Tracking pollution from fur farms using forensic paleolimnology

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Abstract: 212/300 words

Eutrophication, which remains one of the greatest threats to water quality worldwide, is particularly acute in agricultural areas. Here we assessed long-term drivers of potential pollution inputs to lakes in southwest Nova Scotia (Canada), a region marked by fur farming (mainly mink) and other agricultural activities. We used a BACI (before-after-control-impact) study design with sediment cores collected from 14 lakes selected based on their proximity to mink farms. We combined economic data, mink faecal samples, and a series of geochemical markers in dated sediment cores, including sterols, $\delta^{15}N$, visible reflectance spectroscopy (VRS)-inferred chlorophyll-*a*, and heavy metals, to relate changes in sediment geochemistry to the growth of mink farms in the region. Sterol biomarkers (cholesterol and β-sitosterol) measured in a range of samples (i.e. mink faeces and feed, aquaculture feed), were elevated where mink farms were located close to each study lake. Mink-related sterols (cholesterol, β-sitoserol), $\delta^{15}N$ measurements, VRS chlorophyll-*a*, and heavy metals As, Cu, Sr increased in the 1980s coeval with a ~400% increase of mink farms in the region, especially near Nowlans Lake. Agricultural impacts were subtler in other lakes. Our study expands on prior applications of geochemical fingerprinting in forensic paleolimnology when direct monitoring data are incomplete. This multi-proxy approach has promising applications for environmental pollution assessments in other lake ecosystems experiencing water quality issues.

1.0 Introduction

Mink farming is a ~$1 billion industry in Canada (Canadian Mink Breeders, 2022) and has traditionally been a major agricultural producer in Nova Scotia, second only to dairy production in that province (Statistics Canada, 2019). Commercial mink farming in Nova Scotia began in the 1950s, intensified in the 1970s -1980s, and increased ~4-fold between 1980 and 2014 (Statistics Canada, 2019, 2021, Gregory et al., 2022). Despite its economic benefits, mink farming is associated with environmental and public health risks. In 2021, the World Health Organization concluded that mink farms pose a COVID-19 risk to humans (WHO, 2021). Furthermore, fur farming is associated with elevated bacterial, virus, and pathogen abundance in nearby waterways (Tyagi et al., 2008, Nituch et al., 2011). For example, the rise of mink farming in Asia resulted in outbreaks of Aleutian disease (Kashtanov et al., 2017, Cuthbertson, 2020), a disease that is difficult to eradicate and often requires elimination of infected animals (Farid et al., 2010, 2012).

Fur farming also has the potential to introduce excess nutrients to nearby lakes and rivers, in addition to elevated bacteria, viruses, and other pathogens (Tyagi et al., 2008, Nituch et al., 2011). Excess nutrients can lead to toxic algal blooms and water-column oxygen depletion resulting from organic matter decomposition. Eutrophication (and associated algal toxins) can be lethal to freshwater and land-dwelling organisms, with implications for ecosystems and public health (Nova Scotia Department of the Environment, 1998, Government of Canada, 2017, Rashidi et al., 2021, Kelly et al., 2021).

Mink farming is central to polarized debates among southwestern Nova Scotian communities, federal and provincial governments, watershed groups, and NGOs in recent years over declining water quality, odour issues, algal blooms, and the presence of nuisance gulls and flies. In attempts to resolve the controversy, several studies have investigated the impacts of agricultural farming on aquatic systems in southwest Nova Scotia. Following reports of excessive algal blooms, studies (Taylor, 2010, Brylinsky, 2012; Brylinsky and Sollows, 2014, Stantec, 2017) determined that mink farms were the likely source of nutrients to lakes showing signs of eutrophication. Recent watershed modelling supports these findings, indicating lakes would not reach their current state of eutrophication without including nutrient loading from fur farms (Van Heyst et al., 2022). Paleolimnological assessments using benthic invertebrates and zooplankton showed a decades-long trend towards eutrophication coeval with fur farming and

aquaculture activity in the region (Campbell et al., 2022a, 2022b; Jones et al., 2022). Additionally, southwestern Nova Scotian lakes with fur farming in their catchments exhibited elevated total mercury in sediment, suggesting the possibility of fur-farming related metal contamination (Gregory et al., 2022). In addition to affecting local watersheds, mink farming may also influence distant environments indirectly. For example, mink farms in southwest Nova Scotia provide stopover sites for breeding herring gulls (Gutowsky et al., 2021; McIntyre et al., 2022). Gulls foraging on mink farms act as biological vectors carrying persistent, bioaccumulative pollutants (e.g., metals, pesticides, etc.) to distant ecosystems (McIntyre et al., 2022). Applying novel geochemical methods to assess the potential link between fur farming activity and ongoing eutrophication in southwestern Nova Scotian lakes provides additional insight into the underlying cause of water quality issues.

Paleolimnology is an established and effective approach to track contamination sources in lakes through time (Blais et al., 2015). Stable nitrogen isotopes are a commonly used geochemical proxy for waste inputs from humans or animals because the enrichment of $\delta^{15}N$ values with increasing trophic level distinguishes source from background levels of aquatic production. Several geochemical proxies measured in lake sediments have also proven useful in tracking nutrient inputs to lakes and streams from humans, livestock, and wildlife, including sterols, faecal stanols and heavy metals. For example, Gallant et al., (2020) reconstructed decades of eutrophication in lakes from the Canadian Arctic using $\delta^{15}N$, sterols, and heavy metals as indicators of the timing and magnitude of eutrophication. Meanwhile, White et al., (2018) used faecal stanols in a sediment core from a lake near Cahokia Illinois to reconstruct human population levels and determined sterol abundance in sediment cores could be linked to population fluxes over the past ~1000 years. Clear differences between pre- and post-industrialized sediments were observed in lake sediment cores from northern Manitoba (Canada) in which human-derived sterols (coprostanol) reconstructed long-term trends of municipal sewage effluents prior to discharge regulations commencing in 1951 and plant-related sterols indicated increased organic matter inputs due to regional logging and fire deforestation (Tse et al., 2014). D'Anjou et al., (2012) used faecal stanols to reconstruct human migration and agricultural activities affected by climatic changes in Norway throughout the Little Ice Age, early Iron Age, and Medieval Period. Duda et al., (2020a, 2020b) highlighted the need for long-term biodiversity monitoring practices to establish pre-industrialization baselines including

isotopes, sterols, heavy metals, and chlorophyll-*a* in order to assess the impact of anthropogenic influences on avian populations.

