprostanol + epicoprostanol and E: 5β -stigmastanol + epi- 5β -stigmastanol, F-I: stanol ratios to identify faecal input and sources (equations and threshold values in Table 4). F-G: C_{27} -stanol and C_{29} -stanol faecal input indices respectively, where 0.3 to 0.7 are considered as a possible faecal input, >0.7 = confirmed faecal input. H: Stanol ratio 3, herbivore differentiation index, where <1.2 = grazers and intermediate herbivore feeders, 1.2 to 2.6 = herbivore browsers, >2.75 = no robust modern reference match, some overlap with elk; I: Stanol ratio 4, dietary differentiation index, where >1 = omnivore faeces (Shillito et al., 2011) and <0.07 = moose faeces (this study; Fig. 6E); J-M: accumulation rates of individual bile acids. Coloured bar below the x axis represents a summary of main historical changes in Chisholm Lake occupation: blue: seasonal occupation of nearby Birch Lake by Salcha Dene-Athabaskan peoples; red: mining activity, small communities and trail nearby; purple: cabins constructed on edge of lake, green: increased construction and use of cabins from 1950s and Boy Scout camp activity since early 1960s.



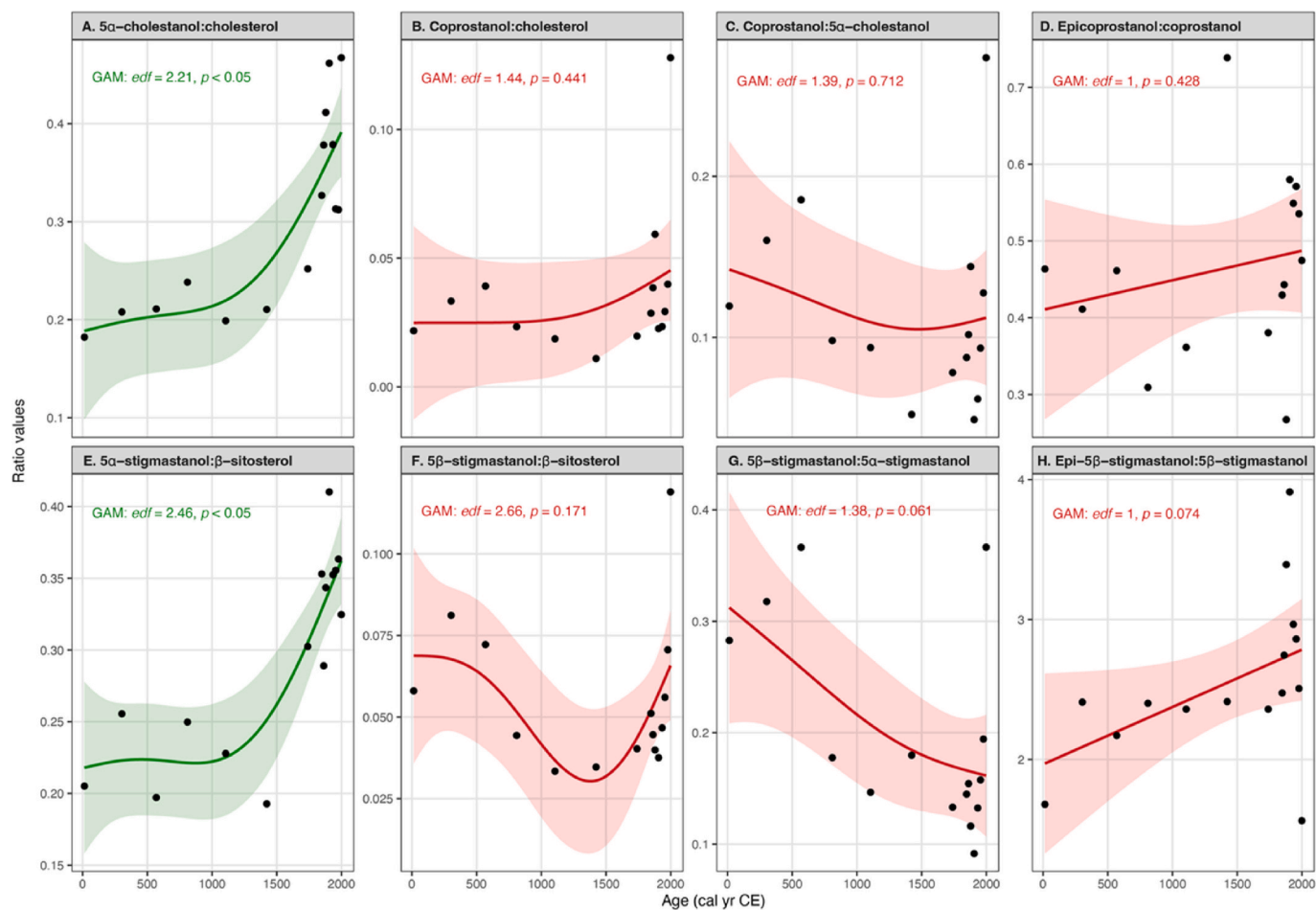
Appendix A5. Chisholm Lake steroid concentrations and ratios over the last 2000 years. A-C: concentrations of different groups of sterols: A: Δ^5 -sterols (cholesterol +, β -sitosterol + stigmasterol), B: 5α -stanols (5α -cholestanol + 5α -stigmastanol) and C: 5β -stanols (coprostanol + epicoprostanol + 5β -stigmastanol + epi- 5β -stigmastanol); D: coprostanol + epicoprostanol and E: 5β -stigmastanol + epi- 5β -stigmastanol, F-I: stanol ratios to identify faecal input and sources (equations and threshold values in Table 4). F-G: C_{27} -stanol and C_{29} -stanol faecal input indices respectively, where 0.3 to 0.7 are considered as a possible faecal input, >0.7 = confirmed faecal input. H: Stanol ratio 3, herbivore differentiation index, where <1.2 = grazers and intermediate herbivore feeders, 1.2 to 2.6 = herbivore browsers, >2.75 = no robust modern reference match, some overlap with elk; I: Stanol ratio 4, dietary differentiation index, where >1 = omnivore faeces (Shillito et al., 2011) and <0.07 = moose faeces (this study; Fig. 6E); J-M: concentrations of individual bile acids. Steroid fluxes are provided in Fig. 8 for comparative purposes.



