

Calibration and Application of Biomarkers in Sediment to Track Paleoclimate and Large-scale Migratory Animal Population Dynamics

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ABSTRACT

The primary objective of this thesis was to develop and apply lipid biomarker and isotope-based methods for reconstructing large-scale migratory animal populations and climatic histories in western North America from lake sediments. Specifically, this thesis aimed to (1) develop sterol and stanol biomarkers as quantitative proxies for Sockeye salmon escapement alongside the traditional lake sediment proxies ($\delta^{15}\text{N}$); (2) utilize sterols, stanols, and $\delta^{15}\text{N}$ to track changes on large cormorant colonies on Lake Ontario Islands; (3) develop and validate lake-specific branched glycerol dialkyl glycerol tetraether (brGDGT) calibrations to refine paleotemperature estimates for Cultus Lake, British Columbia (BC); and (4) to reconstruct past temperatures using lake-specific brGDGT calibrations and assess their relationship with large-scale climate oscillations (Pacific Decadal Oscillation (PDO) and El Niño Southern Oscillation (ENSO)). I will then integrate these results with salmon escapement records and sedimentary $\delta^{15}\text{N}$ dynamics from dated Cultus Lake sediment cores to evaluate how climate variability has influenced long-term salmon production. In Chapter Two, I applied sterol, stanols, and $\delta^{15}\text{N}$ markers in sediment to track a sharp rise in cormorant population on Lake Ontario Islands. In Chapter Three, I developed novel sterol indices that strongly correlated with historical Sockeye salmon returns record in Alaska and BC, providing robust tools for reconstructing long-term Sockeye salmon population dynamics. In Chapter Four, I calibrated brGDGT distributions in soils, sediments, and water column samples from Cultus Lake, BC, establishing the first lake-specific temperature transfer functions in coastal BC. Chapter Five integrated lake-specific brGDGT-based temperature reconstructions with isotope ($\delta^{15}\text{N}$) based salmon proxies, revealing linkages between large-scale climate systems such as PDO, and ENSO variability, regional climate forcing, and salmon escapement trends over the past century. Together, these chapters demonstrated that lipid biomarkers and isotopes preserved in sediments provide high-resolution ecological and climatic archives, offering tools to disentangle the effects of climate oscillations and anthropogenic pressures on migratory animal populations and freshwater ecosystems. The overall conclusions of this thesis are: (1) sterol and stanol biomarkers provide reliable, independent proxies of Sockeye salmon escapement and can be used alongside traditional salmon proxies; (2) sterol, stanols, and $\delta^{15}\text{N}$ signatures enabled the reconstruction of cormorant population and their ecosystem impacts from pond sediments; (3) lake-specific brGDGT

calibrations significantly improved the accuracy and ecological relevance of paleotemperature estimates at Cultus lake; and (4) integrating biomarkers, isotopes, and climate indices strengthens our ability to assess the coupled dynamics of climate variability, nutrient cycling, and salmon populations across timescales.

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DEDICATION

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LIST OF ABBREVIATIONS

ABA	Acid-Base-Acid pretreatment
AIC	Akaike Information Criterion
AMS	Accelerator Mass Spectrometry
APCI	Atmospheric Pressure Chemical Ionization
ASE	Accelerated Solvent Extractor
BC	British Columbia
BrGDGTs	Branched Glycerol Dialkyl Glycerol Tetraethers
CBT	Cyclization of Branched Tetraethers index
CI	Calf Island
CLSRL	Cultus Lake Salmon Research Laboratory
C/N	Carbon-to-Nitrogen ratio
CRS	Constant Rate of Supply (^{210}Pb dating model)
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DFO	Department of Fisheries and Oceans Canada
DO	Dissolved Oxygen
EA-IRMS	Elemental Analyzer-Isotope Ratio Mass Spectrometer
EBI	East Brother Island
EBSI	Expanded Bird Sterol Index
ENSO	El Niño-Southern Oscillation
ESI	Expanded Sterol Index
FDR	False Discovery Rate

GAM	Generalized Additive Model
GC-MS	Gas Chromatography-Mass Spectrometry
GDGTs	Glycerol Dialkyl Glycerol Tetraethers
HC3	Heteroskedasticity-Consistent Covariance Matrix Estimator (Type 3)
Hex	Hexane
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High-Performance Liquid Chromatography
IDL	Instrument Detection Limit
IPA	Isopropanol
IRMS	Isotope Ratio Mass Spectrometer
LC-MS	Liquid Chromatography-Mass Spectrometry
LOI	Loss on Ignition
LOOCV	Leave-One-Out Cross-Validation
MAAT	Mean Annual Air Temperature
MAF	Months Above Freezing
MBT	Methylation of Branched Tetraethers index
MBT' _{5ME}	Methylation of Branched Tetraethers index based on 5-methyl
MCMC	Markov Chain Monte Carlo
MDL	Method Detection Limit
MeOH	Methanol
MLR	Multiple Linear Regression
NCEI	National Centers for Environmental Information
NPGO	North Pacific Gyre Oscillation
PCBs	Polychlorinated Biphenyls
PCoA	Principal Coordinates Analysis
PDO	Pacific Decadal Oscillation

PEARL	Palaeoecological Environmental Assessment and Research Laboratory
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Analysis of Multivariate Dispersion
PLS	Partial Least Squares
REML	Restricted Maximum Likelihood
RMSE	Root Mean Square Error
SEE	Standard Error of Estimate
SIMPER	Similarity Percentage Analysis
SPM	Suspended Particulate Matter
SST	Sea Surface Temperature
SSI	Salmon Sterol Index
SS Ia	Salmon Sterol Index a
SS Ib	Salmon Sterol Index b
SS Ic	Salmon Sterol Index c
SS Id	Salmon Sterol Index d
SS Ie	Salmon Sterol Index e
SS If	Salmon Sterol Index f
SS Ig	Salmon Sterol Index g
TLE	Total Lipid Extract
UPLC	Ultra-Performance Liquid Chromatography
VIP	Variable Importance in Projection

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PREFACE

This thesis is presented as a collection of manuscripts and is formatted in accordance with the guidelines of the University of Ottawa's Faculty of Graduate and Postdoctoral Studies. Chapter One provides a general introduction that outlines the background, scientific context, and objectives of the research. Chapter Two is presented as a manuscript prepared for publication in *Palaeogeography, Palaeoclimatology, Palaeoecology*. Chapter Three is presented as a manuscript prepared for publication in the *Journal of Geophysical Research: Biogeosciences*. Chapters Four and Five are presented as manuscripts prepared for publication in *Organic Geochemistry*. The final chapter, Chapter Six, integrates the main findings from the preceding chapters and presents the overall conclusions of the thesis, as well as the broader implications of the research.

1 CHAPTER 1: General Introduction

1.0 Large Migratory Animals and Ecosystem Dynamics

Large migratory animals are fundamental components of global ecosystems because they connect distant habitats and regulate ecological processes across broad spatial and temporal scales. Every year, vast numbers of animals embark on long and challenging migrations in search of richer feeding areas, favourable climates, suitable breeding grounds, and a safer environment for survival (Alerstam et al., 2003; Bauer & Hoyer, 2014).

By feeding, growing, and accumulating resources in one environment and moving to another, they redistribute nutrients, energy, and biomass across ecosystem boundaries (Bauer & Hoyer, 2014; Bilby et al., 2003). In doing so, they act as critical agents of connectivity, creating nutrient hotspots that enhance productivity and shape the structure and functioning of both terrestrial and aquatic systems (James & Naiman, 2001). Their migrations also influence food-web dynamics by mediating predator-prey interactions, supporting higher trophic levels, and enhancing ecosystem resilience under changing environmental conditions (Michelutti et al. 2009, Moore et al., 2004).

Importantly, the ecological contributions of migratory animals are not short-lived. Because of their large scale and ecological influence, fluctuations in the abundance or behaviour of these animals reverberate throughout entire ecosystems (Caut et al., 2012; Finney et al., 2000, 2002). Their seasonal or cyclical returns leave lasting biogeochemical imprints on nutrient budgets, food-web stability, and long-term ecosystem trajectories that can be detected in soils and sediments (Brimble et. al., 2009; Finney et al., 2000, 2002). Population declines, whether caused by anthropogenic pressures such as overharvesting and habitat degradation or by natural climate variability, disrupt nutrient pathways and alter ecosystem function (Benkwitt et al., 2022; Mantua et al., 1997). Conversely, periods of high abundance amplify cross-ecosystem connectivity and productivity, with cascading effects on biodiversity and ecological services. Understanding the population dynamics of large migratory animals requires attention to both present conditions and long-term histories, extending beyond the limits of instrumental records. Reconstructing these historical trajectories provides critical baselines for assessing how populations have responded to past climatic variability and human pressures, how current

abundances compare with natural baselines, and how future trajectories may unfold under ongoing environmental change (Finney et al., 2000, 2002). As keystone connectors, migratory animals offer a powerful lens through which to examine the links between biodiversity, ecosystem function, and climate variability across timescales, thereby strengthening our ability to connect past population dynamics with present challenges and anticipate future ecosystem responses.

Within this framework, freshwater birds such as cormorants and Pacific salmon serve as focal examples. Their migrations redistribute nutrients across ecosystem boundaries and leave distinctive molecular and isotopic signatures preserved in lake sediments. Reconstructing these signatures provides a unique opportunity to trace long-term population dynamics beyond the limits of observational records and to evaluate how climate variability and human activities have shaped their trajectories. The following subsections examine cormorants and Pacific salmon in detail, outlining their ecological roles, population histories, and the use of sedimentary archives to track past population dynamics.

2.0 Double-crested Cormorant Ecology and Foraging Behaviour

The Double-crested cormorant (*Phalacrocorax auritus*) is a piscivorous freshwater bird that is widely distributed across North America (Devault et al., 2012). Acting as a biovector, it plays a critical role in linking aquatic and terrestrial nutrient cycles through its foraging and nesting activities, thereby influencing ecosystem structure and function. Cormorants typically breed in large colonies on islands near freshwater bodies that provide access to fish-based diets (McDonald et al., 2018). They are proficient diving predators capable of capturing a wide range of fish species like the three-spined stickleback (*Gasterosteus aculeatus*), alewife (*Alosa pseudoharengus*), and yellow perch (*Perca flavescens*) from nearshore and pelagic zones (DeBruyne et al., 2013). Cormorants exhibit flexible feeding strategies, alternating between solitary pursuit and cooperative foraging depending on prey density and habitat conditions. This behavioural versatility allows them to exploit spatially and temporally variable prey resources and adapt to shifting environmental conditions (DeBruyne et al., 2013).

2.1 Double-crested Cormorant Nutrient Transfer and Ecosystem Effects

Cormorants function as a major biogeochemical vector, facilitating the transfer of nutrients, organic matter, and trace elements across ecosystem boundaries. Through their feeding and nesting activities, cormorants transport aquatic-derived materials to terrestrial and nearshore environments, profoundly altering nutrient budgets and ecological processes (Brimble et al., 2009). During the breeding season, adults make repeated foraging trips between aquatic feeding grounds and nesting colonies, where they deposit guano, regurgitated fish remains, and carcasses. These inputs represent concentrated fluxes of bioavailable nitrogen (N), phosphorus (P), and organic carbon (C), often exceeding natural background nutrient deposition by several orders of magnitude (Duda et al., 2021; Hargan et al., 2017).

Cormorant guano is exceptionally rich in uric acid, ammonia, and phosphate minerals, which are readily solubilized during rainfall events. The leaching of these compounds into surrounding soils and waters elevates nutrient concentrations, stimulating algal and microbial productivity (Hargan et al., 2017; 2018). In aquatic systems, such enrichment can enhance primary production, alter phytoplankton community composition, and drive eutrophic conditions. Sedimentary records from colony-impacted sites frequently capture these effects, exhibiting $\delta^{15}\text{N}$ enrichment and chemical signatures that persist for extended periods (Hargan et al., 2018).

The ecological consequences of this nutrient transfer are dual in nature. On one hand, guano deposition creates nutrient-rich “hotspots” that enhance local productivity and sustain diverse microbial and algal assemblages. On the other hand, excessive accumulation of ammonia-rich guano becomes toxic to the vegetation (Boutin et al., 2011). Guano toxicity, coupled with the peeling of leaves for nest making, causes severe vegetation dieback at nesting sites (Boutin et al., 2011). The loss of vegetative cover further accelerates erosion and runoff, enhancing nutrient delivery to nearby aquatic systems.

Lake sediments near colony nesting sites preserve long-term records of nutrient transfer and ecological change driven by recurring cormorant colony activity and management interventions (Hargan et al., 2018). Integrating these archives with modern ecological data enhances understanding of cormorant population dynamics and how cormorant nutrient inputs shape lake and island ecosystems through time.

3.0 Pacific Salmon Ecology in North America

Pacific salmon (*Oncorhynchus* spp.) are among the most ecologically, culturally, and economically significant fish in the North Pacific region. Renowned for their remarkable life cycles and ecological influence, these anadromous species migrate between marine and freshwater environments, linking marine and terrestrial environments through the transfer of nutrients and energy (Schindler et al., 2003). Their endurance, ecological adaptability, and biological complexity have made them enduring symbols of resilience in the dynamic landscapes of western North America (Reid et al., 2022). The genus *Oncorhynchus* comprises five principal species native to North America, including Chinook (*O. tshawytscha*), Coho (*O. kisutch*), Chum (*O. keta*), Pink (*O. gorbuscha*), and Sockeye (*O. nerka*) (Pitman et al., 2020). Sockeye salmon exhibits unique life history traits among Pacific salmon species, relying on lake systems as essential nursery habitats during their juvenile rearing phase.

3.1 Sockeye Salmon Spawning Migration

Sockeye salmon migration represents one of the most extraordinary biological phenomena in nature. After spending one to four years in the ocean, millions of Sockeye salmon navigate thousands of kilometres back to their natal lake, guided by complex environmental cues such as geomagnetic fields and olfactory imprinting (Putman et al., 2014; Ueda et al., 1998). Spawning migration occurs from July to January, but most frequently in August and September. Sockeye salmon spawn by depositing their eggs in gravel beds along stream channels. The female salmon creates a shallow depression, known as a redd, by turning onto her side and rapidly fanning her tail to dislodge fine sediments and expose clean substrate. These redds typically form in areas of moderate flow where turbulence and hyporheic exchange enhance oxygen delivery to the developing embryos. Redds can extend to depths of up to half a metre, depending on gravel composition and local hydraulics. As the female releases her eggs, one or more males concurrently discharge milt to fertilize them. Upon completion of spawning, the female covers the eggs with a layer of gravel to reduce predation risk and prevent siltation. A single female may construct multiple redds before completing her reproductive effort. Following spawning, these millions of male and female salmon undergo severe physiological stress and senescence and die shortly after. Their decomposing carcasses contribute marine-derived

nutrients to the freshwater environment, supporting benthic invertebrates, microbial communities, and riparian productivity (Bilbby et al., 1996, 2003).

3.2 Early Life Stages and Migration

After fertilization, salmon eggs remain buried within gravel nests (redds), protected from direct predation but still vulnerable to environmental disturbance. Their survival depends on the stability of streambed conditions (Levasseur et al., 2006). Eggs may be displaced or suffocated if later-spawning salmon disturb the redds, if fine sediments clog pore spaces, or if flooding and freezing events alter water flow (Zimmermann & Lapointe, 2005). The gravel layer provides both physical shelter and a steady supply of oxygen-rich water, essential for embryonic respiration throughout the winter incubation period. By late winter, the developing embryos hatch into alevins, small translucent fish with large eyes and a prominent yolk sac that supplies nutrients during the early stages of life. Still residing within the gravel, these larvae rely on the protective environment of the redd until the yolk is absorbed. When spring arrives and water temperatures rise, the young fish, now called fry, emerge from the gravel and begin active feeding in the stream or littoral zones of lakes.

Sockeye fry typically stay in their nursery lake for about 1 to 2 years, where they feed on zooplankton and small aquatic invertebrates before migrating to the ocean. As juveniles prepare for ocean entry, they undergo smoltification, a complex physiological transition that enables them to survive in saltwater. During this transformation, the fish develop silvery scales that serve as camouflage against aquatic predators and as partial protection from changing salinity. Smolts migrate downstream toward estuaries in spring, guided by rising discharge and temperature cues (Clark et al., 2016). Many pause in the brackish waters of river mouths, where they gradually acclimate to higher salinity levels and take advantage of nutrient-rich estuarine feeding grounds before continuing along coastal migration routes (Clark et al., 2016).

3.3 Distribution and Ocean Migration

Sockeye salmon exhibit one of the most extensive oceanic distributions among Pacific salmon species, spanning from the coastal waters of North America to those of eastern Asia across the North Pacific Ocean. During this marine phase, immature individuals occupy productive offshore regions such as the Gulf of Alaska and the Bering Sea, where nutrient-rich

upwelling zones support rapid growth and energy accumulation (Habicht et al., 2010). Sockeye salmon primarily feed on planktonic crustaceans such as copepods and euphausiids, as well as small forage fish. This pelagic diet supports the accumulation of substantial lipid and protein reserves essential for reproduction and survival during their subsequent freshwater migration. After spending one to four years in the ocean, millions of Sockeye salmon are guided by a combination of environmental cues and geomagnetic imprinting to navigate to their natal waters for spawning (Putman et al., 2014). The return of millions of salmon to their natal freshwater systems represents major ecological feedback between marine and terrestrial environments. Following spawning, the mass mortality of adult salmon results in the deposition of nutrient-rich carcasses, which decompose and release essential elements such as nitrogen, phosphorus, and carbon into lake and stream ecosystems. These marine-derived nutrients become incorporated into sediments, serving as lasting geochemical and molecular fingerprints of salmon presence. The magnitude and variability of these sedimentary signals can therefore provide an understanding of historical salmon population dynamics.