Here we used a series of geochemical indicators to assess sources of lacustrine eutrophication to lakes in southwestern Nova Scotia. We analyzed five complete sediment core profiles and the 'tops' and 'bottoms' of nine additional sediment cores for sterols, $\delta^{15}N$, chlorophyll *a*, and heavy metals to track potential sources and the trajectory of pollution linked to eutrophication in reference and impact lakes from southwest Nova Scotia. Our overall goal was to use forensic paleolimnology to identify and track the major source(s) of nutrients in southwest Nova Scotia lakes. We hypothesized that chemical fingerprints related especially to mink farms ($\delta^{15}N$, zoosterols, biogenic metals) will indicate the presence and historical impact of mink farms based on sediment core records.

2.0 Methods

2.1 Site description

The study area is part of the Carleton, Meteghan, Sissiboo, and Tusket River watersheds, which flow into St. Mary's Bay near the Bay of Fundy (Figure 1). The watersheds are west of Kejimkujik National Park and much of the headwaters lay within the Tobeatic Wilderness Area, which is part of the Southwest Nova Biosphere Reserve (NS Department of the Environment, 1998). The geology of southwestern Nova Scotia is part of the Meguma terrane and is primarily comprised of granitoid igneous rocks, and slate, quartzite, greywacke, gneiss, and schist (White and Vaccaro, 2019, White and Barr, 2012, NS Department of the Environment, 1998). The land in the region is largely rural and forested with the exception of some residential and agricultural developments. The area gradually slopes upwards from the Atlantic Coast and is considered its own climatic region with mild winters and higher rainfall and generally warmer temperatures than eastern Nova Scotia. The average air temperature in the summer is 15 ^{o}C and in the winter temperatures fall to -1 ^{o}C on average (Environment and Climate Change Canada (ECCC) 2021), with lakes experiencing seasonal ice cover and thaws. The average annual precipitation in the region is 1270 mm (ECCC, 2021). This area is also subjected to some acidic precipitation that can create surface waters with low pH values from aerial deposition of sulphates from northeastern US and eastern Canada (Kerekes et al., 1986, Stantec, 2017). There is a mean

maximum wind gust direction of approximately S30°W, with prevailing winds from the southwest in the summer and northwest in the winter (Kerekes et al., 1989, Neily et al., 2005, ECCC, 2021, Gregory et al., 2022).

2.2 Field Methods and study design

Sediment cores were collected in May and August 2018 from the deepest basin of each lake. Coring location was determined by bathymetric maps and use of a handheld depth sounder. Cores were collected using a UWITEC (Mondsee, Austria) or Glew gravity corer, sectioned at 0.5-cm intervals on site using a vertical extruder (Glew et al., 2001) and placed into Whirlpak® bags. We froze sediment intervals in the field for storage within 24 hours at -4°C until shipment to the University of Ottawa (Ottawa, ON, Canada) to await geochemical analysis.

We applied a before-after-control-impact (BACI) as well as an impact and reference category design to sediment cores in 14 lakes sampled in May and August of 2018 in the southwest region of Nova Scotia (Figure 1). The study lakes were divided *a priori* into one of three categories based on catchment borders outlined by Stantec, (2017): (1) Group 1 (Hourglass, Nowlans, Oakleaf, Placides, Porcupine) lakes have mink farms located within the catchment boundaries or within the catchment of a receiving inlet stream; (2) Group 2 (Fanning, Parr, Ogden, Provost) have mink farms located outside the lake catchment boundary or are downstream from a lake with mink farms in the catchment; and (3) Reference lakes (Clearwater, Comeau, Delaps, Janes, Sloans) have no notable sources of excess nutrient inputs to the watershed. Table S1 provides details of the lake group categories. Five lakes were selected for full sediment core profile resolution in Nowlans, Placides, Porcupine, Comeau, Clearwater. Nine additional lakes were selected for surface (recent sediments) and bottom (pre-industrial sediments) sterol and stanol biomarker analyses (Hourglass, Oakleaf, Fanning, Ogden, Parr, Provost, Delaps, Janes, and Sloans).

We collected terrestrial samples from the southwest Nova Scotia region. Mink waste and mink feed were supplied by a mink farm in the region. Fish feed and aquaculture effluent samples were collected at the aquaculture facility next to Hourglass Lake.

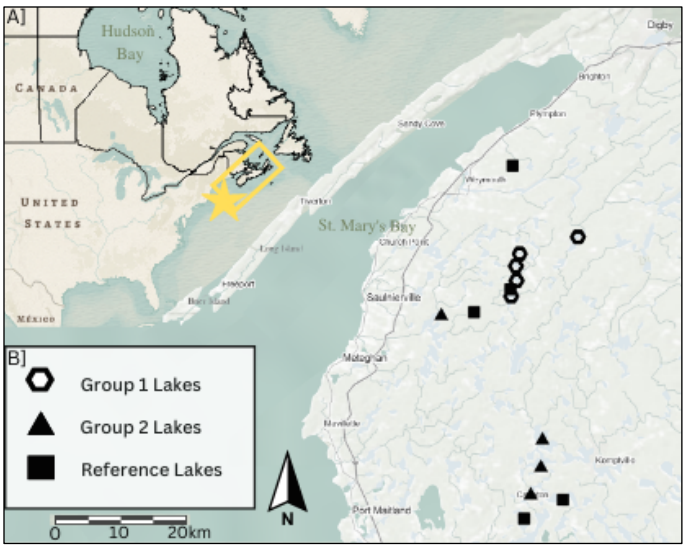

Figure 1: A] Location of our study sites in Nova Scotia, Canada. B] 14 lakes included in our study (Group 1, Group 2, and Reference Lakes group) are located in the southwest region of Nova Scotia, Canada.

2.3 Sediment dating, sterol biomarkers, isotopes, chlorophyll-*a*, heavy metals

Dating by ^{210}Pb was conducted using an Ortec High Purity Germanium Gamma Spectrometer (Oak Ridge, Tennessee, USA.) at the University of Ottawa (Ottawa, ON, Canada) or Queen's University (Kingston, ON, Canada). The dating profiles were analyzed using ScienTissiMe software (Sheer Software Solutions, Barry's Bay, ON, Canada). The constant rate of supply (CRS) model was used to estimate dates for the sediment cores, and ^{137}Cs activity was measured simultaneously to corroborate the ^{210}Pb dating profile (Blais et al., 1995). Detailed radiometric dating for these cores were published in Gregory et al., (2022), and we included all ^{210}Pb, ^{214}Pb, and ^{137}Cs results in Table S5.