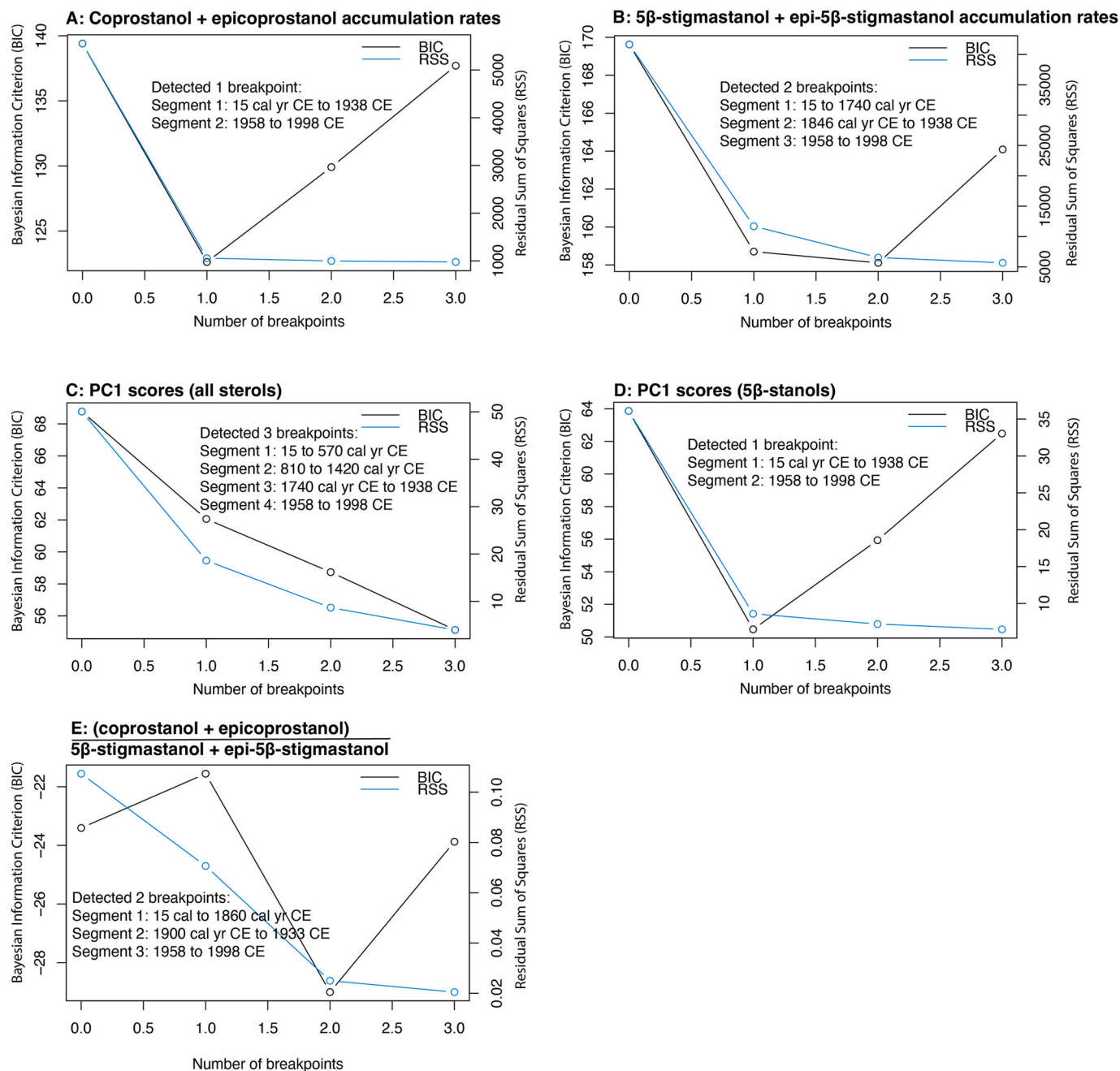
Appendix A6. Generalised additive models (GAMs) of C_{27} - and C_{29} - sterol diagenetic pathways and sources. Diagenesis and source are represented by Δ^5 to 5α ; Δ^5 to 5β ; while 5α to 5β represents source only. A-D: C_{27} -stanols; A: cholesterol (Δ^5): 5α -cholestanol; B: 5α -cholestanol:coprostanol (5β); C: cholesterol (Δ^5):coprostanol (5β); D: coprostanol (5β):epicoprostanol (epi- 5β). E-H: C_{29} -stanols; E: β -sitosterol (Δ^5): 5α -stigmastanol; F: 5α -stigmastanol: 5β -stigmastanol; G: β -sitosterol (Δ^5): 5β -stigmastanol and H: 5β -stigmastanol:epi- 5β -stigmastanol. p -values in green are significant at a 95% confidence level. The outlying surface sample (0.5 cm, c. 1998 CE; 2 σ) is annotated by red circles.