3.4 Decline in Sockeye Salmon Populations

Sockeye salmon have experienced substantial population declines across much of their historical range over the past century, reflecting the cumulative impact of human activities and environmental change (Finney et al., 2000, 2002; Mantua et al., 1997; Mantua & Hare, 2002). Once abundant in the rivers and coastal waters of British Columbia, Alaska, and the U.S. Pacific Northwest, many populations are now reduced or locally extirpated. The factors responsible for this decline are multifaceted, encompassing habitat degradation, overexploitation, and the increasing impact of climate-driven stressors. Together, these pressures have altered migration routes, disrupted spawning success, and reduced the overall resilience of Sockeye salmon populations.

3.4.1 Habitat Alteration and Fragmentation

Habitat alteration and fragmentation have exerted profound and long-lasting effects on salmon production due to their complex anadromous life cycle and reliance on diverse, interconnected freshwater and marine habitats. Each stage of the salmon life cycle, spawning, incubation, rearing, migration, and oceanic maturation, depends on specific environmental conditions, making these species highly sensitive to both physical and climatic disturbances (Healey, 2011).

Human-induced landscape modifications such as dam construction, channelization, deforestation, and urban development have altered natural hydrological regimes, degraded spawning substrates, and reduced habitat connectivity. Barriers such as dams and culverts fragment river networks, preventing salmon from reaching historical spawning and rearing areas that are essential for sustaining viable populations (Finn et al., 2021; Pess et al., 2008). The resulting loss of access to natal habitats reduces gene flow, impairs recolonization, and diminishes the resilience of regional metapopulations (Finn et al., 2021).

One of the most dramatic historical examples of physical habitat disruption is the Hells Gate landslide in the Fraser River Valley, British Columbia, in 1914 (Pess et al., 2012). Triggered by railroad construction, the massive rockfall constricted the river channel, drastically increasing flow velocity and creating a physical barrier to salmon migration. This event severely impeded the passage of migrating Sockeye and other salmon species to their spawning grounds, leading to catastrophic population declines that persisted for decades (Pess et al., 2012). Despite subsequent engineering efforts, including fishways constructed in the 1940s, the Hells Gate slide stands as a pivotal reminder of how human activities can irreversibly alter key migration corridors and disrupt population recovery.

Beyond direct blockages, habitat degradation from logging, mining, and agriculture continues to reduce the quality and function of salmon habitats. Deforestation destabilizes riparian zones, increases sedimentation, and elevates stream temperature conditions that smother spawning gravels and impair egg incubation. Agricultural runoff introduces excessive nutrients, pesticides, and sediments that degrade water quality, while mining activities contribute heavy metal contamination and acidification. Estuarine and coastal modifications from aquaculture and dredging further impact juvenile salmon by disturbing critical nursery habitats necessary for smolt survival and physiological transition to saltwater (Simenstad and Fresh, 1995).

The ecological repercussions of habitat alteration extend beyond physical habitat loss. Spawning salmon deliver substantial marine-derived nutrients to freshwater systems through carcass decomposition, enriching benthic and riparian food webs. When spawning sites are destroyed or rendered inaccessible, this nutrient feedback diminishes, reducing productivity and biodiversity in both aquatic and terrestrial ecosystems. Consequently, disruptions in spawning and migration cycles reverberate across trophic levels, weakening ecosystem resilience and altering long-term nutrient cycling dynamics.

3.4.2 Overexploitation and Harvest Pressure

The overexploitation of Pacific salmon across North America represents one of the most consequential examples of human-induced resource decline in modern ecological history. For millennia, Indigenous nations along the Pacific coast from California to Alaska and across British Columbia managed salmon harvests through culturally embedded governance systems that emphasized balance, restraint, and ecological reciprocity (Atlas et al., 2021; Carothers et al., 2021). Traditional practices such as selective weir fishing, seasonal closures, and community-based stewardship ensured the maintenance of salmon diversity and population stability across river systems. These Indigenous frameworks sustained both human livelihoods and ecological productivity by aligning harvest intensity with natural salmon abundance cycles (Atlas et al., 2021).

The arrival of European settlers in the late 18th and early 19th centuries marked a turning point in the exploitation of salmon resources. Colonial expansion, industrialization, and the introduction of commercial canneries transformed salmon from a locally managed subsistence resource into a globally traded commodity. By the mid-1800s, large-scale canning operations along the Fraser, Skeena, and Columbia Rivers had intensified harvest pressure beyond sustainable limits. The early 20th century witnessed steep declines in key runs, particularly Sockeye in the Fraser River, attributable to overharvesting combined with habitat disturbance from industrial development. In this period, technological advances in fishing methods, including gillnets, purse seines, and trolling gear, greatly increased harvest efficiency, but often at the expense of ecological sustainability. The expansion of ocean fisheries compounded pressures on declining riverine populations, as returning adults were intercepted before reaching their natal

spawning grounds. These practices disrupted the natural variability in escapement, undermining population resilience and genetic diversity.

Efforts to mitigate overexploitation have evolved through progressive policy frameworks emphasizing ecosystem-based and conservation-first management. In Canada, the Wild Salmon Policy (2005) formalized a shift toward protecting genetic diversity, life-history variation, and habitat integrity for Pacific salmon. Similarly, contemporary management employs advanced molecular and statistical tools such as genetic stock identification and parentage-based tagging to refine stock assessments and regulate harvest more precisely (Beacham et al., 2018; Beacham et al., 2021). However, despite these advances, historical overfishing has left a legacy of depleted populations, altered age structures, and reduced resilience to environmental stressors.

3.4.3 Pacific Decadal Oscillation (PDO), El Niño Southern Oscillation (ENSO), and Oceanographic Shifts Affecting Salmon Productivity

The productivity and distribution of Pacific salmon across North America are profoundly shaped by large-scale climatic and oceanographic oscillations, particularly the Pacific Decadal Oscillation (PDO) and the El Niño Southern Oscillation (ENSO). These long- and short-term climate systems modulate ocean temperatures, circulation patterns, and nutrient availability factors that collectively determine prey abundance, juvenile survival, recruitment success, and salmon catch (Mantua et al., 1997; Mantua & Hare, 2002). Because salmon integrate these oceanic signals throughout their marine life stages, variability in these climate systems directly influences population trajectories, migration patterns, and long-term productivity trends (Mantua et al., 1997; Mantua & Hare, 2002).

The PDO represents a multi-decadal pattern of sea surface temperature (SST) anomalies in the North Pacific, alternating between warm (positive) and cool (negative) phases roughly every 20 to 30 years (Mantua et al., 1997; Beamish et al., 1997; Mantua & Hare, 2002). During warm PDO phases, SSTs in the eastern and central North Pacific are elevated, accompanied by weakened upwelling and reduced nutrient supply along the North American coast. Conversely, cool PDO phases are characterized by enhanced upwelling and colder, more productive coastal waters. These alternating climatic regimes exert contrasting influences on salmon populations across North America. Mantua et al. (1997) demonstrated, based on long-term catch records, that Alaskan salmon stocks typically thrive during warm phases of the PDO when marine

productivity is enhanced, whereas populations in BC stocks tend to decline. Conversely, during cool PDO phases, BC stocks generally experience increased abundance, while Alaskan populations decline (Mantua et al., 1997; Schindler et al., 2008).

Superimposed on the PDO, the ENSO introduces shorter-term (2 - 7 year) variability that also shapes salmon productivity through its effects on ocean temperature, stratification, and upwelling intensity. El Niño events, characterized by warmer SSTs in the equatorial Pacific, typically lead to reduced nutrient availability and diminished plankton productivity in the northeast Pacific, thereby constraining salmon feeding opportunities and reducing marine survival (Zhang et al., 1997). In contrast, La Niña events, with cooler SSTs and stronger upwelling, often enhance ocean productivity and improve juvenile growth and survival during early marine residence (Zhang et al., 1997). The interaction between ENSO and PDO phases can amplify or dampen these effects. For example, a warm-phase PDO coinciding with El Niño tends to exacerbate negative impacts on BC salmon productivity, while a cool-phase PDO coupled with La Niña can yield exceptional survival conditions (Mantua et al., 1997; Mantua & Hare, 2002; Zhang et al., 1997).

Beyond PDO and ENSO, broader oceanographic shifts, such as the North Pacific Gyre Oscillation (NPGO) and variations in the North Pacific Current, further modulate salmon productivity by influencing ocean circulation and nutrient transport (Malick et al., 2016). A northward-shifted current bifurcation and strengthened NPGO are associated with increased primary productivity and improved survival for salmon originating from Washington and BC (Malick et al., 2016). These circulation changes influence the availability of planktonic prey and the spatial structure of marine habitats, contributing to interannual and regional variability in salmon growth and return rates.

Overall, salmon productivity in the North Pacific reflects a dynamic balance between climate forcing, oceanographic processes, and ecological interactions. The recurring oscillations of the PDO and ENSO act as primary climate drivers that alternately favour northern and southern salmon stocks. Understanding how these multi-scale processes interact and shape past salmon productivity is essential for forecasting salmon responses to future environmental change and for developing adaptive management strategies that align with the realities of a rapidly shifting ocean-climate system.

4.0 Lake Sediments Biomarkers for Tracking Migratory Animals Population and Climate Trends

Lake sediment biomarkers are organic compounds that preserve the structural and isotopic characteristics of their biological sources and are sufficiently resistant to degradation to persist in soils, sediments, and aquatic systems over geological and ecological timescales (Bull et al., 2002; Duda et al., 2021; Finney et al., 2000). These molecular fossils act as direct chemical archives of biological activity and environmental conditions, providing critical understanding of the sources, transformations, and pathways of organic matter within ecosystems.

Migratory animals such as Pacific salmon and cormorants leave behind distinctive physical, chemical, and biological signals in the environments they inhabit, from nutrient-rich guano and decomposing salmon carcasses to lipid molecules and isotopic enrichments within sediments (Hargan et al., 2018; Finney et al., 2000; 2002). Once deposited into lake water or sedimentary layers, these signatures are incorporated into the stratigraphic record, serving as long-term indicators of population dynamics, trophic interactions, and ecosystem health. By decoding these molecular and isotopic imprints, researchers can reconstruct migratory animal populations' dynamics and how they have shaped nutrient cycling, primary productivity, and ecosystem function over centennial to millennial timescales (Hargan et al., 2018; Finney et al., 2000; 2002).

Similarly, climatic variability, particularly fluctuations in temperature, drives physiological and community-level adaptations in aquatic microorganisms (Zhao et al. 2025; Pearson et al., 2025). In response to changing thermal regimes, bacteria adjust the composition and structure of their membrane lipids to preserve optimal fluidity and functionality, a process known as homoviscous adaptation. This biochemical adjustment enables microorganisms to maintain cellular integrity, enzyme activity, and metabolic efficiency despite environmental temperature shifts. These biochemical adjustments are captured in the molecular composition of lake sediments, soils, and water, where lipid distributions reflect the environmental and thermal conditions prevailing at the time of deposition (Russel et al., 2018; Zhao et al., 2023). Because many microbial lipids are chemically stable over long timescales, they form a durable record of past climatic variability. Consequently, shifts in these molecular signatures act as sensitive indicators of historical temperature change and ecosystem response, offering a powerful means of reconstructing environmental dynamics well beyond the span of instrumental observations.

4.1 Nitrogen Isotopes ($\delta^{15}\text{N}$) as Tracers of Population and Nutrient Dynamics

Nitrogen stable isotopes ($\delta^{15}\text{N}$) are among the most powerful geochemical tools for reconstructing nutrient dynamics, trophic interactions, and animal population histories in aquatic ecosystems (Finney et al., 2000; 2002). $\delta^{15}\text{N}$ represents the ratio of the heavy isotope (^{15}N) to the light isotope (^{14}N), expressed relative to atmospheric nitrogen (air- N_2). Because nitrogen is a fundamental component of organic matter, its isotopic composition becomes incorporated into sediments, providing a continuous record of ecosystem change that integrates both biological processes and climatic influences.

In lake systems influenced by migratory animals, $\delta^{15}\text{N}$ serves as a sensitive tracer of nutrient transfer and ecological coupling between ecosystems. Pacific salmon exhibit elevated $\delta^{15}\text{N}$ values due to their high trophic position in marine food webs, where successive trophic transfers enrich ^{15}N relative to ^{14}N . As a result, salmon tissues are several per mil higher in $\delta^{15}\text{N}$ than freshwater producers (Mathison et al., 1998). During spawning migrations, salmon transport large quantities of marine-derived nitrogen from the ocean to freshwater systems. Following their death and decomposition, this nitrogen-enriched ^{15}N enters aquatic and benthic food webs, ultimately becoming preserved in sediments as an isotopic fingerprint of salmon-derived nutrient input (Finney et al. 2000; 2002). Empirical studies have demonstrated this effect clearly. For instance, comparisons of $\delta^{15}\text{N}$ values across trophic levels in salmon-bearing and control lakes show enrichments of approximately +1 to +3‰ in systems receiving salmon carcasses (Mathison et al., 1998). This isotopic signal confirms that returning salmon substantially augment the nitrogen pool in nursery lakes. The process is particularly important for Sockeye salmon, whose juveniles rely on these enriched lake environments and the resulting zooplankton productivity during early development. Consequently, variations in sedimentary $\delta^{15}\text{N}$ over time can serve as reliable proxies for historical escapement, reflecting fluctuations in salmon abundance, spawning intensity, and nutrient delivery to freshwater ecosystems (Finney et al., 2000; 2002).

Similarly, cormorants congregate in large colonies in trees near waterbodies where thousands of individuals roost and breed over several seasons. Through their foraging and nesting activities, cormorants transport large amounts of aquatic-derived nutrients from feeding areas to their nesting colonies on nearby islands. From these sites, nutrient-rich guano is

subsequently washed or leached back into the lake, serving as a major source of nitrogen (N) and phosphorus (P) enrichment to the aquatic environment (Hargan et al., 2018).

Cormorant guano is typically enriched in ^{15}N for two key reasons. First, cormorants occupy high trophic positions as piscivorous predators, feeding mainly on fish that are themselves isotopically enriched relative to lower trophic levels. Second, isotopic fractionation occurs during the cormorants' metabolic processing and excretion of nitrogenous waste, mainly uric acid, resulting in further ^{15}N enrichment of guano relative to their diet. The combined effect of these processes produces guano $\delta^{15}\text{N}$ values several per mil higher than those of surrounding terrestrial or lacustrine organic matter, providing a distinct isotopic fingerprint of avian nutrient input (Hargan et al., 2018).

4.2 Carbon-to-Nitrogen Ratios (C/N) as Indicators of Organic Matter Sources

In lake ecosystems, the C/N ratio serves as a diagnostic tool for distinguishing among terrestrial, aquatic, and animal-derived organic inputs. Organic matter from vascular land plants typically exhibits high C/N values (>20) due to the predominance of cellulose and lignin, carbon-rich but nitrogen-poor structural polymers. In contrast, algal and bacterial organic matter, which is richer in proteins and nitrogenous compounds, tends to display low C/N ratios (4 - 10) (Meyers & Ishiwatari, 1993).

In lake ecosystems influenced by migratory animals such as cormorants, C/N ratios provide an additional line of evidence for interpreting nutrient transfer and population dynamics. The deposition of nitrogen-rich cormorant guano typically depresses sedimentary C/N ratios while concurrently enriching $\delta^{15}\text{N}$ values. This coupled geochemical signal represents a distinctive fingerprint of animal-derived nutrient input, reflecting enhanced nitrogen loading and increased aquatic productivity. Periods of high salmon return or prolonged cormorant colony occupation can thus be inferred from synchronous decreases in C/N and increases in $\delta^{15}\text{N}$.

Cormorant colonies exert further influence on sedimentary C/N ratios through vegetation modification and habitat disturbance. Nesting cormorants often strip leaves and branches from surrounding trees for nest construction and deposit large amounts of guano beneath roosting sites (Hargan et al., 2018). This dual process of defoliation and nutrient enrichment can alter the balance of organic inputs to nearshore sediments. The loss of terrestrial vegetation reduces the influx of high-C, low-N plant detritus, while guano deposition enhances nitrogen inputs, together

driving a notable decline in C/N ratios in sediments underlying or adjacent to colony sites. Over time, these changes create a persistent biogeochemical imprint that reflects both the physical and chemical impacts of colonial nesting activity. When interpreted alongside $\delta^{15}\text{N}$ and complementary molecular biomarkers such as sterols, stanols, and lipid-based compounds, the C/N ratio provides a multidimensional perspective on past ecosystem function.