To prepare sediment sub-samples and livestock and aquaculture feed/waste for isotopic analyses, we freeze-dried and homogenized the sediment subsamples. Lake sediments were isotopically analyzed at the Jan Veizer Stable Isotope Lab at the University of Ottawa. Samples were first analyzed for percent nitrogen and carbon using an Elementar Isotope Cube (Langenselbold, Germany) then δ^{15}N was analyzed using an elemental analyzer Vario EL Cube (Elementar, Germany) interfaced with a Conflo IV (Thermo, Germany) to an isotope ratio mass spectrometer (IRMS) Delta Advantage (Thermo, Germany). The δ^{15}N measurements were reported using delta (δ) notation in parts per thousand (‰). Isotopes were normalized to internal

standards and calibrated to International standards IAEA-N1 (0.4‰), IAEA-N2 (20.3‰), USGS-40 (-4.52‰) and USGS-41 (47.57‰).

Sterols were analyzed in sediment cores, mink faeces, mink feed, and fish feed after minor modifications of methods outlined by Hargan et al., (2019). In preparation of sterol extraction, 0.1 g of copper (Fisher C434-500, laboratory grade Cu powder; CAS 7440-50-8, Fisher Scientific, Ottawa, ON, Canada) was subsampled into glass scintillation vials. 10 mL of dichloromethane (DCM, high-grade Optima®, Fisher Scientific) was added and the scintillation vials were sonicated for 10 minutes; these steps were repeated twice. The Cu was subsequently air-dried. Approximately 0.1 g of freeze-dried sample was then added to the Cu in a 1:1 ratio and spiked with 50 µL of 10,000 ng mL^{-1} deuterated cholesterol (d_6-cholesterol; Cambridge Isotope Laboratories, Tewksbury, MA, USA). Extraction was completed with a 10 ml addition of DCM sonicated for 10 min, and the DCM transferred to an evaporation flask. The extraction was repeated three more times producing a final combined sample volume of ~40 ml DCM. For method blanks, d6 cholesterol was spiked directly onto the Cu. For extraction, 10 mL of DCM was added to samples, then samples were sonicated for 10 min, these steps were repeated thrice more. Extracts were then evaporated to 1 mL under nitrogen at 35°C, 0.4 bar/6 p.s.i., and subsequently cleaned using 1 g Supelclean™ (Sigma-Aldrich Co. St. Louis, MO, USA) LC-Si® solid-phase extraction cartridges that were preconditioned with 6 mL DCM. Samples were eluted with 20 mL DCM and evaporated to 1 mL under nitrogen, and then transferred to a 2 mL GC vial. For derivatization, samples were evaporated to complete dryness under nitrogen, and derivatized with 0.1 mL BSTFA + 1% TCMS (N,O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane; Sigma Aldrich, St. Louis, MO, USA) and subsequently placed on a dry heating block (Thermo Scientific, Waltham, MA, USA) at 60 °C for 2 hours. Samples were taken off the block and cooled for 15 minutes, followed by the addition of 0.9 mL toluene and spiked with *p*-terphenyl-d_{14} (Cambridge Isotope Laboratories, Tewksbury, MA, USA) as an internal standard.

Following Hargan et al., (2019), samples were quantified for sterol analyses by gas-chromatography-mass spectrometry (GC-MSD) using an Agilent 6890 gas chromatograph (GC) - 5973 mass selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA) in electron impact and selected ion monitoring. An Agilent 19091J-433 HP-5 5% phenyl methyl siloxane column (30 m x 250 µm x 0.25 mm) was used with the following GC conditions using He carrier

gas: a pulsed splitless injection at 250°C at 17.75 psi, DB-5MS (Agilent, Santa Clara, CA, USA), oven start at 150°C, ramp 1 at 8°C/min to 250°C, ramp 2 at 12°C/min to 300°C held for 8 minutes. MSD conditions were as follows: transfer line 300°C, source 230°C, quad 150°C. Method blanks of Cu powder were extracted and derivatized using the same protocols. Sterol concentrations were normalized to dry weight and expressed in $\mu g\ g^{-1}$. Eleven sterols were measured in all samples: coprostanol (5β-cholestan-3β-ol), epicoprostanol (5β-cholestan-3α-ol), coprostanone (5β-cholestan-3-one), cholesterol (cholest-5-en-3β-ol), 5α-cholestanol (5α-cholestan-3β-ol), cholestanone (5α- cholestan-3-one), desmosterol (3b-cholesta-5,24-dien-3-ol), campesterol (campest-5-en-3b-ol), fucosterol (stigmasta-5,24-dien-3β-ol), sitosterol (β-sitosterol), and stigmastanol (5α-stigmastano-3β-ol), all standards were purchased through Sigma-Aldrich.

Metals were analyzed at 2 cm resolution for select sediment cores and livestock and aquaculture samples at the University of Ottawa. Freeze-dried samples were weighed into Digi-Tubes (SCP Science, Baie D'Urfé, QC, Canada): Metals were then extracted by digesting freeze-dried sediment (0.5g), mink faecal matter, mink, and fish feed samples by adding 10 ml of 1:1 HNO_3 (Omni-Trace, Fisher Scientific) was added to samples and placed in a graphite heating block. The sample was then refluxed at 95°C for 10 min in the DigiPrep (company). Test tubes were then removed from the block and 5mL of pure HNO_3 was added to reflux the sample again at 95°C for 30 min; this step was repeated until the brown fumes dissipated. After cooling to room temperature, 2 mL of deionized water and 3 mL of H_2O_2 (Fisher Scientific) were added to samples, which were placed in the graphite block and heated at 95°C for 3 hr in the heated graphite block. After cooling to room temperature, sample volumes were normalized to 50 mL with deionized water and filtered with a DigiFILTER. Prepared samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies) following methods outlined in Gallant et al., (2020). Nine metals were quantified: Ti, Cr, Ni, Cu, Zn, As, Sr, Cd, Pb. Certified Reference Materials (PACS-3, National Research Council Canada, Ottawa, ON, Canada) and procedural blanks were analyzed alongside the sediment samples to provide a measure of methodological accuracy and to quantify the recovery of biogenic elements studied. All analyses were completed at the University of Ottawa.