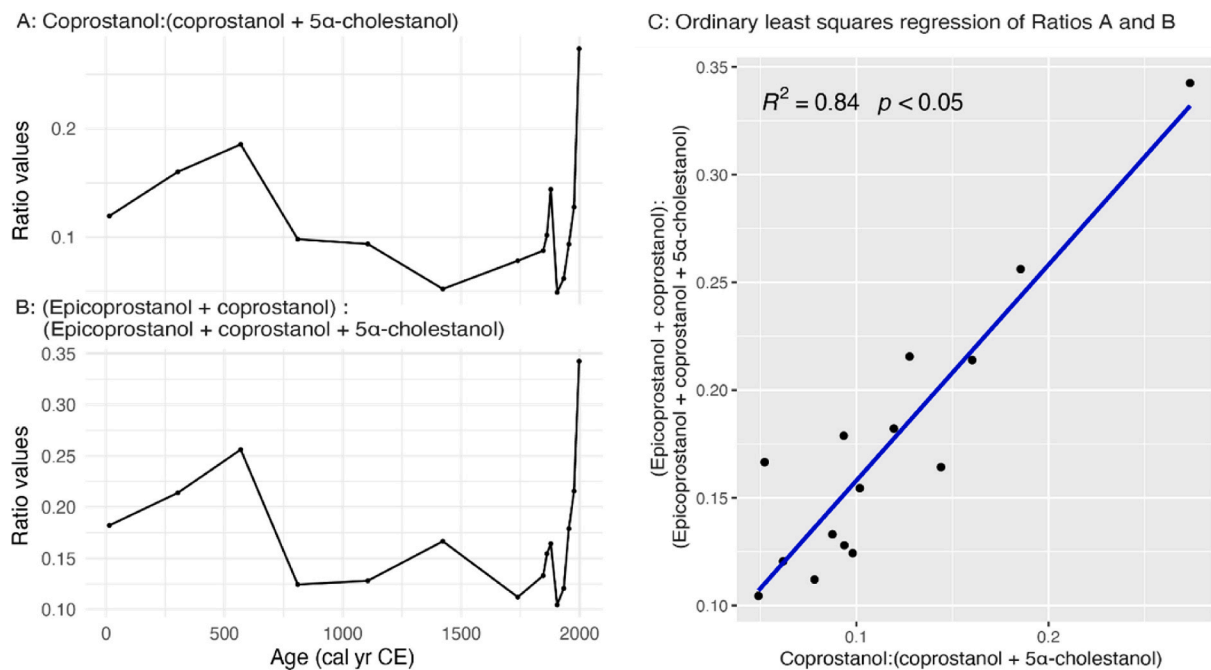
Appendix A7. Generalised additive models (GAMs) of C_{27} - and C_{29} - sterol diagenetic pathways and sources following removal of the outlying surface sample (2 σ ; Supplementary, [Appendix A6](#)). Diagenesis and source are represented by Δ^5 to 5α ; Δ^5 to 5β ; while 5α to 5β represents source only. A-D: C_{27} -stanols; A: cholesterol (Δ^5): 5α -cholestanol; B: 5α -cholestanol:coprostanol (5β); C: cholesterol (Δ^5):coprostanol (5β); D: coprostanol (5β):epicoprostanol (epi- 5β). E-H: C_{29} -stanols; E: β -sitosterol (Δ^5): 5α -stigmastanol; F: 5α -stigmastanol: 5β -stigmastanol; G: β -sitosterol (Δ^5): 5β -stigmastanol and H: 5β -stigmastanol:epi- 5β -stigmastanol. p -values in green (red) are significant (not significant) at a 95% confidence level.