4.3 Sterols and Stanols as Indicators of Animal and Plant Inputs

Sterols and stanols are important lipid biomarkers that provide understanding of the biological sources, diagenetic transformations, and depositional environments of organic matter preserved in sediments. Originating primarily from the cell membranes of plants, animals, algae, and microorganisms, these compounds retain source-specific structural characteristics that allow for the identification of their biological origins long after deposition (Bull et al., 2002; Hargan et al., 2018). Because of their relative stability and persistence in sediments, sterols and their hydrogenated counterparts, stanols, have become fundamental tools for reconstructing past populations, ecological and biogeochemical processes in aquatic systems.

The molecular diversity of sterols and stanols enables their use as tracers of specific organic matter sources. For example, cholesterol (cholest-5-en-3 β -ol) is the dominant sterol in animal tissues and serves as an indicator of zooplankton, fish, or other faunal contributions, whereas sitosterol (β -sitosterol) and campesterol (campest-5-en-3 β -ol) are characteristic of plant organic matter input. Fucosterol (stigmasta-5,24-dien-3 β -ol) and desmosterol (3 β -cholesta-5,24-dien-3-ol), on the other hand, are primarily associated with algal and diatom productivity and indicate autochthonous aquatic production. In contrast, compounds such as coprostanol (5 β -cholestan-3 β -ol), epicoprostanol (5 β -cholestan-3 α -ol), and coprostanone (5 β -cholestan-3-one) are formed through microbial reduction and oxidation of cholesterol in the digestive tracts of higher animals, making them reliable molecular markers of fecal input (Bull et al., 2002).

Because of these distinct biological origins, the relative abundances and ratios of sterols and stanols can be used to differentiate between plant, aquatic, and animal-derived sources of organic matter in sediments. In lake systems influenced by large migratory animals, such as Pacific salmon or cormorants, the occurrence of animal-derived sterols, particularly coprostanol and cholesterol, within sedimentary profiles can thus be interpreted as evidence of animal presence or nutrient enrichment, while plant- and algal-derived sterols provide complementary

information about terrestrial input and in-lake productivity from nutrient enrichment (Finney et al., 2000, 2002; Hargan et al., 2018). Moreover, the ratio of specific sterols, such as the sum of cholesterol and cholestanol to the sum of cholesterol, cholestanol, sitosterol, and stigmastanol, could track the population dynamics of cormorants (Hargan et al., 2018).

In paleolimnological studies, the integration of independent proxies such as sterol and stanol with isotopic and elemental proxies, such as $\delta^{15}\text{N}$ and C/N ratios, allows for multiple lines of evidence for reconstructing past migratory animal population dynamics. This multi-proxy approach enables researchers to identify shifts in nutrient sources, organic matter input, and biological productivity through time, offering a molecular-level understanding of how terrestrial and aquatic processes shape sedimentary records. Whereas these biomarkers provide understanding of ecological and biogeochemical processes, other lipid classes capture the changing climatic conditions in which migratory animals live. Among these, glycerol dialkyl glycerol tetraethers (GDGTs) synthesized by aquatic and soil bacteria have emerged as powerful molecular thermometers, capable of recording historical variations in temperature and climate.

4.4 Branched Glycerol Dialkyl Glycerol Tetraethers (brGDGTs) as Temperature Proxies

Glycerol dialkyl glycerol tetraethers (GDGTs) are membrane lipids produced by archaea and bacteria that modify their chemical structures in response to environmental conditions, particularly temperature and pH. GDGTs occur in two principal structural groups: isoprenoid GDGTs (isoGDGTs), primarily synthesized by archaea in marine and some freshwater systems, and branched GDGTs (brGDGTs), predominantly produced by bacteria, especially Acidobacteria, in soils, peats, and lacustrine environments (Pearson et al., 2025; Zhao et al., 2020).

Structurally, brGDGTs consist of two alkyl chains ether-linked to a glycerol backbone. Variations arise from the number and position of methyl branches and the occurrence of zero, one, or two cyclopentane moieties. These modifications produce tetra-, penta-, and hexamethylated homologues with methyl substituents commonly located at the 5' and 6' carbon positions of the alkyl chains. The recent discovery of 7'-methyl isomers has added further structural diversity and refined analytical resolution (Ding et al., 2016). These compositional adjustments are ecophysiological responses that allow microorganisms to maintain membrane

stability under changing thermal and pH conditions, establishing the biochemical foundation for GDGT-based environmental proxies.

Empirical relationships between GDGT structures and environmental parameters have been formalized through indices such as the Methylation of Branched Tetraethers (MBT) and Cyclization of Branched Tetraethers (CBT) (Damsté et al., 2008; Weijers et al., 2007a; Weijers et al., 2007b). The degree of methylation generally increases with temperature, while the degree of cyclization is regulated by pH. Advances in chromatographic separation, particularly the distinction between 5- and 6-methyl isomers, have led to the development of refined indices, including MBT'_{5ME} and CBT_{5ME}, which enhance the precision of temperature and pH reconstructions (De Jong et al., 2014; Hopmans et al., 2016). These indices have been successfully applied to a wide range of terrestrial and aquatic environments, providing quantitative reconstructions of temperature across spatial and temporal gradients from tropical to polar regions. Integrating these quantitative paleothermometers can complement biogeochemical indicators such as $\delta^{15}\text{N}$, C/N ratios, sterols, and stanols, enabling a holistic reconstruction of both migratory animal population dynamics and environmental processes, linking biological activity to underlying climatic variability.

5.0 Knowledge Gaps

Despite decades of research on the ecology and environmental influence of large migratory animals such as Pacific salmon and cormorants, critical knowledge gaps persist that limit our understanding of their long-term population dynamics, nutrient transfer pathways, and responses to climate variability. These uncertainties arise from the relatively short duration of instrumental and monitoring records, the episodic nature of ecological surveys, and the scarcity of independent biomarkers capable of distinguishing among overlapping nutrient sources to complement the traditional $\delta^{15}\text{N}$ tracers. Equally important are gaps in the application of paleoclimate proxies such as brGDGTs that capture historical temperature and environmental variability shaping these migratory animals and their freshwater habitats. The lack of well-calibrated, site-specific paleoclimate records constrains our ability to link population dynamics to long-term climatic forcing, necessitating the need for integrated, multi-proxy reconstructions that combine biomarker evidence of animal influence with geochemical indicators of past climate conditions.

5.1 Knowledge Gaps in Biomarker Applications for Tracking Large Migratory Animals

Despite major advances in biomarker and isotopic applications, substantial uncertainties remain in applying these tools to quantify the ecological influence and population histories of migratory species. For Sockeye salmon, $\delta^{15}\text{N}$ has long been used to trace marine-derived nutrient subsidies from decomposing carcasses. However, interpretation of $\delta^{15}\text{N}$ enrichment is complicated by baseline isotopic variability, watershed land use, and post-depositional microbial processes such as nitrification and denitrification. In addition, terrestrial organic inputs and variations in lake productivity can alter sedimentary $\delta^{15}\text{N}$ values, limiting their reliability as a direct and unambiguous proxy for salmon escapement or nutrient loading.

Sterol and stanol biomarkers such as cholesterol, cholestanol, fucosterol, and sitosterol offer molecular specificity that complements isotopic data by providing source-specific fingerprints of organic matter. These compounds can distinguish between animal, algal, and terrestrial inputs, improving the capacity to isolate salmon-derived contributions (typically reflected in elevated cholesterol) from plant-derived inputs (evident in higher sitosterol). Pacific salmon are characterized by high cholesterol content, and their post-spawning die-off delivers a significant flux of sterol-rich organic material to nursery lakes. Yet, quantitative calibration of these sterols and stanols against historical escapement records remains limited. Establishing robust calibrations between sterol biomarkers and known Sockeye escapement records would enable reliable reconstruction of salmon population histories extending beyond the period of instrumental monitoring.

For cormorants, biomarker applications are comparatively more developed but remain incomplete. Guano deposition is known to elevate sedimentary $\delta^{15}\text{N}$ through isotopic fractionation during excretion, and recent studies have demonstrated that sterols and stanols, particularly cholesterol, sitosterol, and coprostanol corroborate isotopic evidence of waterbird nutrient input. However, significant gaps persist in understanding the temporal and spatial variability of these biomarkers across cormorant-affected and reference sites, especially from longer sediment cores that capture colony establishment and decline. This limits our ability to determine the precise timing of colony formation, the duration and intensity of occupation, and whether modern cormorant expansion represents recovery to pre-disturbance levels or a novel population increase driven by ecological and anthropogenic change. The absence of multi-site,

multi-proxy reconstructions encompassing colonies of contrasting intensity further constrains efforts to assess long-term ecological effects and management outcomes.

These challenges underscore the need for standardized biomarker indices and cross-system calibration frameworks that link molecular and isotopic signals to known ecological baselines. Developing integrated multi-proxy approaches combining sterol/stanol profiles with $\delta^{15}\text{N}$ and C/N elemental data will be essential for disentangling overlapping nutrient sources and accurately reconstructing the historical dynamics of salmon escapement, cormorant colony expansion, and nutrient transfer through time.

5.2 Knowledge Gaps in Paleotemperature Reconstructions and Proxy Integration

BrGDGTs have emerged as powerful molecular tools for reconstructing past temperature and pH conditions, yet most existing calibrations are derived from global or regional datasets that fail to capture lake-specific variability. Parameters such as redox gradients, hydrological regime, and microbial community composition strongly influence brGDGT distributions, often producing systematic biases when global models are applied to individual systems.

In warm monomictic lakes like Cultus Lake, seasonal temperature fluctuations strongly influence brGDGT composition within the epilimnion, with these compounds eventually settling into sediments. However, no studies have yet developed lake-specific calibrations that directly link brGDGT compositions to in-situ temperature and pH measurements of the Cultus Lake. The absence of such locally constrained models restricts the accuracy of paleotemperature reconstructions and hinders the evaluation of thermal variability in relation to major climate oscillations like the PDO and ENSO.

Equally critical is the lack of integration between ecological and climatic archives. While isotopic and biomarker proxies (e.g., $\delta^{15}\text{N}$, C/N, sterols, stanols) document nutrient dynamics and population histories, they are rarely interpreted alongside paleotemperature indicators such as brGDGTs. This disconnect prevents a mechanistic understanding of how long-term climate variability influences migratory species, nutrient cycling, and ecosystem structure. Without coupled reconstructions of biological and climatic signals, it remains difficult to distinguish climate-driven ecological change from intrinsic population or biogeochemical processes.

Addressing these limitations requires the development of lake-specific brGDGT calibrations, validated against modern temperature and pH gradients, and their integration with

ecological biomarkers and isotopic tracers. Such a framework will enable direct linkage of salmon escapement and cormorant colony histories to historical temperature variability, advancing a more comprehensive understanding of climate-ecosystem feedbacks in North American lake systems.

6.0 Research Objectives and Thesis Overview

To address the knowledge gaps identified in the preceding sections, this thesis integrates molecular, isotopic, and paleotemperature approaches to reconstruct long-term population dynamics of large migratory animals and climatic interactions in North Pacific Lake ecosystems. The overarching aim is to bridge the temporal gap between modern ecological monitoring and pre-instrumental climate records by developing and applying sediment-based proxies capable of capturing both biological and environmental variability at high temporal resolution. In doing so, this research advances the capacity to quantify the ecological influence of key migratory animals, including Sockeye salmon and cormorants and to link their historical population dynamics to climatic forcing across centennial timescales.

The specific objectives of this research are threefold. First, to develop and validate quantitative molecular indices, using sterol and stanol biomarkers in conjunction with isotopic tracers, for reconstructing the long-term population histories of Sockeye salmon and cormorants in freshwater systems. Second, to establish a lake-specific calibration of brGDGTs from Cultus Lake, BC, thereby refining the accuracy of paleotemperature reconstructions and addressing limitations associated with the use of generalized global models. Third, to integrate these molecular and isotopic archives with instrumental records of salmon escapement and large-scale climatic indices, specifically the PDO and the ENSO, to evaluate how climatic variability has influenced salmon productivity and ecosystem structure through time.

Through this integrated multi-proxy approach, the study enhances the interpretive resolution of sedimentary records and provides one of the first comprehensive molecular frameworks linking large migratory animal population dynamics, nutrient transfer, and climatic variability in North American lake ecosystems. Beyond its methodological innovation, this research contributes to a deeper understanding of how biological and climatic processes co-evolve, offering valuable tools for both paleoecological reconstruction and contemporary conservation management.

7.0 Thesis Structure

This thesis adopts a manuscript-based format, comprising six interrelated chapters that collectively address the overarching objective of developing and applying sedimentary biomarkers to reconstruct past migratory animal populations and climate dynamics.

Chapter One introduces the ecological and climatic context of the study, emphasizing the importance of migratory species such as cormorants and Pacific salmon as biovectors of nutrients across ecosystems. It also discusses the influence of large-scale climatic oscillations (PDO and ENSO) on their population dynamics and outlines the existing knowledge gaps that this research aims to address.

Chapter Two focuses on the long-term ecological impacts of cormorants by analyzing sterols and stanol biomarkers and isotopic tracers in sediments from contrasting Lake Ontario islands. Through the integration of sterols, stanols, $\delta^{15}\text{N}$, and C/N data, this chapter reconstructs cormorant population dynamics and their ecological impact in freshwater island ecosystems.

Chapter Three presents the development and validation of sterol and stanol biomarkers as quantitative indicators of Sockeye salmon escapement. Using sediment cores from nine Alaskan nursery lakes with well-documented historical records, this chapter establishes empirical relationships between cholesterol and other diagnostic sterols (e.g., sitosterol, fucosterol) and salmon return density. The resulting “Salmon Sterol Index” is applied to reconstruct long-term escapement trends and could extend fishery baselines beyond the onset of instrumental monitoring.

Chapter Four develops and validates a Cultus Lake-specific calibration for brGDGT-based temperature reconstruction. By relating seasonal brGDGT distributions in the water column to measured water temperature and pH profiles, this chapter demonstrates the advantages of site-specific calibration in improving the accuracy and ecological relevance of paleotemperature estimates compared to existing regional and global models.

Chapter Five synthesizes isotopic and paleotemperature records to reconstruct coupled salmon-climate interactions in Cultus Lake. By integrating $\delta^{15}\text{N}$ and brGDGT proxies with historical escapement data and climate indices (PDO and ENSO), this chapter elucidates how decadal-scale variations in temperature and nutrient cycling have influenced salmon productivity and lake ecosystem function over the past century.

Finally, Chapter Six presents a synthesis of the thesis findings, emphasizing their broader implications for paleolimnology, biomarker calibration, and climate-ecosystem interaction studies. It discusses the methodological advances achieved through the integration of molecular and isotopic proxies, highlights the potential applications of these tools for long-term ecological monitoring, and identifies future research directions aimed at improving biomarker-based reconstructions in dynamic freshwater systems.

In summary, this thesis demonstrates the power of combining molecular biomarkers, isotopic tracers, and paleotemperature proxies to reconstruct the intertwined histories of migratory animals and climate in North Pacific lakes. By linking ecological and climatic processes across multiple temporal scales, it provides a cohesive and data-rich framework for understanding ecosystem resilience under changing environmental conditions.

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2 CHAPTER 2: Sharp rise in cormorant (*Phalacrocorax auritus*) populations on Lake Ontario islands inferred from analyses of pond sediment cores

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STATEMENT OF CONTRIBUTION

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Abstract

We measured biomarkers in pond sediment cores to infer population dynamics and track the ecological impacts of cormorants at East Brother Island (EBI), a cormorant-affected site, and Calf Island (CI), a reference site, in Lake Ontario over the past ~170 years. Pronounced synchronous increases in cholesterol, cholestanol, cholestanone, coprostanol, epicoprostanol concentrations, expanded bird sterol index (EBSI), and $\delta^{15}\text{N}$ values in the 1970s mark the establishment and rapid expansion of cormorant colonies at EBI. That time coincides with human-mediated environmental changes such as the regulation of organochlorine contaminants (e.g. DDT, PCBs), later introduction of invasive fish species, and reduced human disturbances. Declining C:N ratios and an initial increase in the plant stanol stigmastanol at EBI, following the initial colony establishment reflect short-term enhancement of terrestrial primary productivity driven by guano deposition. However, stigmastanol concentrations declined after ca. 1990, likely from vegetation degradation caused by the toxic effects of ammonia-rich guano and physical disturbances from bird nesting activities. We document the transformative impacts of cormorant populations on nutrient cycling, sediment processes, and vegetation dynamics at their nesting sites, providing a detailed record of how waterbird populations modify ecosystems over time, following their arrival. The findings highlight the value of integrating long-term ecological monitoring and adaptive management strategies to address the impacts of expanding waterbird populations, particularly in sensitive lacustrine environments.

1.0 Introduction

The double-crested cormorant (*Phalacrocorax auritus*) is a prominent freshwater avian species that has significantly impacted North American ecosystems. This large, widely distributed bird has adapted successfully to diverse aquatic habitats across the continent (Clark et al., 2006; DeVault et al., 2012). During breeding seasons, cormorants form large colonies, typically nesting in trees near water bodies where they can access their fish-based diet (McDonald et al., 2018; Wires and Cuthbert, 2006). Their diet is composed predominantly of fish, encompassing a size range from small species such as the three-spined stickleback (*Gasterosteus aculeatus*) to larger species like yellow perch (*Perca flavescens*) (DeBruyne et al., 2013; Johnson et al., 2010). The ecological influence of double-crested cormorants extends

beyond their role as predators in aquatic food webs, as their presence can significantly alter local ecosystems through the deposition of ammonia-rich guano, which can induce changes in soil chemistry, resulting in the loss of ground cover, and potentially leading to tree mortality (Boutin et al., 2011; Lafferty et al., 2016; Moran Veum et al., 2019).