Sedimentary chlorophyll *a* (which includes its isomers and main diagenetic products; Michelutti and Smol, 2016) was measured at Queen's University using visible near-infrared

reflectance spectroscopy with a Model 6500 series Rapid Content Analyzer (FOSS NIRSystems Inc. Eden Prairie, MN, USA). VRS-inferred chlorophyll a concentrations were determined from characteristic troughs at 650-700 nm in the absorption spectra of freeze-dried sediment samples (Michelutti et al., 2010).

A principal components analysis (PCA) was performed using XLSTAT 2023.1.6(1410) in Excel 16.0.16529 (64 bit) (Lumivero, 2023) to visualize variation in sterol composition in sediment cores.

2.3 Biogenic enrichment factors

We developed biogenic enrichment factors, a metric used to rank elements based on the likelihood they are enriched by mink waste, as described in Brimble et al., (2009). These enrichment factors are calculated as the mean concentration of an element in mink waste divided by the mean concentration of the same element in background (pre-industrial) sediment from the region. We calculated these enrichment factors for Nowlans, Placides, Porcupine, Clearwater, and Comeau lakes to identify which elements in the sediments are more likely to be enriched by mink waste (Table S3). When biogenic enrichment factors are > 1, the concentration of the element is higher in the mink waste compared to background sediment concentrations and is therefore more likely to be enriched from mink farming practices. Elements were normalized to Ti prior to statistical analysis to minimize the influence of geogenic factors on sediment accumulation in lakes. Elements were selected as showing a potential increase related to mink farming on the basis of enrichment factors and through visual inspection of trends in element concentration (i.e., a difference between pre- and during- mink farming).

3.0 Results & Discussion

3.1 Terrestrial samples

Mink waste was most enriched $\delta^{15}N$ (15.01 ± 0.10‰, n=2), compared to mink feed (6.38 ± 0.18‰, n=2), and fish feed (10.43‰, n=1), Table S2. Cholesterol was the sole sterol in mink food samples (900 µg/g DW), whereas mink faecal samples were highest in cholesterol (3500

μg/g DW) and β-sitosterol (145 μg/g DW, Table S2), making them a likely source of sterols to lake sediments with mink farms near the lake shore. The presence of β-sitosterol in faecal samples suggests another plant-based source of food to the mink's diet. Farmed mink diet is high-protein and consists predominately of products and byproducts from fish and meat (poultry, pork, beef), as well as cereals, and vitamin mixes to optimize fur pelt quality and to meet the mink's nutritional requirements (Canada Mink Breeder's Association, 2013). Mink in southwest Nova Scotia fur farms may receive nutritional supplements in the form of plant sterols because β-sitosterol has been shown in previous studies to enhance reproductive performance of fur-producing carnivores (Nieminen et al., 2008, 2010; Bin Sayeed et al., 2016). Fish food samples were also high in cholesterol (708 μg/g DW) and β-sitosterol (256 μg/g DW). In the aquaculture effluent, which we collected from an outflow into Hourglass Lake, only cholesterol and stigmastanol were observed in minor amounts above detection limits, making it an unlikely source of excess inputs to Hourglass Lake (Table S2).

3.2 Sediment core profiles

Sedimentary $\delta^{15}N$ is used here as a proxy to identify where excess N inputs from mink farms or aquaculture to a lake occur over time. We also examined sterols to identify potential organic C sources to lakes. Cholesterol and β-sitosterol were the dominant sterols in mink waste and fish feed (Table S2). Notably, a mink processing facility is located in the Nowlans Lake catchment near the shore, while only the mink farms at that site have been cited in previous studies as contributing to excess phosphorus inputs, which may be partly due to inputs of superphosphate, an additive to increase the storage life of mink feed (Brylinsky et al., 2012). Coprostanol and its microbially reduced product in the soil, epicoprostanol, are associated predominately with human sources, while other animals with similar diets and microbiomes produce these biomarkers in comparatively lesser amounts (Cheng et al., 2016, Harrault et al., 2019).

We used stigmastanol in this study as an indicator of plant-sourced inputs including herbivorous faecal sources, plant agricultural activities, or forestry activities (Lerch et al., 2022). We also used VRS-inferred chlorophyll *a* as a proxy of productivity in all study lakes. Our independent paleolimnological proxies in the sediment cores, namely sterol biomarkers, $\delta^{15}N$,

VRS-inferred chlorophyll *a*, and heavy metals, increased further in the 1980s in Nowlans Lake (Figure 2), with the introduction and acceleration of mink farming practices to the southwest region of Nova Scotia in the 1950s – 1980s.

Cholesterol and β-sitosterol, which were the predominant sterols in mink faecal samples, were also the dominant sterols in Nowlans Lake sediment, exceeding 100 µg/g dw in sediments deposited after the mink farming industry was introduced to the region (Figure 2, Table S2). The sterols recorded in mink faecal samples increased coeval with a ~400% increase of the mink farm industry in the region, suggesting a link between mink fur farming and the concentrations of these sterols. Coprostanol and epicoprostanol, traditionally used to indicate the source of impacts from human sewage pollution and also found in minor amounts above detection limits in the mink waste and fish feed we collected (Table S2), were comparatively low or absent in the studied lakes, indicating human waste was not a major source of contamination (Figure 2, Table S2). Lerch et al., (2022) and Bull et al., (1999a), used a sterol ratio (coprostanol+epicoprostanol)/(coprostanol+epicoprostanol+5*a*-cholestanol) to detect human sewage pollution in sediments, where low human faecal input produced a ratio < 0.7, and a ratio of 0.7-1 indicated high human faecal deposition. Nowlans Lake sediments had a ratio ranging from 0.05 (bottom interval, <1980s) to 0.23 (top interval, >1980s). Stigmastanol, representative of herbivorous faeces (Lerch et al., 2022) or other anthropogenic impacts such as silviculture, did not suggest additional sources of inputs; nor did cholestanol, a microbially-reduced product in the soil of cholesterol and b-sitosterol (Björkhem and Gustafsson, 1971, Lerch et al., 2022). We used principal component analysis (PCA) to track the progression of sterol abundance in Nowlans (Figure S1), Placides (Figure S2), and Porcupine (Figure S3), which further corroborated our observations that the most apparent changes in sterol abundance were seen in sediment intervals dated after mink farms were established in Nowlans Lake.