Appendix A8. Generalised additive models (GAMs) of ratios of sterol diagenesis and source for cholesterol (C_{27}) and β -sitosterol (C_{29}) pathways against sediment core age for the Chisholm Lake record. 5 α : Δ^5 (A and E) 5 β : Δ^5 (B and F) and Epi-5 β :5 β (D and H) representing diagenesis and sterol sources. 5 β :5 α (C and G) represent sterol sources. Significant (non-significant) relationships highlighted in green (red) at a 95% confidence level.



Appendix A9. Breakpoints in Chisholm Lake steroid fluxes, ratios and PCA Axis 1 scores over the last 2000-years. The number of breakpoints (based on lowest BIC values) and age of segment lengths identified are noted. BIC: Bayesian Information Criterion; RSS: residual sum of squares.



Appendix A10. Comparisons of stanol ratio 1 (C_{27} -based faecal index; Equation (1)) with (A) and without (B) the 5β -epimer (epicoprostanol). C: Ordinary least squares regression of ratios shows a significant linear relationship ($R^2 = 0.84$, $p < 0.05$).

Data availability

Data generated in this study are publicly available through PANGAEA - Data Publisher for Earth and Environmental Science (Felden et al., 2023); the ^{14}C age determinations for the Chisholm Lake core CHIS19 (Mackay et al., 2026a; <https://doi.org/10.1594/PANGAEA.993067>); ^{210}Pb age determinations for CHIS19 (Mackay et al., 2026b; <https://doi.org/10.1594/PANGAEA.993069>); faecal steroid data from CHIS19 based on the ^{210}Pb chronology (Mackay et al., 2026c; <https://doi.org/10.1594/PANGAEA.993065>); faecal steroid data from CHIS19 based on the combined ^{14}C and ^{210}Pb chronology (Mackay et al., 2026d; <https://doi.org/10.1594/PANGAEA.993066>); and modern faecal steroid data (Mackay et al., 2026e; <https://doi.org/10.1594/PANGAEA.993064>). The datasets are licensed under the Creative Commons Attribution 4.0 International licence (CC BY 4.0).

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