In the mid-20th century, cormorant populations in Lake Ontario declined sharply because of the widespread use of organochlorine compounds, particularly dichlorodiphenyltrichloroethane (DDT) insecticide and polychlorinated biphenyls (PCBs), which caused eggshell thinning and reduced reproductive success (Weseloh et al., 1983). However, following the ban of these contaminants in the 1970s and subsequent conservation efforts, cormorant populations began to recover rapidly. Lake Ontario colonies, for instance, grew at an average rate of 42.9% between 1978 and 1992 (Weseloh et al., 1995). The recovery and subsequent expansion of cormorant populations were further facilitated by their adaptive feeding behaviour (Dias et al., 2012). Cormorants shifted their diet to include invasive fish species in the Great Lakes, such as the round goby (*Neogobius melanostomus*) and alewife (*Alosa pseudoharengus*) (Coleman et al., 2012; Somers et al., 2003). This dietary adaptation not only supported population growth but also altered their seasonal behaviour, potentially extending their presence at breeding colonies (Craig et al., 2016).

Despite their ecological importance, the expanding cormorant populations have intensified conflicts with humans, particularly in areas where these freshwater birds are perceived as competitors for commercial or recreational fish stocks and as agents of environmental degradation (Dorr and Fielder, 2017; Weseloh et al., 2012). The lack of historical population data, especially before the 1960s, has hindered our understanding of whether current population numbers represent a recovery to historical norms or an unprecedented expansion. Recently, paleolimnological approaches have emerged as valuable tools for reconstructing past ecological conditions and bird population dynamics (Duda et al., 2021; Hargan et al., 2018). These methods enable researchers to analyze dated lake sediment cores to infer long-term environmental changes and species interactions. Whereas previous studies utilized this approach to examine the pre- and post-ecological impact of cormorants on aquatic ecosystems, a more comprehensive analysis of their historical population dynamics and long-term ecological effects is needed (Hargan et al., 2018; Stewart et al., 2015). This study expanded upon existing research by examining sediment cores collected from both heavily cormorant-impacted and less bird-

affected sites. Through a multi-proxy approach that utilized stable N isotope values ($\delta^{15}\text{N}$), C:N ratios, and lipid biomarkers, this investigation sought to reconstruct long-term patterns of cormorant colonization, population fluctuations, and associated environmental impacts on islands in Lake Ontario.

Stable isotope measurements, particularly $\delta^{15}\text{N}$, have proven to be effective for identifying the impact of waterbird activities on terrestrial and aquatic ecosystems (Blais et al., 2005; Brimble et al., 2009; Hargan et al., 2017, Hargan et al., 2018; Huang et al., 2016; Stewart et al., 2015). However, elevated $\delta^{15}\text{N}$ values in sediment may also result from other factors, including anthropogenic inputs, such as sewage (Vane et al., 2010). To avoid the effects of this and other potential factors, the study sites were selected to minimize the chance that alternative factors may have caused elevated $\delta^{15}\text{N}$ values. Comparison of stratigraphic shifts in $\delta^{15}\text{N}$ values with those of avian sterols and stanols in sediment cores provides a more compelling record of temporal changes in past double-crested cormorant populations. Moreover, a ratio that includes cholesterol, sitosterol, and their reduced products was found to correlate significantly with sediment $\delta^{15}\text{N}$ values and best explained cormorant abundance across Lake Ontario islands (Hargan et al., 2018).

The C:N ratio and plant sterol profiles in sediment provide valuable insights into the environmental impact of cormorants on their habitats. The C:N ratio in lacustrine sediment serves as an indicator of organic matter sources in lakes, with terrestrial vascular plants typically exhibiting greater ratios (≥ 20) due to their high cellulose content, whereas algae have lower ratios (4–10) because of their high-protein composition (Meyers and Ishiwatari, 1993). Cormorants alter their environment through the production of nitrogen-rich guano, which is toxic to terrestrial plants, and their destructive nesting behaviours that lead to tree loss (Boutin et al., 2011; Lafferty et al., 2016). These activities are expected to influence the C:N ratios and sitosterol profiles in lake sediments over time. We hypothesized that cormorant colonization leads to concurrent increases in sediment $\delta^{15}\text{N}$, zoosterols such as cholesterol, and fecal sterols like coprostanol, enabling the tracking of bird colony establishment and subsequent population fluctuations over time. Additionally, stratigraphic changes in plant sterols and C:N ratios were expected to reflect the impact of cormorants on the local vegetation. This research contributes to the understanding of freshwater bird ecology, ecosystem dynamics, and the long-term effects of

colonial waterbirds on island environments and informs conservation and management strategies for affected areas.

2.0 Materials and Methods

2.1 Study sites

As summarized by Stewart et al. (2015), for this study, we selected two small islands in eastern Lake Ontario with different size populations of double-crested cormorants, including predominantly large and small colonies (**Error! Reference source not found.**). The highly affected site, East Brother Island (EBI) pond (44°12'18.4" N, 76°37'28.3" W, $Z_m = 0.5$ m), hosted a large double-crested cormorant colony with approximately 1300 nests between 2003 and 2012 (Stewart et al., 2015). Cormorants were also seen on the island during sample collection in 2016. There was very little to no living terrestrial vegetation at EBI at the time of sample collection (**Error! Reference source not found.**) (Hargan et al., 2018).

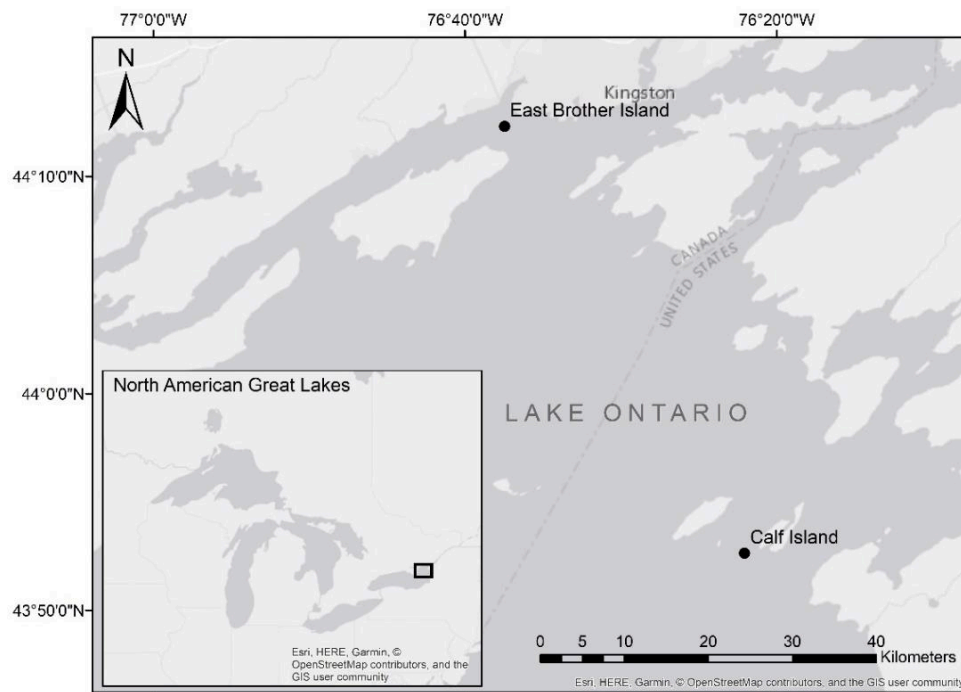


Figure 2.1 Map of study sites: East Brother Island (cormorant affected site) and Calf Island (reference site). The rectangle in the inset map shows the location of the study area in relation to the Great Lakes region.

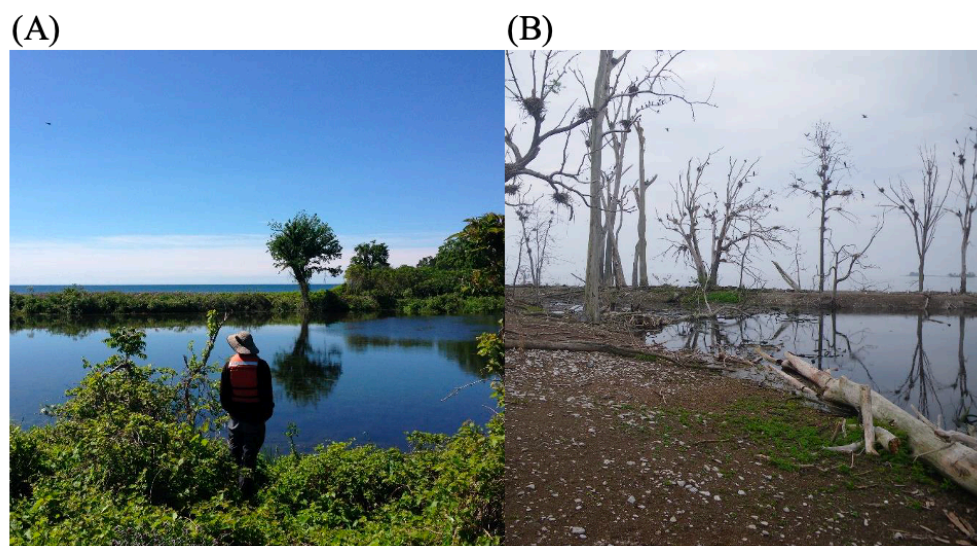


Figure 2.2 The island ponds from which sediment cores were collected: (A) Calf Island (reference site) and (B) East Brother Island (affected site). (Photos courtesy of Neal Michelutti).

The Calf Island Pond, located at coordinates 43°52'37.6"N, 76°22'04.3"W, served as a reference site for this study (Stewart et al., 2015). This small water body, with a maximum depth of ~1.0 m, is situated on an island that has a history of light, intermittent cormorant nesting. Calf Island supported a modest cormorant population in the recent past, with 170 nests recorded in 2008 and 24 nests in 2009 (I. Maazzocchi 2016–2017, personal communication as cited in (Hargan et al., 2018)). However, since 2009, cormorants have ceased nesting on the island, rendering it a suitable reference site for comparative studies. The landscape of Calf Island is characterized by its abundant vegetation and thriving tree population. These living plants constitute a significant component of the island's biota and contribute to its biodiversity. The island is small in size, and the pond occupies a substantial portion of its area (Hargan et al., 2018).

2.2 Sediment core collection and dating (^{210}Pb)

Sediment cores were collected from the EBI and CI ponds by collaborating researchers at the Queen's University PEARL lab utilizing a Glew gravity corer (Glew, 1988). The EBI core was collected on 28 June 2016, and extruded at 0.5-cm intervals for the topmost 10 cm, followed

by 1-cm intervals for the remainder of the core. The CI core was collected on 21 July 2016, and extruded at 0.5-cm intervals throughout the entire core. Samples from both cores were subsequently stored at 4 °C until analysis. The two sediment cores were freeze-dried and subsequently dated using excess ^{210}Pb activity and verified with ^{137}Cs employing an Ortec High Purity Germanium Gamma Spectrometer (Oak Ridge, TN, USA). Dates were calculated using ScienTissiME (Barry's Bay, ON, Canada) software in accordance with the Constant Rate of Supply (CRS) model (Appleby, 2001). Profiles of ^{210}Pb activity versus depth and the resulting age-depth model were generated using the CRS model including validation with ^{137}Cs .

2.3 Element and stable isotope analysis ($\delta^{15}\text{N}$)

Element and stable isotope analyses were performed at the Jan Veizer Stable Isotope Laboratory (Ottawa, Ontario). For each sediment interval, 3–5 mg of freeze-dried, untreated material was weighed into tin capsules, accompanied by approximately 10 mg of tungsten trioxide (WO_3) to enhance combustion efficiency (Talbot, 2001). Samples were analyzed for total carbon (C), total nitrogen (N), and nitrogen isotope composition using a continuous-flow elemental analyzer (Elementar Vario EL Cube, Elementar, Germany) coupled to an isotope ratio mass spectrometer (Delta Advantage IRMS, Thermo Fisher Scientific, Germany) via a ConFlo IV interface. Samples were flash combusted at 1800 °C, and the resulting CO_2 and N_2 gases were purified and separated before IRMS analysis. $\delta^{15}\text{N}$ values are expressed in per mil (‰) relative to atmospheric nitrogen (Air- N_2) and normalized using certified international reference standards: IAEA-N1 (+0.4‰), IAEA-N2 (+20.3‰), USGS-40 (-4.52‰), and USGS-41 (+47.57‰).

2.4 Sterols and stanols extraction and analysis

Sterol and stanol compounds were extracted and analyzed from freeze-dried sediment core samples collected from EBI and CI. The targeted analytes included 5β -cholestan- 3β -ol (coprostanol), 5β -cholestan- 3α -ol (epicoprostanol), 5β -cholestan-3-one (coprostanone), cholest-5-en- 3β -ol (cholesterol), 5α -cholestan- 3β -ol (cholestanol), 5α -cholestan-3-one (cholestanone), 3β -cholesta-5,24-dien- 3β -ol (desmosterol), campest-5-en- 3β -ol (campesterol), stigmasta-5,24-dien- 3β -ol (fucosterol), β -sitosterol (sitosterol), and 5α -stigmastan- 3β -ol (stigmastanol). Prior to extraction, 0.1 g of copper powder was cleaned using dichloromethane (DCM). Approximately

0.1 g of dry sediment was then combined with the cleaned copper powder. To evaluate extraction efficiency, each sample was spiked with 50 µL of cholesterol-d6 (10 µg/mL; Cambridge Isotope Laboratories, Tewksbury, MA, USA) as a recovery standard. Samples were extracted by sonication with 10 mL DCM, repeated three times. The combined extracts were reduced to a volume of 1 mL under a stream of nitrogen at 35 °C and 0.4 bar pressure. Extract purification was performed using 1 g Supelclean™ LC-Si solid-phase extraction cartridges (Sigma-Aldrich, St. Louis, MO, USA) preconditioned with 6 mL DCM. Extracts were loaded and eluted with 30 mL of DCM. The eluents were dried under nitrogen and derivatized with 0.1 mL of BSTFA +1% TMS (*N,O*-bis(trimethylsilyl)trifluoroacetamide +1% trimethylchlorosilane; Sigma-Aldrich, St. Louis, MO, USA) at 60 °C for 2 h. Following derivatization, 0.4 mL of toluene was added, and samples were spiked with 10 µL of *p*-terphenyl-d14 (10 µg/mL; Cambridge Isotope Laboratories, Tewksbury, MA, USA) as an internal standard. Prepared samples were analyzed by gas chromatography–mass spectrometry (GC–MS) following the method of Cheng et al. (2016). All concentrations were corrected for recovery and adjusted based on procedural blanks. Method detection limits (MDLs) were determined following Loconto (2005), with calculation details provided in the Supplementary Information.

2.5 Statistical analysis

We used the expanded bird sterol index (EBSI) developed by Hargan et al. (2018) to examine avian subsidies to these ponds. The EBSI was initially developed from the bird sterol index that tracked the impact of waterbirds on High Arctic ponds on Devon Island (Nunavut) (i.e., waterbird index = cholesterol / (cholesterol + sitosterol) (Cheng et al., 2016). Previous analysis of sterols and stanols across different ponds on the islands in eastern Lake Ontario showed substantial, measurable amounts of cholesterol and sitosterol, hence the expansion of the initial bird sterols to include cholesterol and sitosterol and their microbial degradation products under aerobic conditions in the natural environment (Hargan et al., 2018). In the natural environment, cholesterol and sitosterol are reduced to 5 α -cholestanol and stigmastanol, respectively (through 5 α -stanone intermediates) (Bull et al., 2002; Leeming et al., 1996). By considering both the parent sterols and their degradation products, the EBSI offers a more robust method for quantifying the impact of cormorants on the local ecosystem.

$$\text{EBSI} = \frac{\text{cholesterol} + 5\text{a-cholestanol}}{\text{cholesterol} + 5\text{a-cholestanol} + \text{sitosterol} + \text{stigmastanol}} \quad (\text{Eq. 1})$$

The relative abundance of sterols and stanols in dated sediments from CI and EBI were calculated and represented using bar plots. We modelled the dynamics of the concentration of sterols and stanols, $\delta^{15}\text{N}$ values, and C:N ratios in dated pond sediment cores using generalized additive models (GAMs) (Simpson, 2024). GAMs were chosen for their effectiveness in modelling complex and non-linear relationships while accounting for model uncertainty. They enable the identification of significant temporal changes in sterol and stanol profiles, C:N ratios, and $\delta^{15}\text{N}$ values by detecting periods when the first derivative of the GAM exhibits a nonzero rate of change. These statistical analyses were conducted utilizing R workspace's packages *gratia* v.0.9.2 (Simpson, 2024) and *mgcv* v.1.9.1 (Wood, 2017).