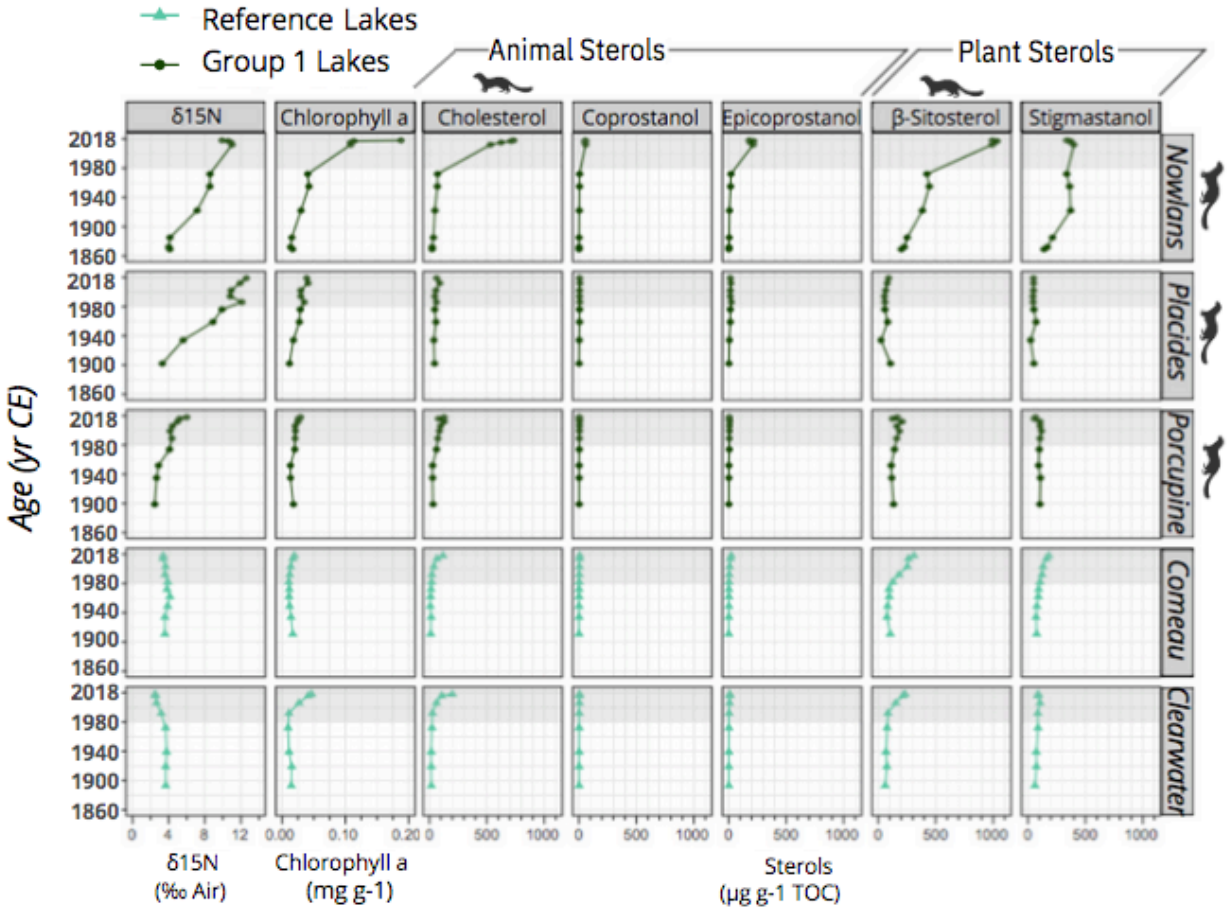

Figure 2: Geochemical proxies relative to sediment age. Group 1 lakes (circles) have mink farms within the lake catchment boundaries (Nowlans, Placides, and Porcupine lakes), and Reference Lakes (triangles) do not have mink farms within, near, or upstream to lake catchment boundaries (Comeau and Clearwater lakes). Sterol concentrations are normalized to total organic content (TOC). Grey shading indicates when the mink farming industry in southwest Nova Scotia experienced a 400% growth between the 1980s to present.

Trends in VRS-inferred sedimentary chlorophyll *a* estimates, which importantly includes both primary chlorophyll *a* and its degradation products (Michelutti et al., 2010, Michelutti and Smol, 2016), increased substantially in Nowlans Lake (bottom chlorophyll *a* = 0.0164 mg g^{-1} DW, top VRS-inferred chlorophyll *a* = 0.1878 mg g^{-1} DW) (Figure 2) when the mink farm industry was introduced to the southwest region, coeval with enrichment of $\delta^{15}N$ and increases in cholesterol, and β-sitosterol (Figure 2). This increase would be expected with enhanced primary production