3.0 Results

3.1 Sediment chronologies and age-depth relationships

Sediment chronologies for CI and EBI ponds were developed using unsupported ^{210}Pb activities and the Constant Rate of Supply (CRS) model, with ^{137}Cs used solely for independent verification (Figure 2.3). Unsupported ^{210}Pb activities declined systematically with cumulative dry mass in both cores (Figure 2.3A & B), indicating progressive burial and justifying CRS-based age-depth modelling under temporally variable sediment accumulation. The CI core showed measurable ^{137}Cs but no distinct peak (Figure 2.3C). At EBI, a distinct ^{137}Cs activity maximum occurred at ~14.8 and corresponded closely with CRS-derived ages centered on ca. 1963, providing independent validation of the CRS chronology (Figure 2.3D). Together, these results indicated continuous sediment accumulation at both sites over the past ~150 years.

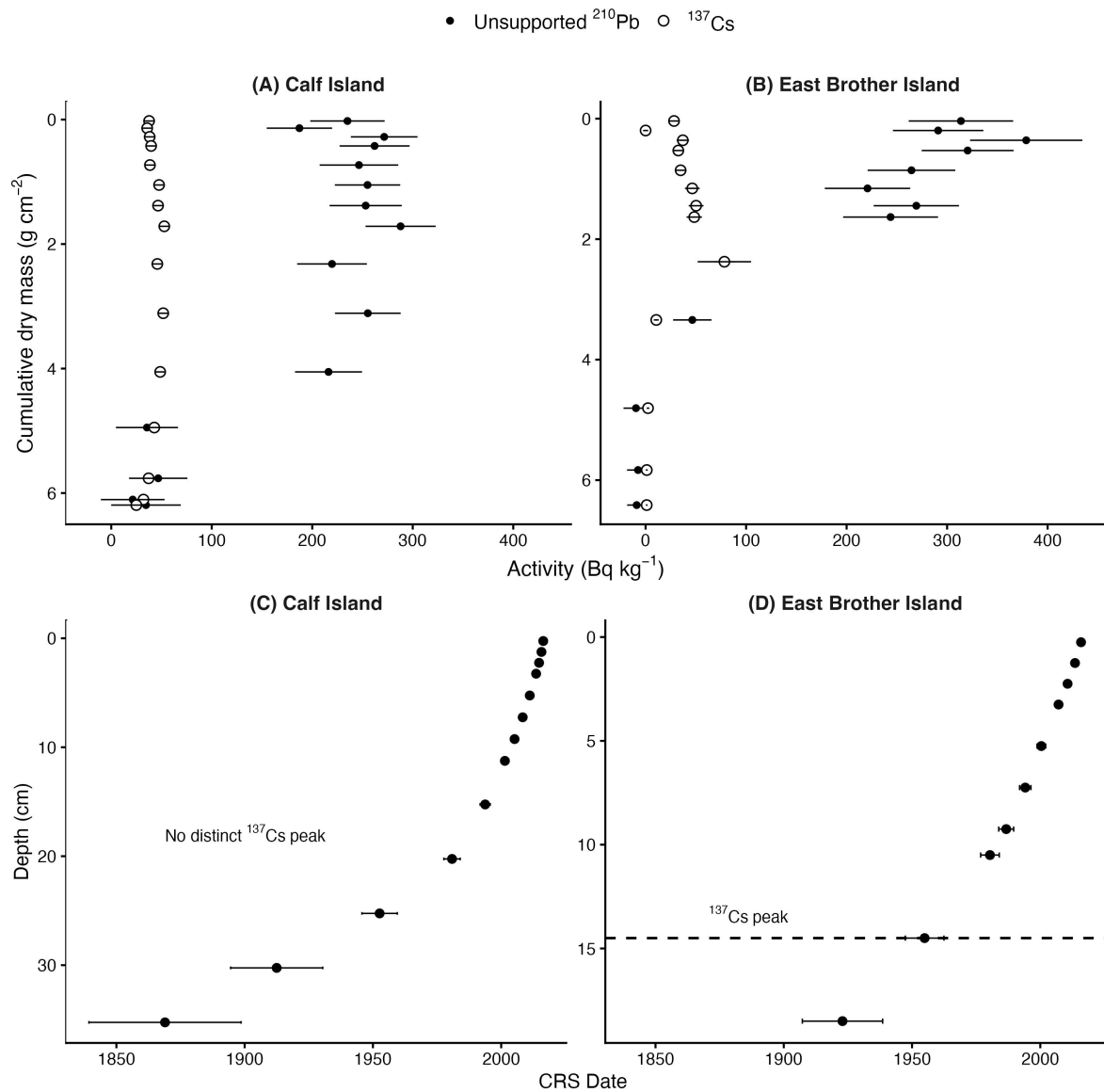


Figure 2.3 Sediment radioisotope profiles and Constant Rate of Supply (CRS)- based chronologies for Calf Island Pond and East Brother Island Pond. (A-B) Measured unsupported ^{210}Pb (filled circles) and ^{137}Cs (open circles) activities plotted against cumulative dry mass for (A) Calf Island and (B) East Brother Island. Horizontal error bars represent analytical uncertainties. C-D, CRS age-depth models for (C) CI and (D) EBI, showing CRS calendar ages with associated uncertainties plotted against sediment depth. The dashed horizontal line in panel D indicates the depth of the maximum measured ^{137}Cs activity. Depth and cumulative dry mass are plotted increasing downward.

3.2 Percent relative abundance of sterols and stanols at EBI and CI

Analysis of dated sediment cores from a reference site (CI) and a cormorant-impacted site (EBI) revealed different sterol and stanol relative abundances. Most of the sterols, including cholesterol, cholestanol, coprostanol, epicoprostanol, campesterol, stigmasterol, and sitosterol, had high relative abundances throughout the sediment cores from both locations (Figure 2.4). However, desmosterol was not detected at either site, and cholestanone, coprostanone and fucosterol were only prevalent near the EBI core's upper sediments. Prior to ca. 1970, sterol profiles at both sites indicated baseline conditions, with consistently low cholesterol relative abundances ($< 5\%$), suggesting background levels before cormorant expansion. Post-1970, dramatic changes were observed, particularly at EBI. Cholesterol showed a notable increase in abundance, reaching a peak before declining slightly around 2005. Cholesterol metabolites, such as cholestanol and cholestanone, also displayed substantial increases in abundance from 1970 to the core surface. Fecal sterol biomarkers, including coprostanol, coprostanone, and epicoprostanol, exhibited large increases in abundance after ca. 1970, further indicating a direct link to cormorant activity at EBI. In contrast, plant-derived sterols, such as campesterol, sitosterol, and stigmasterol, showed a general decrease in relative abundance from ca. 1970 to the top of the core at the cormorant-impacted site, suggesting a potential shift in organic matter inputs following cormorant colonization. At the reference site, campesterol exhibited relatively consistent abundance throughout the sediment core. In contrast, sitosterol increased near the surface, while stigmasterol declined in the upper layers. After ca. 1970, both stigmasterol and sitosterol displayed higher relative abundances at the reference site (CI) compared to the affected site (EBI).

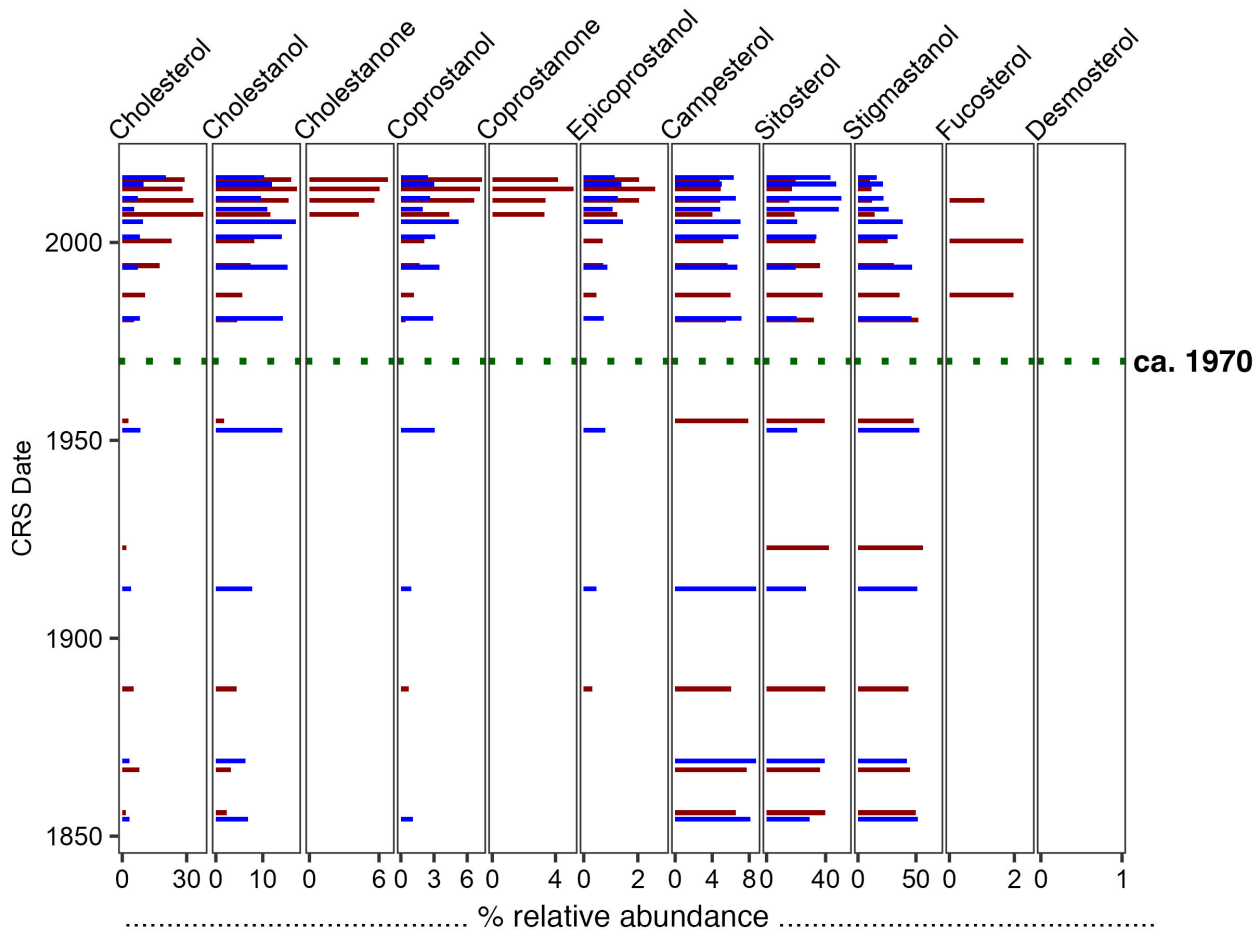


Figure 2.4 Percent relative abundances of sterols and stanols in ^{210}Pb -dated pond sediments from EBI (shown in red) and CI (shown in blue). The dashed green horizontal line represents our estimated year of cormorant colonization (ca. 1970 CE). The data show the distribution of sterols and stanols, demonstrating the compositional differences in sterol and stanol abundance in sediment cores from the two sites over time.

3.3 Concentrations of sterols and stanols, bird sterol index, $\delta^{15}\text{N}$ values, and C:N ratio, at EBI and CI

Analysis of dated sediment cores from CI and EBI also revealed different temporal patterns in the concentrations of sterols and stanols within the pond sediments (Figure 2.5). At EBI, a marked increase in cholesterol concentrations was observed beginning around 1970, coinciding with the recorded expansion of cormorants. This increase was paralleled by its metabolites, cholestanol, and fecal biomarkers such as epicoprostanol and coprostanol. The plant sterols campesterol and stigmastanol exhibited a sharp initial increase following cormorant

colonization ca. 1970, followed by a significant decline after ca. 1990. Prior to 1970, both sterols displayed similar patterns of fluctuation in concentration. However, the concentrations of both sterols during pre-1970 periods of increase were slightly lower than those observed after ca. 1970, when the fluctuations in concentration were more pronounced. The EBSI, representing various zoosterols and phytosterols, displayed a strong relationship with the substantial rise in cholesterol and its derivatives post-1970, further supporting the inference for a notable shift in ecosystem dynamics driven by cormorant activity. This shift was corroborated by a sharp increase in $\delta^{15}\text{N}$ values, indicative of greater nitrogen input consistent with expanding cormorant populations. A multi-proxy approach, integrating $\delta^{15}\text{N}$, a suite of sterols and stanols, and a sterol index provided robust evidence for a rise in cormorant populations beginning in ca. 1970. Moreover, cormorant activity at EBI induced substantial changes in nutrient dynamics, as evidenced by a continuous decline in the C:N ratio after 1900. In contrast, the CI site displayed typical C:N ratio fluctuations, indicative of background conditions unaffected by cormorant activity.

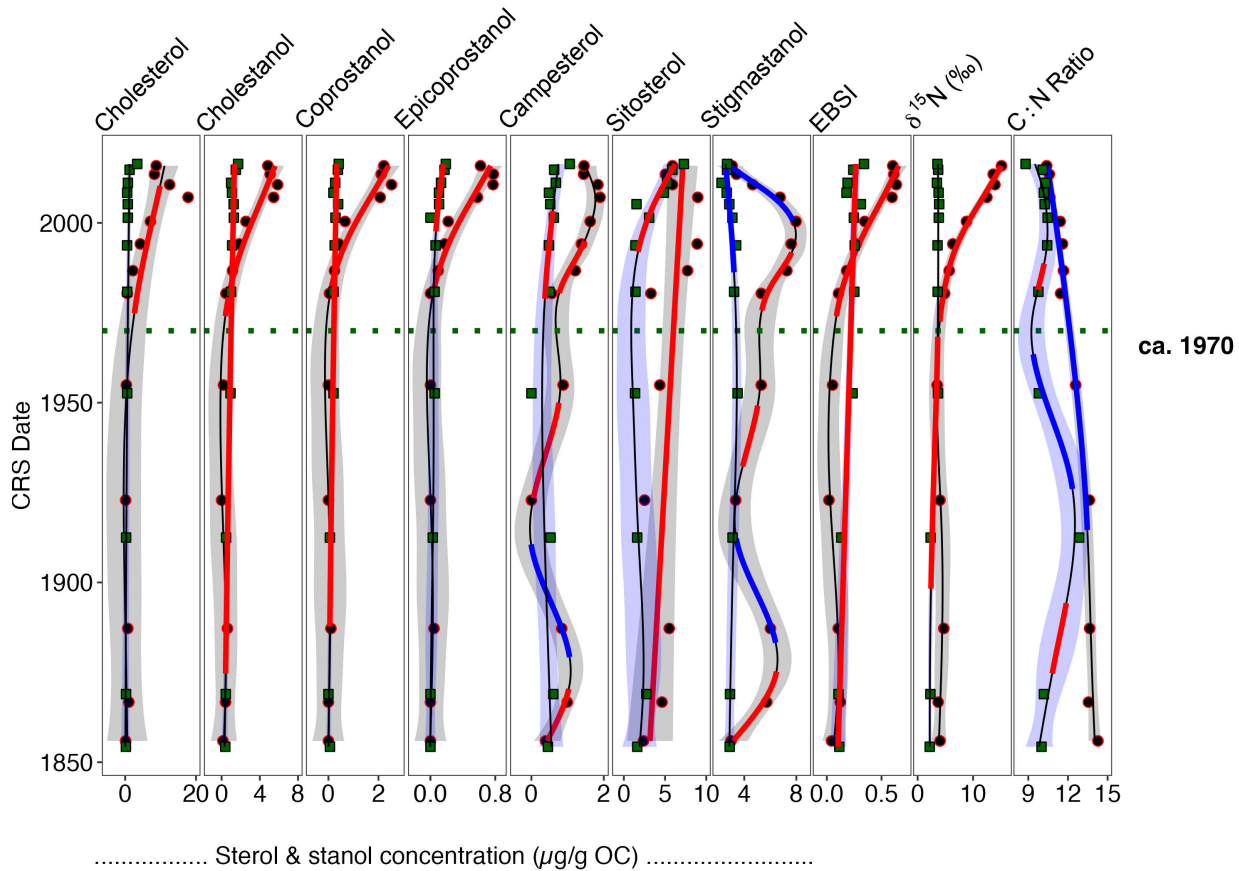


Figure 2.5 Sterol and stanol concentrations versus ^{210}Pb dates for the EBI and CI cores. The generalized additive models (GAMs) for the impacted site, EBI, are represented by black circles with gray shading (confidence interval), whereas the reference site, CI, is shown by green squares with blue shading (confidence interval). The dashed green horizontal line represents our estimated year of cormorant expansion (1970). Significant periods of increase and decrease are indicated by solid red and blue lines, respectively. The sterol profile was compared with the expanded bird sterol index (EBSI), $\delta^{15}\text{N}$, and carbon-nitrogen (C: N) ratio. The years shown were calculated using the constant rate of supply (CRS) model.

4.0 Discussion

Cormorants are recognized as influential drivers of nutrient cycling and ecological dynamics in lacustrine systems, primarily through the deposition of guano. Previous studies showed that sterol and stanol concentrations in the guano of double-crested cormorants display a large range of values, with cholesterol as the dominant sterol ($205 \pm 144 \mu\text{g/g}$, $n = 3$), reflecting their piscivorous diet (Hargan et al., 2018). The birds' expelled guano, characterized by high

nitrogen content and elevated $\delta^{15}\text{N}$ values due to their fish-based diet, acts as a nutrient pulse into local ecosystems, influencing microbial and biogeochemical processes (Kameda et al., 2006). At nesting sites, guano deposition stimulates microbial processes such as nitrification and denitrification, with subsequent volatilization of ammonia and N_2 enriching $\delta^{15}\text{N}$ in residual guano as temperatures rise and soil surfaces dry (Mizutani et al., 1986; Talbot, 2001). This nitrogen ultimately enters aquatic systems, altering the nutrient dynamics and ecosystem structure.

At EBI, a cormorant nesting site, $\delta^{15}\text{N}$ values in sediments showed a marked enrichment over time, consistent with guano deposition. Nitrogen cycling alterations caused by guano input are evident from a steep increase in $\delta^{15}\text{N}$ beginning around 1970, rising by approximately 10‰ to the present. In contrast, $\delta^{15}\text{N}$ values at Calf Island, a reference site unaffected by cormorants, remained stable ($\sim 3\text{‰}$) throughout the sediment record. This disparity highlights the localized impact of cormorant colonies and confirms that ^{15}N enrichment at EBI is primarily driven by the establishment of a local cormorant colony rather than broader environmental changes. These findings align with earlier studies that linked ^{15}N enrichment to bird population size and guano inputs (Hargan et al., 2018; Stewart et al., 2015).

The period before ca. 1970 provides a historical baseline for EBI, marked by relatively low $\delta^{15}\text{N}$ values and relatively lower cholesterol, cholestanol, cholestanone, epicoprostanol, coprostanol concentrations and EBSI values. During that time, the widespread use of organochlorine pesticides such as DDT and PCBs in North America led to significant declines in cormorant populations, caused by reduced reproductive success and eggshell thinning (Weseloh et al., 1983). The absence or low abundance of sterol biomarkers, including cholesterol and its microbial transformation products (cholestanol, coprostanol, and epicoprostanol), during that period reflects the limited cormorant presence and the adverse effects of those contaminants. Reports of cormorants returning to EBI after 2001 (Stewart et al., 2015) likely represent a recovery period following the bans on DDT and PCBs in the 1970s (Environment and Climate Change Canada, 2013).

Using sterols and $\delta^{15}\text{N}$ as proxies for historic bird numbers, this study identified a significant ecological shift at EBI beginning in the 1970s. The synchronous increase in $\delta^{15}\text{N}$ and the slight increase in the relative abundance of the algal sterol, fucosterol, during that time, indicates a shift toward algal-dominated organic matter in response to nutrient enrichment. This

observation is further corroborated by the plant-derived sterols, campesterol and stigmasterol, which showed an initial rise post-1970, reflecting enhanced nutrient inputs that temporarily supported terrestrial vegetation. However, concentrations declined after ca. 1990, perhaps because of guano toxicity, vegetation destruction for nest-building, or shifts in plant communities (Hargan et al., 2018; Stewart et al., 2015).

Cormorant guano not only alters nitrogen cycling but also impacts sediment organic matter composition, as evidenced by changes in the C:N ratio. The C:N ratio is a reliable indicator of organic matter sources in sediments, with terrestrial vascular plants exhibiting higher ratios (≥ 20) due to their lignin and cellulose content, and algae displaying lower ratios (4–10) because of their protein-rich composition (Meyers and Ishiwatari, 1993). At EBI, the steady decline in C:N ratio since ca. 1900 reflects an increasing influence of algal-derived organic matter. Presence of the algal sterol, fucosterol, absent before ca. 1970 but present thereafter, further supports the shift toward algal dominance in sediment organic matter at EBI. The comparative stability in sediment composition at Calf Island highlights the localized nature of cormorant impacts. At EBI, water chemistry analyses documented elevated nitrogen and phosphorus concentrations, driven by guano deposition (Stewart et al., 2015). Such nutrient enrichment suppresses terrestrial vegetation due to guano toxicity while promoting algal blooms, further driving the observed shifts in sediment organic matter composition. The differences between the two sites demonstrate that the magnitude of cormorant impacts is strongly influenced by bird colony size and density, as well as the susceptibility of local ecosystems to nutrient loading.

Recovery and subsequent expansion of cormorant populations in Lake Ontario after the 1970s were facilitated by multiple factors, including the ban on organochlorine contaminants, legal protections under the U.S. Migratory Bird Treaty Act, and the availability of abundant food sources such as invasive fish species (e.g., alewife, round goby) (Johnson et al., 2010; Madura and Jones, 2016; Wires and Cuthbert, 2006). These factors combined to create favorable conditions for cormorant resurgence, with profound implications for nutrient cycling, primary productivity, and sediment composition in ponds.

5.0 Conclusions

Historical changes in populations of double-crested cormorants and their ecological effects on islands in Lake Ontario were inferred from analyses of sediment cores from two ponds, which span approximately the last 170 years: Calf Island (CI), a reference site, and East Brother Island (EBI), a cormorant-affected site. By combining sterol biomarkers, $\delta^{15}\text{N}$ values, and sediment organic matter composition (C:N ratios), we documented ecosystem alterations linked to cormorant activities. At EBI, data revealed an unprecedented concurrent increase in sterol concentrations and $\delta^{15}\text{N}$ values starting around 1970, a pattern unrecorded in earlier deposits from the pond. The shift indicates a substantial rise in guano inputs, coinciding with the arrival and expansion of cormorant populations, and coincides with the regulation of organochlorine contaminants, the introduction of non-native fish species, climate warming, and other ecosystem changes. These factors collectively created optimal conditions for cormorant colony establishment and expansion in Lake Ontario. Furthermore, a general decrease in C:N ratios was observed in EBI sediments, suggesting reduced input of terrestrial plant material. That decline was likely due to vegetation degradation caused by ammonia-rich guano deposition and the removal of leaves for nest construction. This observation is corroborated by the general decline in stigmasterol concentrations following cormorant colony establishment. These results highlight the ecological changes associated with cormorant colonization, leading to cascading effects on nutrient cycling, sediment composition, and vegetation dynamics. The study underscores the value of long-term ecological information to better comprehend the drivers and impacts of rapidly changing waterbird populations.

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Conflicts of Interest

The authors declare no conflict of interest.

Open Research

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Appendix

Table S2.1 Detection limits for the eleven sterols and stanols analyzed in this study, expressed on a dry weight (DW) basis. Method detection limits (MDLs) were calculated based on a standard sample mass of 0.1 g, using the approach outlined by Loconto (2005). The MDL was computed as: $MDL = \frac{(IDL \times VE \times 100)}{(VS \times \% \text{ recovery})}$ where IDL is the instrument detection limit, VE is the final extract volume, VS is the sample mass, and % recovery is the analyte recovery efficiency. IDLs were derived by adding three times the standard deviation to the minimum detectable signal and dividing by the slope of the calibration curve.

Sterols and stanols	Average blank (ng mL ⁻¹)	Standard deviation	Minimal detectable signal (ng mL ⁻¹)	Slope of calibration curve	Instrument detection limit (ng mL ⁻¹)	Method detection limit (µg g ⁻¹ DW)
Coprostanol	0.02	0.02	0.07	0.36	0.14	0.166
Epicoprostanol	0.02	0.01	0.06	0.35	0.10	0.118
Coprostanone	1.08	0.36	2.15	0.05	21.9	26.021
Cholesterol	0.43	0.21	1.06	0.09	6.76	8.032
Cholestanol	0.31	0.10	0.62	0.11	2.83	3.363
Cholestanone	0.91	0.04	1.03	0.06	2.18	2.590
Desmosterol	5.64	0.33	6.61	0.06	15.9	18.892
Campesterol	1.12	0.13	1.50	0.15	2.50	2.970
Fucosterol	0.73	0.14	1.15	0.09	4.64	5.513
β-Sitosterol	6.52	0.40	7.72	0.03	44.5	52.874
Stigmastanol	3.43	0.45	4.77	0.02	57.1	67.845

3 CHAPTER 3: Tracking long-term trends in Sockeye salmon (*Oncorhynchus nerka*) population dynamics using sterol and stanol biomarkers in lake sediments

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Abstract

We analyzed biomarkers, including sterols, stanols, and $\delta^{15}\text{N}$, in sediment cores from lakes with well-documented sockeye salmon return histories. Our goal was to improve estimates of past changes in salmon escapement, that is, the population that return to their freshwater nursery lakes, inferred using sediment biogeochemical markers. Cholesterol, the predominant sterol in

adult sockeye salmon muscle tissue, displayed a strong positive relationship with escapement ($R^2 = 0.8$, $p = 0.001$, and $F_{1,8} = 28.3$). Sediment concentrations of the plant-derived sitosterol and algal-derived fucosterol, absent in salmon muscle tissue also related positively with salmon escapement, suggesting that salmon-derived nutrients from decomposing fish promote the production of these lipids by primary producers in the lakes. We developed a novel salmon sterol index (SSI_a) from values in surface sediments of the nine Alaskan lakes $[(\text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol})/(\text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol} + \text{fucosterol} + \text{sitosterol} + \text{stigmastanol})]$ that was strongly related with salmon return density (pseudo $R^2 = 0.86$ and RMSE = 0.071). This index also tracked historical sockeye escapement patterns and $\delta^{15}\text{N}$ values in ^{210}Pb -dated lake sediment cores from Frazer, Karluk, Red, and Kinaskan lakes that span more than a century of salmon population history, suggesting that the index has potential as a proxy for tracking historical salmon populations, particularly when used in combination with independent biomarkers of salmon-derived nutrient inputs. SSI_a and the other salmon sterol indices we developed show promise for improving and extending long-term sockeye salmon population estimates using lake sediment records, which will help inform salmon conservation and management efforts.

Plain Language Summary

Sockeye salmon are vital to both ecosystems and fisheries, but their populations have fluctuated over time as a consequence of both climate changes and human activities. To better understand these long-term population trends, we analyzed biomarkers in lake sediments, which serve as natural archives of past salmon populations. Biomarkers preserved in sediments from nine Alaskan lakes revealed that certain lipids (sterols and stanols) and stable nitrogen isotopes ($\delta^{15}\text{N}$) are reliable indicators of past salmon population numbers based on historical records. Cholesterol, a lipid found in salmon tissue, was strongly linked to historical salmon returns. Sterols from higher plants and algae also increased with salmon abundance, suggesting that nutrients from decomposing salmon boost aquatic plant and algal growth. We developed a new salmon sterol index that combines multiple sterols associated with salmon and will improve estimates of past salmon population numbers based on lake sediment records. This index closely tracked known sockeye salmon return trends over the past century, illustrating its value for inferring historical salmon populations from lake sediments. These derived salmon sterol indices

offer a new way to study long-term salmon population dynamics, which will help scientists and resource managers better protect and manage sockeye salmon populations in a changing environment.

1.0 Introduction

Five species of Pacific salmon inhabit the North Pacific waters off the coasts of Canada and the United States: coho (*Oncorhynchus kisutch*), pink (*Oncorhynchus gorbuscha*), chinook (*Oncorhynchus tshawytscha*), chum (*Oncorhynchus keta*) and sockeye salmon (*Oncorhynchus nerka*) (Pitman et al., 2020; Stachura et al., 2014). These species are vital to coastal ecosystems and human populations, providing essential nutrients such as high-quality protein, Omega-3 fatty acids, vitamins, and minerals (Ardren et al., 2014; Gladyshev et al., 2012; Porter et al., 1992). For Pacific West Coast Indigenous communities, often referred to as "Salmon People," salmon are central to cultural identity, subsistence, and spiritual practices (Reid et al., 2022). These communities have established complex salmon management and stewardship systems rooted in traditional ecological knowledge (Atlas et al., 2021; Carothers et al., 2021; Westley et al., 2021). Beyond their nutritional, and cultural significance, Pacific salmon help drive regional economies in Alaska, British Columbia, Washington, and Oregon, where commercial and recreational fisheries contribute millions of dollars annually to gross domestic product (Watson et al., 2021).

Pacific salmon also play a crucial ecological role, transferring marine-derived nutrients (e.g., nitrogen, carbon, phosphorus) into freshwater systems through post-spawning decomposition (Bilby et al., 1996; Schindler et al., 2003; Walsh et al., 2020). This nutrient transfer benefits not only aquatic systems but also riparian ecosystems, where predators such as bears, wolves, and eagles redistribute salmon-derived nutrients, enhancing terrestrial primary productivity (Bilby et al., 2003; Clinton et al., 2010; Gende et al., 2001). Studies have shown that trees and shrubs near salmon nursery lakes may derive 22 - 24% of their foliar nitrogen from salmon (James & Naiman, 2001). Thus, salmon populations influence both aquatic and terrestrial ecosystems.

Despite their ecological and economic importance, sockeye salmon populations along Canada's west coast are in decline, influenced by human pressures and natural environmental (climate) changes (Schoen et al., 2017; Shelton et al., 2021). A major driver of sockeye productivity in the North Pacific is the Pacific Decadal Oscillation (PDO), a multidecadal

climate phenomenon characterized by alternating warm and cool phases of sea surface temperature (SST) (Beamish et al., 1997; Mantua et al., 1997; Mantua & Hare, 2002; Schindler et al., 2008). Historical records suggest that during the warm phases of the PDO (e.g., 1925–1946, 1977–2006), Alaska’s sockeye salmon stocks were highly abundant, whereas BC stocks declined, with the reverse occurring during cool phases (Mantua et al., 1997). Whereas PDO influences on salmon productivity have been established using historical catch records, the relationship between PDO phases and escapement patterns requires further investigation. Understanding historical Sockeye salmon population dynamics is critical for developing effective conservation and management strategies, particularly in the context of climate change and increasing human impacts (Bottom et al., 2009; Walsh et al., 2020).

Paleolimnology has emerged as a powerful tool to reconstruct past sockeye salmon populations through lake sediment core analysis (Finney et al., 2002, 2000). Stable nitrogen isotopes ($\delta^{15}\text{N}$) and biological markers such as diatoms in lake sediments were utilized to track salmon-derived nutrient inputs from decomposing salmon carcasses over time (Finney et al., 2002). Finney et al. (2002) found that marine-derived nitrogen from decomposing sockeye salmon in lake sediments is associated with elevated $\delta^{15}\text{N}$ values, which correlate with salmon escapement. Paleoecological studies utilizing $\delta^{15}\text{N}$ in nursery lakes have elucidated salmon abundance patterns and the effects of natural factors (e.g., SST) and stressors (e.g., commercial fishing, hydroelectric dams, species introductions) (Finney et al., 2002; Rogers et al., 2013; Schindler et al., 2005). However, terrestrial inputs and lake productivity changes can also influence salmon-derived nutrients, complicating the interpretation of $\delta^{15}\text{N}$ as a proxy for escapement (Douglas et al., 2022; Finney et al., 2000). Multiple sediment variables can facilitate disentanglement of the interactions between salmon-derived and other nutrient sources in aquatic environments.

Paleolimnological advances, such as the ability to measure lipid biomarkers, have enhanced our ability to infer migratory species population dynamics from sediments (Cheng et al., 2016; Davidson et al., 2018; Duda et al., 2021, 2022; Hargan et al., 2018, 2019). Sterols and stanols are crucial for biological membrane structure and function (Dufourc, 2008), readily bind to organic matter and particles, and are incorporated into lake sediments, where they are often well preserved (Bartlett, 1987; Benfenati et al., 1994; Korosi et al., 2015). The degradation products of cholesterol, including cholestanol and cholestanone, along with other microbial

transformation products such as coprostanol and epicoprostanol, and plant-derived stanols like stigmasterol, serve as valuable biomarkers in sediments for monitoring biogeochemical processes and environmental changes (Bartlett, 1987; Bull et al., 2002). Previous studies demonstrated that the ratio of cholesterol to the sum of cholesterol and sitosterol, referred to as the "seabird index," was effective for tracking seabird-derived sterol enrichment in pond sediments from a nesting colony of northern fulmars (*Fulmarus glacialis*) in the High Arctic and that this index could be applied more broadly (Cheng et al., 2016, 2021; Hargan et al., 2018). Utilization of lipid biomarkers has been employed extensively in tracking seabird population dynamics; however, this method has not yet been applied to track sockeye salmon return densities. Sockeye salmon, characterized by elevated cholesterol levels, may contribute substantially to lake sediment sterol composition, following their post-spawning mortality. This phenomenon presents a unique opportunity to investigate the potential for using cholesterol concentrations in sediments as a biomarker for tracking sockeye salmon return densities over extended periods (decades to millennia).

We examined the relationship between sterol concentrations in lake surface sediments and known sockeye salmon return densities across nine Alaskan nursery lakes, inhabited predominantly by this species. By measuring lipid biomarkers, in conjunction with the established $\delta^{15}\text{N}$ proxy, we sought to optimize a method to track historical sockeye salmon population dynamics from information in lake sediment cores. The research focused on inferring sockeye salmon population trends during both warm and cool phases of the PDO, demonstrating how salmon return densities fluctuated in response to the large-scale climate system. We hypothesized that cholesterol concentrations in lake sediments would correlate positively with known periods of high sockeye salmon returns. It was our hope that this approach would enhance our ability to track sockeye salmon populations over extended periods, thereby informing conservation efforts and management strategies for this ecologically and economically important species.