329 from high trophic level allochthonous inputs (Michelutti et al., 2010, Michelutti and Smol, 2016,
330 Duda et al., 2020, Gallant et al., 2020) and eutrophication (Kalff and Welch, 1974, Schindler et
331 al., 1974, Gallant et al., 2020). By contrast, trends in VRS-inferred chlorophyll *a* concentrations
332 were low or relatively unchanging in reference lakes such as Comeau (bottom VRS-inferred
333 chlorophyll *a* = 0.0167 mg g^{-1} DW, top chlorophyll *a* = 0.0193 mg g^{-1} DW) (Figure 2).
334 Historically, VRS-inferred chlorophyll *a* has been positively correlated with water temperature,
335 nutrients, and cyanobacteria biomass (Paterson et al., 2017). and these data suggest any potential
336 effects related to climate change would have been enhanced at sites with high phosphorus inputs
337 and with evidence of high nutrient influxes ($\delta^{15}N$, cholesterol, β-sitosterol) such as Nowlans
338 Lake.
339 Porcupine Lake (Group 1, Figure 2) also exhibited enriched $\delta^{15}N$ values, especially in
340 recent sediments (>1980). However, VRS-inferred chlorophyll *a* and sterols in Porcupine Lake
341 more closely resemble reference lake conditions. This may be due to the location of mink farms
342 at this lake, which are approximately 3 km from the lake's shoreline. Not surprisingly, some
343 cores (especially from Nowlans) tracked a stronger enrichment pattern than others and results of
344 each lake should be considered within the context of its catchment boundaries, nutrient input
345 sources, associated geographical influences, and chemical transport and taphonomy.
346 For example, the elevated surface (>1980) sedimentary sterols in Nowlans Lake and
347 enriched $\delta^{15}N$ in both Nowlans and Placides lakes (Figure 2, Figure 3) may reflect differences in
348 the respective lacustrine ecosystem and molecular processes between lipid biomarkers and
349 isotopic markers. Placides Lake has more enriched $\delta^{15}N$ values than Nowlans Lake, yet has
350 lower sterol concentrations. This pattern might be explained by the retention of hydrophobic
351 lipids, like sterols, in the catchment of Placides Lake. While sterols can remain stable in
352 sediments for thousands of years or more, hydrophobic lipids do not usually percolate into
353 deeper soils and leachates (Bull et al., 2001, 2002, Lloyd et al., 2012, Lerch et al., 2022) unlike
354 in a low soil pH environment (<4) where some organic matter can become mobile with metal
355 ions through complexation (Lerch et al., 2022). While Nowlans and Placides lakes are similar in
356 lake area (Nowlans 0.74 km^2, Placides 0.81 km^2), Nowlans Lake has a much smaller catchment
357 area (2.56 km^2) than Placides Lake (12.8 km^2), with Placides exhibiting the second largest
358 catchment to lake area ratio (CA:LA) of all studied lakes (Placides CA:LA = 14.9, Nowlans
359 CA:LA = 2.44) (Gregory et al., 2022). In addition, Nowlans is a headwater lake that receives

direct overland run-off from mink farms in the catchment (Brylinksy et al., 2012; Campbell et al., 2021; Jones et al., 2021). The overland run-off is located near a water saturated ravine on the eastern shoreline characterized by high cattail growth and, is also near a high phosphorus stream which originates from near a mink farm facility (Brylinsky et al., 2012). Near the southeastern shoreline, Nowlans Lake also receives inputs directly from a discharge pipe which was observed by authors to appear to source effluent from a mink farm within the catchment (Brylinsky et al., 2012). Similarly, samples taken in the sole stream that flows into Placides Lake were reported to have high water pH, anoxic soils, a strong sulphur smell, and cattail growth cited by authors as likely due to overland run-off from an unidentified source rather than a nearby aquaculture operation at Hourglass Lake (Brylinksky et al., 2012). This supports our finding from the same aquaculture facility's effluent that the effluent is not likely a source of contamination (Table S2). The water sampling sites along the inlet to Placides Lake, taken by Brylinsky et al., (2012), were in the Carleton River Watershed, which contains the highest concentration of mink farms. Therefore, since Brylinsky et al., (2012) found high phosphorus north of Placides Lake along the inlet stream rather than at the shoreline, it is plausible that Placides Lake catchment may be receiving inputs from mink farms in the catchment, retaining hydrophobic lipids like sterols, while releasing more hydrophilic constituents like ammonium (NH_4+) and nitrates (NO_3^{-2}).

We compared $\delta^{15}N$ measurements from our recent surface (post-1980) and bottom sediments (pre-1980, representing the past before mink farming activities were introduced to the region) between all 14 lakes -- with mink farms in the catchment (Group 1: Nowlans, Placides, Porcupine, Hourglass, Oakleaf), outside of the catchment or to receiving waters (Group 2: Fanning, Ogden, Parr, Provost), and reference lakes (Sloans, Comeau, Delaps, Janes, Clearwater). Note that this method doesn't reveal the timing of any changes. The results showed significant differences between recent and past sedimentary $\delta^{15}N$ ($p = <0.01$, based on 2-factor ANOVA) when we examined the effect of pre- and post- 1980 (factor 1) and control versus mink-affected site (factor 2). Recent (>1980) sediment $\delta^{15}N$ values are most enriched in lakes with mink farms in the catchment ~6-13‰ (Group 1), followed by mink farms outside of the catchment,~4-6‰ (Group 2), and lake sediments without sources of nearby anthropogenic inputs are 0-4‰ (Reference group), see Figure 3. Recent sediments (>1980) from Nowlans Lake have the most enriched $\delta^{15}N$ values coeval with mink-related sterols (cholesterol and β-sitosterol). Comparatively, Placides Lake has the highest $\delta^{15}N$ of all study lakes but low sterol

concentrations, likely due to geochemical taphonomy, catchment geography, and proximity of mink farms to the catchment as discussed above.