2.0 Materials and Methods

2.1. Site Description

Nine Sockeye salmon nursery lakes in Alaska (Figure 3.1 and Table 3.1) were selected for this study based on a regression design for sockeye salmon impact in lakes that ranged from low to extremely high sockeye return densities. We used sockeye salmon escapement data to identify nine lakes that span a large range of sockeye salmon escapement densities (Frazer, Karluk, Red (aka Ayakulik Lake), Iliamna, Upper Ugashik, Upper Olga, Kijik, Becharof, and Spiridon). We used Kinaskan Lake, BC, as a reference lake because it does not receive sockeye salmon. In these Alaskan lakes, the primary ecological driver of nutrient and lipid input during peak spawning years is the annual deposition of decaying salmon carcasses. These carcasses are widely recognized as a dominant source of organic material, particularly contributing marine-derived nutrients and lipids to freshwater systems (Finney et al., 2002). While we acknowledge that other sources, such as terrestrial runoff, autochthonous algal production, or sporadic animal carcasses, may contribute to the lipid pool, extensive literature supports the predominance of salmon-derived inputs in shaping lake biogeochemistry in these systems (Finney et al., 2000; 2002, Gregory-Eaves et al., 2003; Krümmel et al., 2005). Moreover, these lakes are situated in relatively remote and minimally developed catchments, and there is limited evidence of significant anthropogenic nutrient enrichment or disturbance. Therefore, our analysis focused on tracing salmon-derived biomarkers as the primary ecological signal, with other potential inputs considered negligible in comparison.

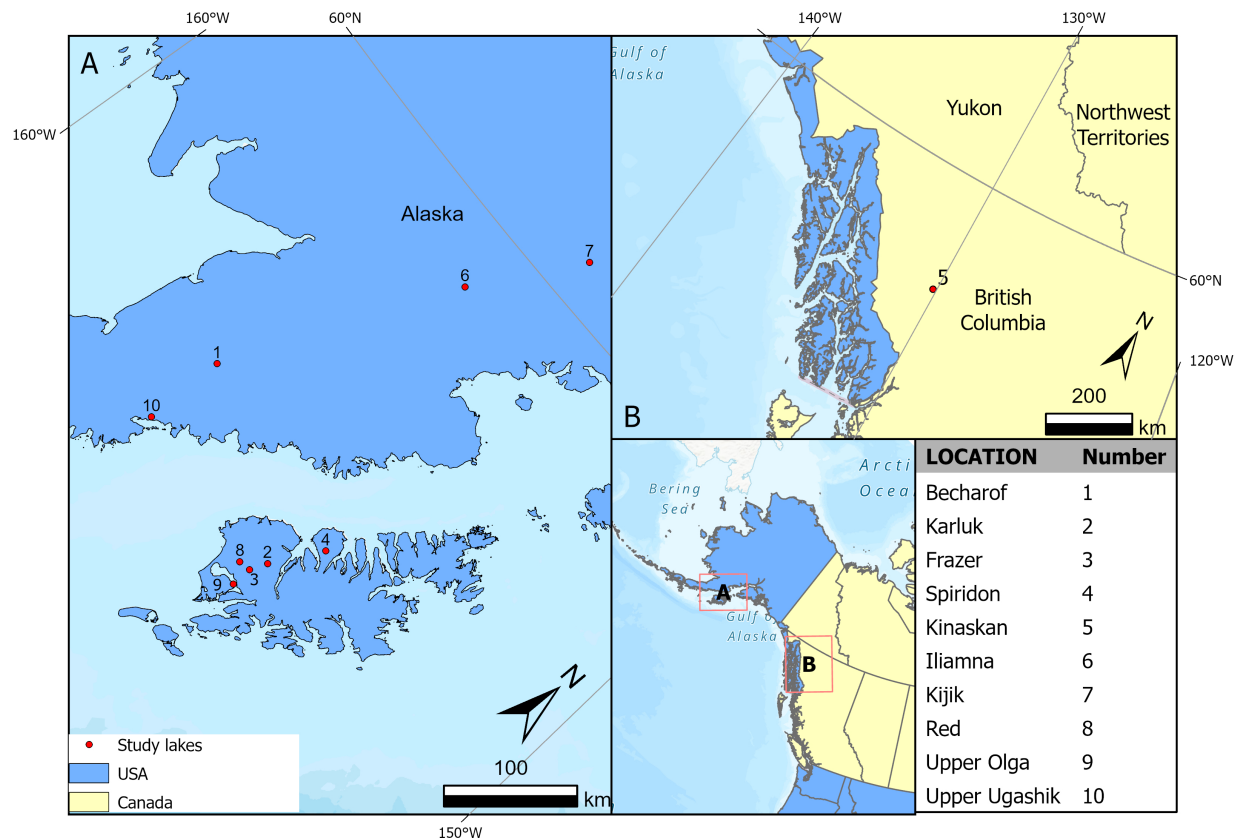


Figure 3.1 Map showing the locations of the nine sockeye salmon nursery lakes sampled in Alaska for this study, along with Kinaskan Lake in British Columbia, which does not receive sockeye salmon and served as a reference lake.

Table 3.1 Site location, coring years, and morphometry of the lakes used in this study.

Site	Location	Lat (N)	Long (W)	Coring year	Lake area (km ²)	Lake depth max (m)	Watershed area (km ²)	Salmon escapement/ lake area (km ²)
Becharof	Alaska Mainland	58°03'	58°03'	1995	1172	181	3379.45	1286
Karluk	KI, Alaska	57°25'	154°05'	1995, 2002	39.4	126	282	20034
Frazer	KI, Alaska	57°16'	154°08'	1998, 2002	16.6	59	181	12149
Spiridon	KI, Alaska	57°72'	153°68'	2002	9.2	80	> 60	204
Kinaskan	BC	57°35'	130°08'	2003	28.7	120	1251	0
Iliamna	Alaska Mainland	59°03'	155°	1997	2662	393	16888.15	2342
Kijik	Alaska Mainland	60°20'	154°22'	1999	7.8	108	> 52	1137
Red	KI, Alaska	57°15'	154°20'	1998, 2002	8.4	48	43.8	42568
Upper Olga	KI, Alaska	57°04'	154°10'	1998, 2002	7.9	70	40.1	19842
Upper Ugashik	Alaska Mainland	57°24'	157°18'	1995	212.71	~150	1291.74	4575

Note. Based on (Krümmel et al., 2005)

KI = Kodiak Island; BC = British Columbia.

2.2. Sediment Core Collection, Dating (^{210}Pb) and Stable Isotope Analysis ($\delta^{15}\text{N}$)

Lake sediment cores were collected from Becharof, Iliamna, Kijik, Upper Ugashik, Upper Olga, Karluk, Frazer, Red, Spiridon, and Kinaskan lakes between 1995 and 2003 (

Figure 3.1 and Table 3.1). Prior to sediment core collection, the bathymetry and surrounding topography of the lakes were determined using bathymetric and topographic maps. Sediment cores were subsequently extracted from the deepest, low-gradient areas of the lakes. Cores were sectioned into fine (0.5 cm) intervals, and the sediment slices were transferred into WhirlPakTM bags, sealed, and immediately frozen.

In the laboratory, wet sediments were weighed, dried at 60°C for 48 hours, and re-weighed to determine their bulk density (g/cm^3 wet) and water content. Subsamples of the sediment from selected intervals were freeze-dried and dated using ^{210}Pb and ^{137}Cs radiometric dating techniques. Sediment chronologies were established using the Constant Rate of Supply (CRS) model (Appleby, 2001). Krümmel et al. (2005) collected and dated these cores and provided the total ^{210}Pb profiles for all the dated lake sediment cores. 3-5 mg of freeze-dried sediment for each sample interval was weighed into tin capsules along with 5 mg of tungsten trioxide (WO_3) to enhance combustion efficiency (Talbot, 2001). The encapsulated samples were analyzed for total nitrogen content and nitrogen isotopic composition using a continuous-flow elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS). $\delta^{15}\text{N}$ values were reported in per mil (‰) relative to atmospheric nitrogen (Air-N_2) and calibrated using C-51 Nicotinamide and Caffeine. Freeze-dried sediments were also analyzed for $\delta^{15}\text{N}$ using two parts of tungsten trioxide. The loss-on-ignition method (Hierl, 2001) was employed to quantify organic matter content in the sediment samples.

2.3. Lake Sediment Core Sterol and Stanol Analysis

Sterols and stanols were extracted and analyzed from lake sediment core samples. They included 5β -cholestan- 3β -ol (commonly referred to as coprostanol), 5β -cholestan- 3α -ol (epicoprostanol), 5β -cholestan-3-one (coprostanone), cholest-5-en- 3β -ol (cholesterol), 5α -cholestan- 3β -ol (cholestanol), 5α -cholestan-3-one (cholestanone), 3β -cholesta-5,24-dien- 3β -ol (desmosterol), campest-5-en- 3β -ol (campesterol), stigmasta-5,24-dien- 3β -ol (fucosterol), β -sitosterol (sitosterol), and 5α -stigmastan- 3β -ol (stigmastanol). The sterol and stanol extraction

procedure began with the preparation of 0.1 g copper powder, cleaned using dichloromethane (DCM). Subsequently, subsamples from selected intervals in the sediment core from all the lakes were freeze-dried, after which approximately 0.1 g of dry sediment was added to the cleaned 0.1 g of copper powder. Samples were then spiked with 50 μ L of deuterated cholesterol- d_6 recovery standard (10 μ g/mL) (Cambridge Isotope Laboratories, Tewksbury, MA, USA) and extracted three times by sonication with 10 mL DCM. Extracts were then evaporated to 1 mL at 35 °C at a nitrogen pressure of 0.4 bar/6 p.s.i. Subsequently, 1 g SupelcleanTM LC-Si solid-phase extraction cartridges (Sigma-Aldrich, St. Louis, MO, USA) were preconditioned with 6 mL of DCM before loading, allowing the extract to adsorb to the column before eluting using 30 mL DCM. For derivatization, samples were evaporated to dryness under a nitrogen stream and then 0.1 mL of the derivative, BSTFA + 1% TMS (N, O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane) (Sigma-Aldrich, St. Louis, MO, USA) was added. Samples were heated for 2 hours at 60 °C and then cooled. Following cooling, 0.4 mL toluene was added prior to spiking with 10 μ L of the internal standard, p-terphenyl- d_{14} (10 μ g/mL) (Cambridge Isotope Laboratories, Tewksbury, MA, USA) as an internal standard and analyzed using gas chromatography/mass spectrometry (Birk et al., 2012; Cheng et al., 2016). Mean recovery of sterols and stanols was 95%. All sterol and stanol concentrations were corrected for recovery and subsequently adjusted for method blanks. The method detection limits (MDLs) were calculated based on the procedure described by Loconto (2005), with details provided in supplementary information (Table S3.1).

2.4. Sockeye Salmon Muscle Tissue Sterol Analysis

Three adult Sockeye salmon specimens from Alaska were analyzed in this study. Sockeye salmon from both BC and Alaska spend the majority of their marine life stage in overlapping regions of the North Pacific Ocean, including the Gulf of Alaska and the Bering Sea, where they share access to similar dietary resources, such as zooplankton and micronekton (Beacham et al., 2014; Graham et al., 2021; Peterman, 1984; Radchenko & Mathisen, 2004). Upon arrival at the laboratory, 3 g of muscle tissue from each specimen were homogenized, pulverized, and analyzed for sterols and stanols following the method described for lake sediment analysis.

2.5. Pacific Decadal Oscillation (PDO) and Sockeye Salmon Escapement Datasets

The PDO dataset for the North Pacific was obtained from the National Oceanic and Atmospheric Administration National Centre for Environmental Information (NCEI, 2024). The data comprise monthly PDO indices from January 1854 to February 2024. The PDO exhibits a positive value (i.e. warm phase) when sea level pressure is below average over the North Pacific and when SSTs are anomalously cool in the interior North Pacific and warm along North America's Pacific Coast. The PDO exhibits a negative value (i.e. cool phase) when the climate anomaly patterns are reversed, with warm SST anomalies in the interior and cool SST anomalies along the North American coast or when sea level pressures over the North Pacific are higher than typical. We calculated and used the mean annual PDO indices spanning the ^{210}Pb -dated age range for our lake sediment cores.

Enumeration of sockeye salmon that return to their natal lake to spawn is conducted using different methods, such as visual observation, sonar technology, and fish traps (Hatch et al., 1994; Holmes et al., 2006; Parsons & Skalski, 2010). For instance, Frazer Lake has a fish pass comprised of a diversion weir and many fish ladders, designed to aid salmon navigate around its 30m high waterfall (Fuerst, 2017). Salmon counts are usually conducted in the upstream section of the fish pass. Information from these monitoring activities is crucial for managing and preserving the lake's salmon populations and ecosystems. We obtained the sockeye salmon escapement dataset for Frazer Lake, Karluk Lake, and Red Lake (Fuerst, 2017) and subsequently extracted the annual Sockeye salmon escapement, which we matched to ^{210}Pb dates (depths) in all the study lakes. Kinaskan Lake, the reference lake, does not have salmon escapement.

2.6. Statistical Analysis

Concentrations of sterols and stanols in the muscle tissue of the three analyzed sockeye salmon specimens were quantified and are presented as bar plots with means and standard deviations. The distribution of sterols and stanols in lake sediments was determined by calculating the percent relative abundance of these compounds in lake surface sediment across the nine studied lakes and subsequently presenting the results using bar plots. We investigated the relationship between sterols and stanol concentrations in lake surface sediments and sockeye

escapement density using simple linear regression. Prior to simple linear regression analysis, concentrations of sterols and stanols that did not have non-detect values (e.g., cholesterol, sitosterol, and epicoprostanol) were log-transformed to stabilize variance and normalize their distribution. However, for sterols and stanols with concentrations below the method detection limits, non-detect values were replaced with values of the detection limit divided by the square root of 2 (Hornung & Reed, 1990; Succop et al., 2004). We then stabilized their variance by subjecting them to a $\log(x+1)$ transformation, where x represents the sterol concentration. To account for the limited sample size and reduce bias in model evaluation, we reported adjusted R^2 values, which incorporate the number of predictors relative to sample size and are more appropriate for small datasets. To further address potential heteroskedasticity and improve the robustness of inference, we applied ordinary least squares regression with heteroskedasticity-consistent standard errors, specifically using the HC3 estimator implemented via the `vcovHC()` function in the sandwich package in R. This method is particularly suited for small-sample contexts and helps reduce sensitivity to influential observations, thereby mitigating the risk of overfitting and improving the reliability of the regression results. All statistical analyses were conducted using R (R Core Team, 2024).

We explored the use of sterol ratios in the lake sediments as a way to track sockeye salmon return density. Previous studies of waterbird impacts on various ponds were conducted on both spatial and temporal scales utilizing multiple sterol ratios. The Seabird index ($\text{cholesterol}/(\text{cholesterol} + \text{sitosterol})$) was employed as an index to monitor seabird influence on High Arctic ponds at Cape Vera, Nunavut, northern Canada (Cheng et al., 2016). Recognizing the limitations of the initial seabird index, researchers developed the expanded sterol index (ESI) to include stanols, $(\text{cholesterol} + \text{cholestanol})/(\text{cholesterol} + \text{cholestanol} + \text{sitosterol} + \text{stigmastanol})$, which reflected the substantial quantities of stigmastanol in sediment samples as well as the inclusion of the aerobic degradation product of cholesterol, 5α -cholestanol, to track the population dynamics of waterbirds in ponds on islands across Lake Ontario, on the Canadian-US border (Hargan et al., 2018). The ESI provided a more comprehensive assessment of waterbird influence by incorporating a broader spectrum of zoosterol and phytosterol biomarkers.

Drawing inspiration from those studies, we considered how we could adapt the approach to our study of sockeye salmon populations and their impact on lake ecosystems. To accomplish

that, we used Partial Least Squares (PLS) regression analysis, given its ability to address multicollinearity among sterols and reduce dimensionality through the construction of new components that maximize the shared variance between sterols and sockeye salmon escapement density. Prior to PLS analysis, zero values in the sterol and stanol dataset, representing concentrations below the detection limits, were replaced with the detection limit divided by the square root of two (Hornung & Reed, 1990; Succop et al., 2004). All sterols and stanols were $\log_{10}$ -transformed to stabilize variance and normalized using z-scores (mean-centering and scaling to unit variance). Model performance and predictive reliability were evaluated using nine-fold cross-validation implemented using the `plsr()` function in R. Cross-validated Q^2 values were used to assess the proportion of variance in sockeye salmon escapement density explained by each component. Through application of PLS, key sterols were identified based on their variable importance for the projection (VIP) score. Sterols with a VIP score > 1 demonstrated the highest capacity to predict sockeye salmon density. Subsequently, selected sterols were validated by their ability to elucidate the variance in sockeye salmon escapement density within the PLS model. Identified sterols were further utilized to develop seven distinct salmon sterol indices (SSIs), which were examined for their ability to track sockeye salmon escapement using data from surface sediments. This was achieved by fitting the function

$$y = a \left(\frac{(x)}{(b+x)} \right) \quad Eq. 1$$

where y = SSI, x = sockeye salmon escapement density, and a and b are fitted constants.