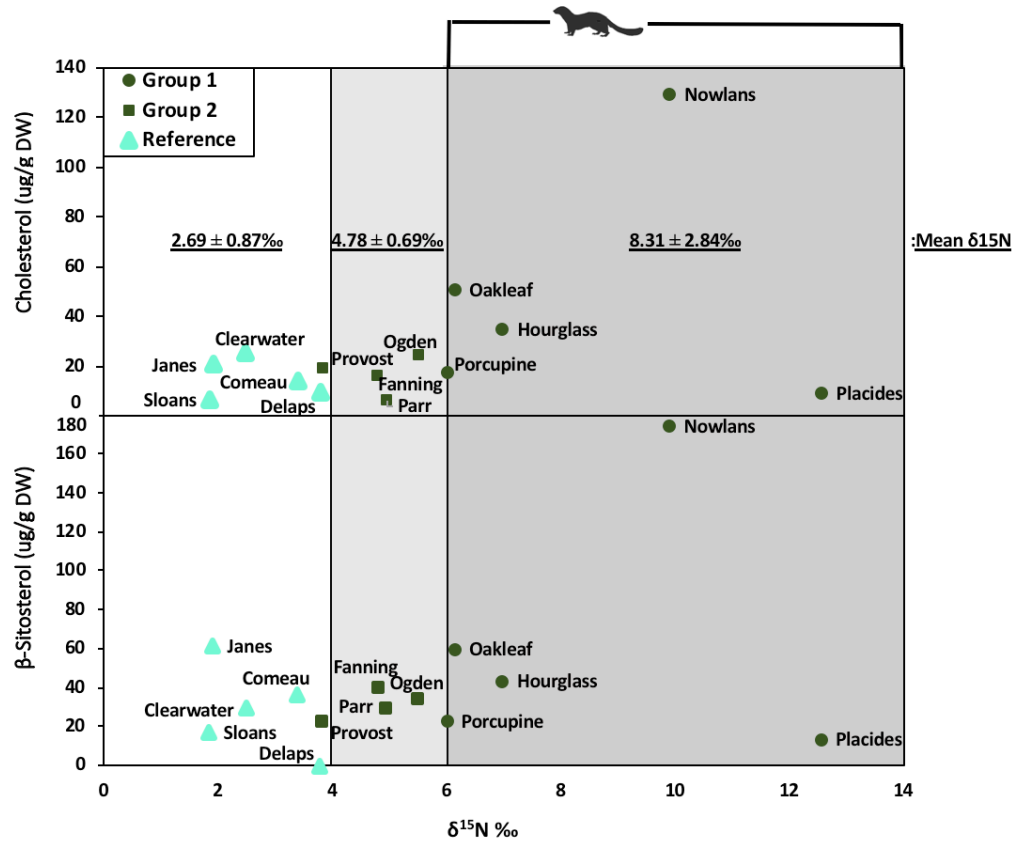

Figure 3: Surface sediment (>1980) samples of sterols representing mink inputs (cholesterol and β-sitosterol) and surface sediment $\delta^{15}N$. Group 1 represents lakes with mink farms in the catchment post-1980 (far right), Group 2 represents lakes with mink farms outside the catchment (middle) post-1980, and reference conditions post-1980 sediments (far left).

Before the 1980s, sedimentary $\delta^{15}N$ values were relatively low (<4 ‰) in all lakes (Group 1, 3.03 ± 0.74‰; Group 2, 3.27 ± 0.81‰; Reference, 3.36 ± 0.88‰). These low $\delta^{15}N$ values from the bottom sediment intervals suggest N sources prior to fur farming (pre-1980) were comparable to reference lake conditions through time. Surface $\delta^{15}N$ sediments (post-1980) were elevated in lakes with mink farms in the catchment (8.31 ± 2.84‰), and in lakes receiving indirect inputs from mink farms (4.78 ± 0.69‰) relative to sediments from reference lakes (2.69 ± 0.87‰). Notably, deforestation activities and increased residential development, which may also impact Group 2 and reference lakes, have been shown to result in enrichment of sedimentary $\delta^{15}N$ by up to 1-3‰ and 2-3‰, respectively, from pre disturbance conditions (Dunnington et al.,

2018). Compared to lakes downstream from a catchment with mink farms and the reference lakes, the effect of enriched $\delta^{15}N$ coeval with increased cholesterol and β-sitosterol is most pronounced in Nowlans Lake (Figure 2), which is currently hypereutrophic and contains the most mink farms near its shoreline and in the catchment (Taylor, 2010; Brylinsky, 2012; Stantec, 2017).

Mink pelt production in southwest Nova Scotia grew by nearly 400% between 1980 and 2014 (Statistics Canada, 2019, 2021, Gregory et al., 2022). Sedimentary $\delta^{15}N$ in the Nowlans Lake core began to increase in the 1950s -1980s during introduction of mink farming to southwest Nova Scotia, and peaked at 12.3‰ between 2001-2006. A depletion of $\delta^{15}N$ values in Nowlans Lake's surface sediment beginning in 2009 (Figure 2, Table S2) may have been due to a rise in nitrogen fixation related to cyanobacteria blooms. In 2009, cyanobacteria blooms were reported at Nowlans Lake exceeding the Health Canada GCRWQ value of 100,000 cells/mL (Taylor, 2010, Stantec Consulting Ltd., 2017), cited as possibly due to nutrient inputs from the nearby mink farms and a mink food processing facility, and in response to excess phosphorus inputs (Brenner et al., 1999, Taylor, 2010, Brylinsky, 2012, Torres et al., 2012, Brylinsky and Sollows, 2014). Some cyanobacteria fix atmospheric N_2, utilizing atmospheric ^{14}N as the primary nitrogen source (atmospheric $^{15}N = 0.4‰$), which would then be incorporated into the sediments upon decomposition, bringing bulk sedimentary $\delta^{15}N$ closer to atmospheric levels as indicated in previous studies (Das et al., 2007, Riedinger-Whitmore et al., 2005, Rosenmeier et al., 2004, Gu, 2009, Gu et al., 1996). Another possible explanation for the depletion of $\delta^{15}N$ in surface sediments is reduced mink waste into Nowlans Lake due to preparation for stricter mink waste regulations enacted in 2013 for the first time in Nova Scotia's history (Fur Industry Act, 2013). However, mink-related sterols (cholesterol, β-sitosterol) in Nowlans Lake continued to increase from 1980 (36.25 cm, cholesterol = 10.32 µg/g DW, sitosterol = 56.55 µg/g DW) until present day (0.25 cm, cholesterol = 129.06 µg/g DW, β-sitosterol = 174.54 µg/g DW), see Figure 2, which suggests longer-term continuous inputs into the lake. Other natural processes may likely contribute to $\delta^{15}N$ including high N availability allowing for preferential discrimination against the ^{15}N isotopes by aquatic plants (Fogal and Cinfuentes, 1993, Torres et al., 2012).

We also analyzed sedimentary and mink faecal samples for concentrations of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), strontium (Sr), titanium (Ti), zinc (Zn) and developed biogenic enrichment factors to identify which elements are more likely

to be enriched by mink farming practices, as expressed by enrichment factors >1 μg/g dw (Brimble et al., 2009) (Table S3). Enrichment factors of <1 indicate environmental background levels, while 1 to <3 indicate minor enrichment, and between 10-25 indicate significant enrichment of sediments (Zahra et al., 2014). Biogenic enrichment factors were developed for Nowlans, Placides, Porcupine, Clearwater, and Comeau sediments and As (1.26 μg/g dw), Cu (2.81 μg/g dw), Sr (20.09 μg/g dw) were determined to be enriched by mink waste (Table S3). These elements were above background levels in the natural ecosystem and were the most likely to undergo bioenrichment in response to direct inputs from mink farming practices. Increased concentrations of As, Cu, and Sr are observed starting in the 1980s and are most apparent in Nowlans Lake, the lake with the most direct input from mink farming activities suggesting that mink farms act a source for these elements to aquatic ecosystems (Figure 4). Other metals like Cd and Ni also increased to a lesser extent in sediments corresponding to the 1980s, though these may be unrelated to mink farming (Table S4).

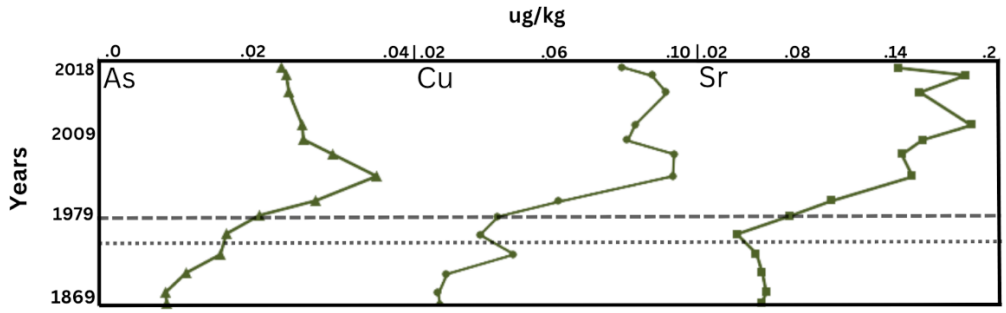

Figure 4: Nowlans Lake profile of paleolimnological proxies from ca. 1869-2018, concentrations of As, Cu, and Sr heavy metals normalized by titanium. As/Ti (left, triangles), Cu/Ti (middle, circles), (C) Sr/Ti (right, squares). Dashed line indicates ca.1980 to present day; dotted line and above represents introduction of the first mink farms to the region around the 1950s; ca. 1869 to the dotted line represent reference baseline conditions.

Heavy metals occur naturally in bedrock and make their way into the soil, sediment, and biota through erosion but delivery to lakes can be accelerated due to anthropogenic influences including agricultural and industrial activities (Bilali et al., 2002, Yang et al., 2009, Gallant et al., 2020), and high trophic level inputs (Duda et al., 2020, Brimble et al., 2009). For example, Ni can be naturally high in background sediments and it can also be deposited into lacustrine ecosystems through industrial processes and waste disposal (Lin et al., 2018). Cd can occur in elevated concentrations near animal or plant farming activities (Zhang et al., 2019) and Cu has

been found to have high enrichment from farming wastewater (Yang et al., 2009) This highlights the importance of a before-after control-impact (BACI) approach to studying historical water pollution. While we acknowledge other forms of agriculture as a potential source of contamination to these lakes, we focused our study design on mink farms. Our results show that sterols enriched in mink waste (cholesterol and β-sitosterol), $\delta^{15}N$, chlorophyll *a*, and heavy metals As, Cu, and Sr in our Nowlans Lake sediment core track one another through the sediment core record (Figure 5), and became proportionately enriched coeval with a 400% expansion of mink farms in the 1980s (Statistics Canada, 2019, 2021, Gregory et al., 2022).

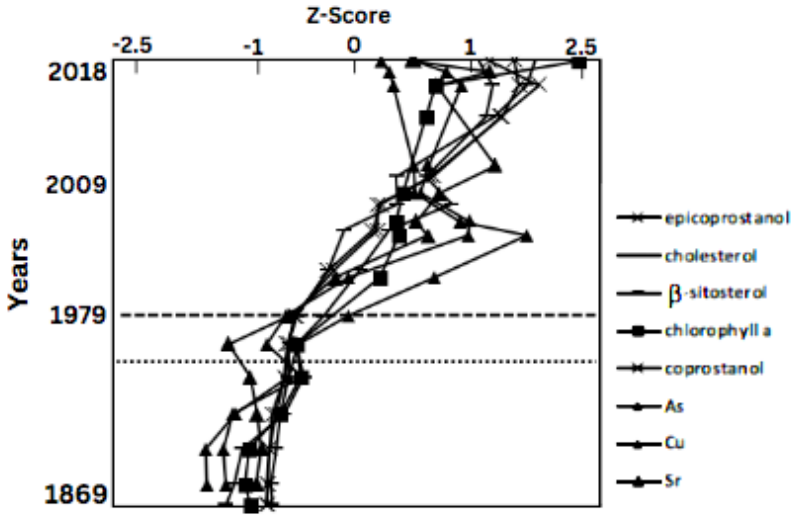

Figure 5: Nowlans Lake profile of paleolimnological proxies from ca.1869 - 2018. Z-scores of heavy metals, sterols, $\delta^{15}N$, and chlorophyll *a*. Dashed line indicates ca. 1980 to present day; dotted line and above represents introduction of the first mink farms to the region around the 1950s; ca. 1869 to the dotted line represent reference baseline conditions.

Conclusion

We used forensic paleolimnology to track sources of water pollution and eutrophication in southwest Nova Scotia lakes central to a pollution debate surrounding waste management from fur farms. Mink-related sterols (cholesterol, β-sitosterol), $\delta^{15}N$, VRS chlorophyll *a*, and heavy

metals As, Cu, Sr increased in the 1980s in Nowlans Lake, the lake with the highest concentration of mink farms in the catchment and near its shoreline, coeval with a ~400% increase of mink farms to the region between 1980 and 2014 (Statistics Canada, 2019, 2021, Gregory et al., 2022). In contrast, impacts were more subtle in other lakes. Catchment size, catchment geography, proximity of contamination sources to the shoreline and receiving waters, and chemical taphonomy appear to play an important role. Our approach expands the applications of forensic paleolimnology and quantifies multiple independent lines of geochemical evidence to determine the source of water pollution in a freshwater ecosystem. Our geochemical toolbox is applicable to other ecosystems facing similar water quality or waste management issues and offers a new perspective for long-term evidence-based environmental monitoring and source tracking, particularly when addressing questions of an environmental forensic nature.

Acknowledgements

We thank our citizen scientists, partners, and local stakeholders including the Environment and Climate Change Canada [ECCC], Tusket River Environmental Protection Association (TREPA), and Mink Farmer's Association (MFA) for invaluable in-kind contributions of monitoring data, logistical support in the field, and continued feedback. We thank Joëlie Lecompte, Kieran Wilkins, and Tatiana Kozbenko for their hard work in the laboratory. Julia Campbell and Abbie Gail Jones, researchers working under this grant number, were instrumental to the study and fieldwork. This study was funded by NSERC-Strategic Partnership Grant (Grant# 401190196; to JPS, JK, JMB).

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