To further elucidate the dynamics of sterols, stanols, SSIs, and $\delta^{15}\text{N}$ measurements in ^{210}Pb -dated lake sediment cores, we incorporated visualization of historical data and employed advanced statistical modelling of the sediment core proxy measurements. All $\delta^{15}\text{N}$ values, as well as sterol and stanol concentrations measured in dated lake sediment cores were square root transformed and subsequently fitted using generalized additive models (GAMs). GAMs were selected for their efficacy as statistical tools for modelling potentially complex and non-linear relationships, providing appropriate accounts of model uncertainty and identifying periods of significant temporal change in sterol and stanol profiles and $\delta^{15}\text{N}$ values by determining when a GAM's first derivative exhibited a non-zero rate of change. These statistical analyses were conducted utilizing the R workspace's packages *gratia* v.0.9.2 (Simpson, 2024) and *mgcv* v.1.9.1 (Wood, 2017).

3.0 Results

3.1. Sterols in Adult Sockeye Salmon Tissue

Analysis of 11 sterols and stanols in sockeye salmon muscle tissue revealed that seven sterols were below the detection limit: fucosterol, campesterol, sitosterol, coprostanol, epicoprostanol, stigmastanol, and cholestanone (Figure S3.1). This suggests that these compounds are either absent or present at extremely low concentrations in the muscle tissue. However, four sterols that were detected displayed a broad range of concentrations. Cholesterol was predominant, measured at $\sim 1270 \pm 4.1$ $\mu\text{g/g}$ dry weight (SD), accounting for $\sim 98\%$ of the total sterol content. Other stanols, stanones, and sterols, detected in lower amounts, included cholestanol (4.2 ± 0.2 $\mu\text{g/g}$ SD), coprostanone (18.3 ± 2.5 $\mu\text{g/g}$ SD), and desmosterol (6.3 ± 0.4 $\mu\text{g/g}$ SD) (Figure S3.1).

3.2. Percent Relative Abundances and Ratios of Sterols and Stanols in Lake Surface Sediments

To examine the ubiquity and potential biological sources of sterols and stanols in the surface sediments of sockeye salmon nursery lakes, we calculated their relative abundances in the surface sediments of the nine nursery lakes (Figure 3.2). Sitosterol emerged as the predominant compound, followed closely by its stanol derivative, stigmastanol. Sitosterol concentrations were notably high in Spiridon, Upper Ugashik, and Becharof lakes. The next most abundant sterols detected were cholesterol and its derivatives. The compounds epicoprostanol, coprostanol, and fucosterol were present in all nine lakes but at consistently low relative abundances.

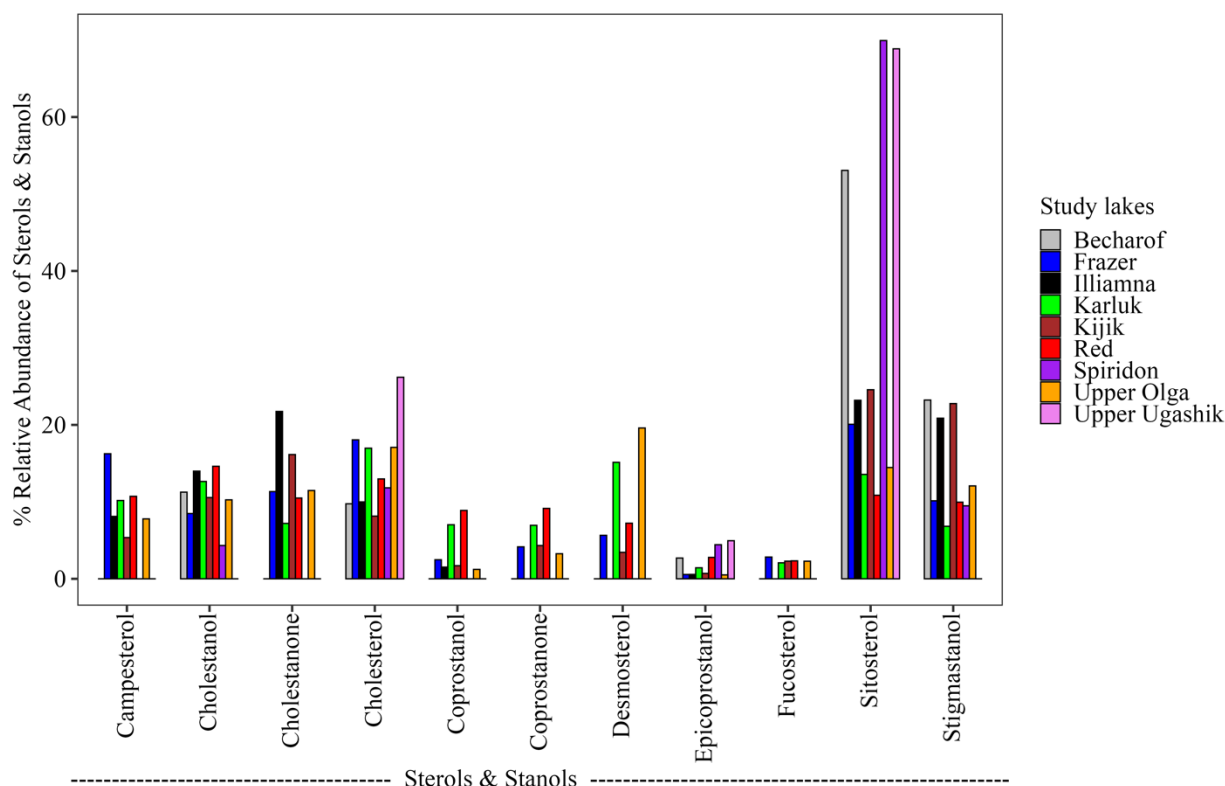


Figure 3.2 Percent relative abundances of sterols and stanols in lake surface sediments ($n = 9$). All sterols and stanols are ubiquitous in surface sediments of sockeye salmon nursery lakes. Sitosterol, cholesterol and their derivatives are highly abundant in the nine study lakes. Kinaskan Lake was excluded from the analysis due to non-detectable levels of sterols.

3.3. Relating Sterol and Stanol Concentrations in Lake Surface Sediments to Sockeye Salmon Escapement/Lake Area

We investigated the utility of sterols and stanols in lake surface sediments as sockeye salmon escapement proxies using simple linear regression (Figure S3.2, Table S3.2). Of the sterols considered here, only cholestanol, cholestanone, and stigmastanol were not significantly related to salmon return density ($p = 0.05$, $p = 0.05$, and $p = 0.10$, respectively) (Figure S3.2, Table S3.2). However, most sterols and stanols in surface sediments of our lake set showed a significant positive relationship with sockeye salmon escapement density. Cholesterol, the predominant sterol in sockeye salmon tissue, showed the strongest relationship with sockeye salmon return density ($R^2 = 0.8$, $p = 0.001$, $F_{1,8} = 28.29$) (Figure 3.3A). Epicoprostanol, a

hydrogenated derivative of cholesterol that is formed through the microbial reduction of cholesterol to coprostanol in the gut and subsequently reduced under anoxic sediment conditions, also exhibited a strong positive relationship with salmon return density ($R^2 = 0.6$, $p = 0.013$, $F_{1,8} = 10.89$) (Figure 3.3C). The main plant sterols in lake surface sediment, sitosterol, ($R^2 = 0.5$, $p = 0.03$, $F_{1,8} = 7.75$), campesterol ($R^2 = 0.5$, $p = 0.02$, $F_{1,8} = 8.49$), and the algal sterol, fucosterol, ($R^2 = 0.7$, $p = 0.005$, $F_{1,8} = 16.73$), which were absent in Sockeye salmon muscle tissue, were also positively related to salmon return densities (Figure 3.3B, Figure S3.2, Table S3.2). We also observed a strong positive relationship ($R^2 = 0.7$, $p = 0.004$, $F_{1,8} = 17.15$) between coprostanone in lake surface sediments and sockeye salmon escapement density (Figure S3.2, Table S3.2).

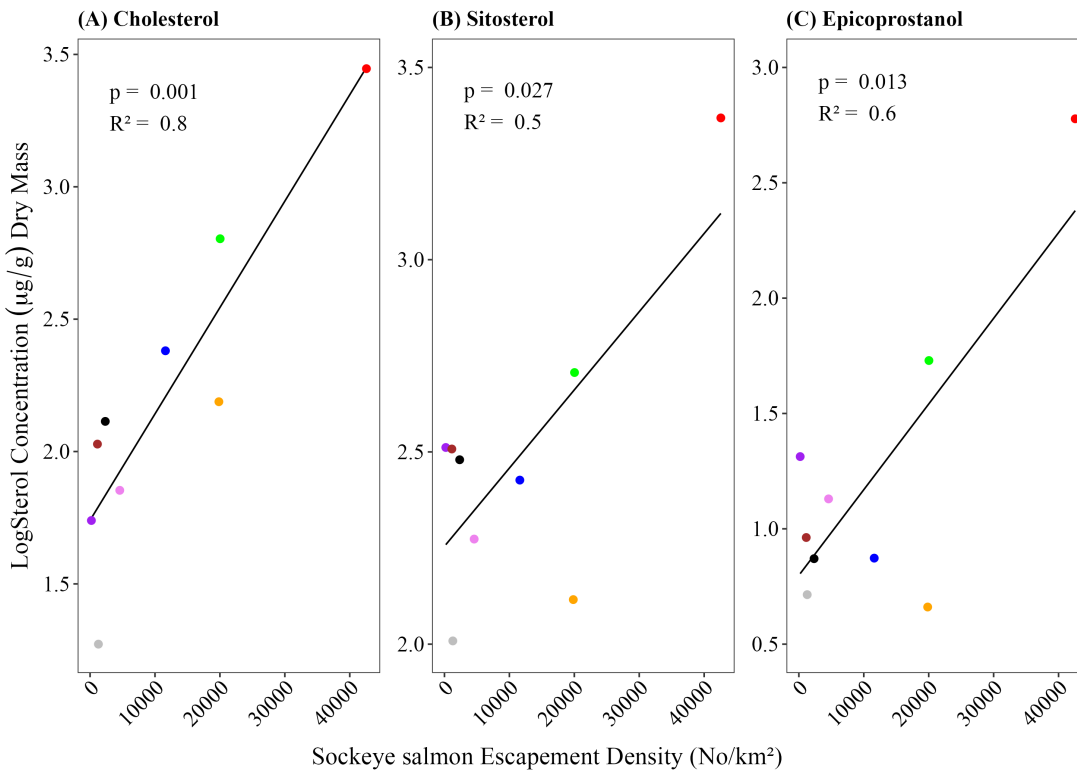


Figure 3.3 Relationships between sterol and stanol concentrations in lake surface sediments and sockeye salmon escapement density. Panels (A) - (C) show the linear relationships between sockeye salmon escapement density and log-transformed concentrations of (A) cholesterol, (B) sitosterol, and (C) epicoprostanol in lake surface sediment, revealing statistically significant ($p < 0.05$) coefficients of determination (R^2) values. Each data point represents one of nine Alaskan lakes: Spiridon (purple), Frazer (blue), Karluk (green), Red (red), Upper Olga (orange), Iliamna (black), Upper Ugashik (violet), Kijik (brown), and Becharof (gray).

3.4. Relating SSIs to Sockeye Salmon Escapement/Lake Area

The PLS model identified a specific sterol subset that is strongly correlated with sockeye salmon return density across the nine study lakes, leading to the development of a novel SSI for lake sediments. In the PLS model, sockeye salmon escapement density across the nine study lakes was explained by PLS component 1 (76%) and PLS component 2 (12%) (Figure 3.4). The PLS model was fitted using absolute concentrations of sterols and stanols as the predictors of sockeye salmon escapement density, that is, the response variable. From the model, we determined the influence of each sterol and stanol in tracking sockeye salmon escapement density. Our results demonstrated that cholesterol, epicoprostanol, coprostanone, desmosterol, and fucosterol had a VIP score > 1 , indicating that these sterols contributed strongly to predicting sockeye escapement density (Figure S3.3). The next most influential sterol in the model was sitosterol, which was present in surface sediments across the nine lakes in high concentrations and exhibited a VIP score of 0.95 in the model.

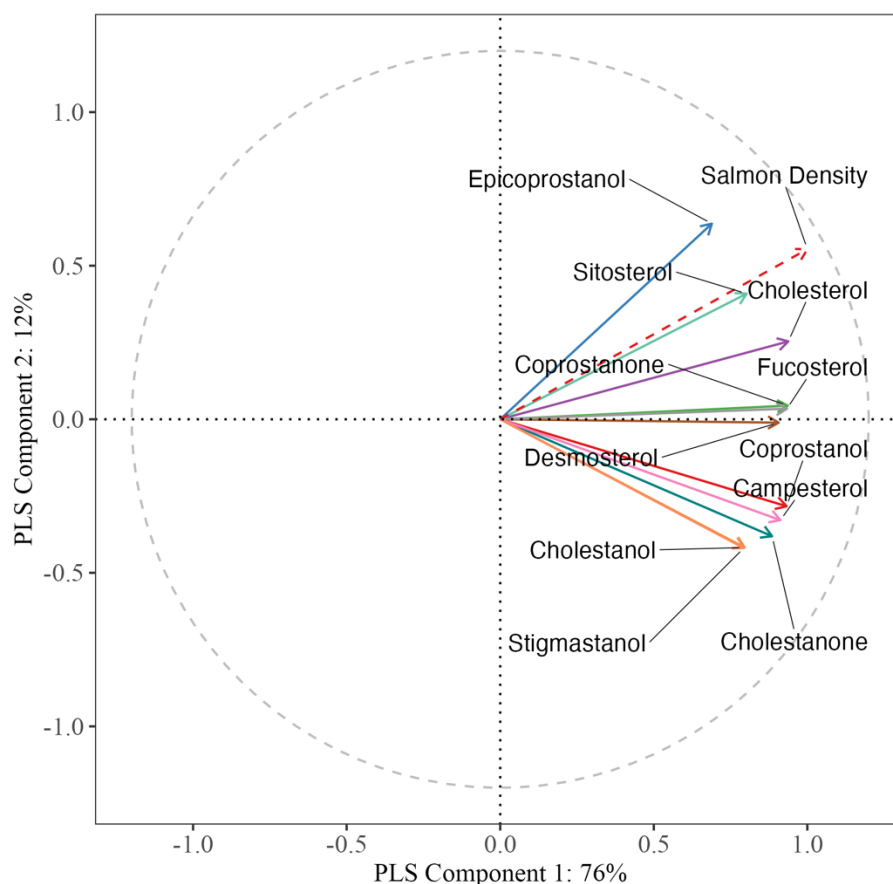


Figure 3.4 Correlation circle of partial least squares (PLS) regression, illustrating the relationships between sterols and stanols and salmon return density. The PLS biplot depicts the loadings of sterols and stanols (arrows) and the first two PLS components, which accounted for 76% and 12% of the total variance, respectively. Cholesterol, epicoprostanol, coprostanone, sitosterol, fucosterol, and desmosterol are situated in closer proximity to the salmon density, indicating a potential positive association.

We identified seven SSIs (i.e. SSI_a ... SSI_g) that were robust in tracking sockeye salmon escapement, using both lake surface sediments and dated sediment cores from lakes with known escapement histories, Frazer, Karluk, and Red Lakes (Table S3.3, Figure S3.4). SSI_a was selected as the optimal SSI, as it effectively incorporated key sterols associated with sockeye salmon. The numerator of SSI_a integrated cholesterol, the dominant sterol in sockeye salmon muscle tissue, alongside its metabolite coprostanone and its precursor desmosterol, all of which were detected in different concentrations across the sockeye salmon muscle tissue samples.

Additionally, the index incorporated the reduction products of cholesterol (coprostanone and epicoprostanol), which are primarily introduced into lake sediments following post-spawning, large-scale sockeye salmon decomposition. Consequently, cholesterol, desmosterol, coprostanone, and epicoprostanol were identified as the primary sterol inputs into lake sediments from sockeye salmon decomposition. Furthermore, although fucosterol and sitosterol were not directly derived from the salmon tissue or its sterol-reduction products, their inclusion in the model was necessary, as their high concentrations in lake sediments were ultimately driven by nutrient inputs from the salmon to the lake system. Although stigmastanol a reduction product of sitosterol, did not exhibit a significant relationship with salmon density in the PLS model, its incorporation into the index was essential because of its high concentrations across sediment samples from the study lakes. Inclusion of fucosterol, sitosterol, and stigmastanol enabled our model to capture a broader spectrum of zoosterol and phytosterol biomarkers, enhancing the accuracy and reliability of SSI_a in tracking sockeye salmon escapement.

We further investigated the relationship between sockeye salmon escapement density and the SSIs using a non-linear regression model based on the function presented in Eq. 1. The fitted model revealed a strong, statistically significant relationship between SSIs and salmon escapement density (Figure 3.5, Figure S3.4, and Table S3.3). Model parameters a and b were statistically significant for all SSIs from SSI_a through SSI_g , except SSI_c , for which the parameter b was not significant. Excluding SSI_c , the results suggest that both parameters a and b were crucial in explaining variation in the SSIs. The scatter plot of the data points, colour-coded by lake, illustrates a clear trend - as escapement density increases, the SSI also increases, following an asymptotic pattern characteristic of the function shown in Eq. 1.

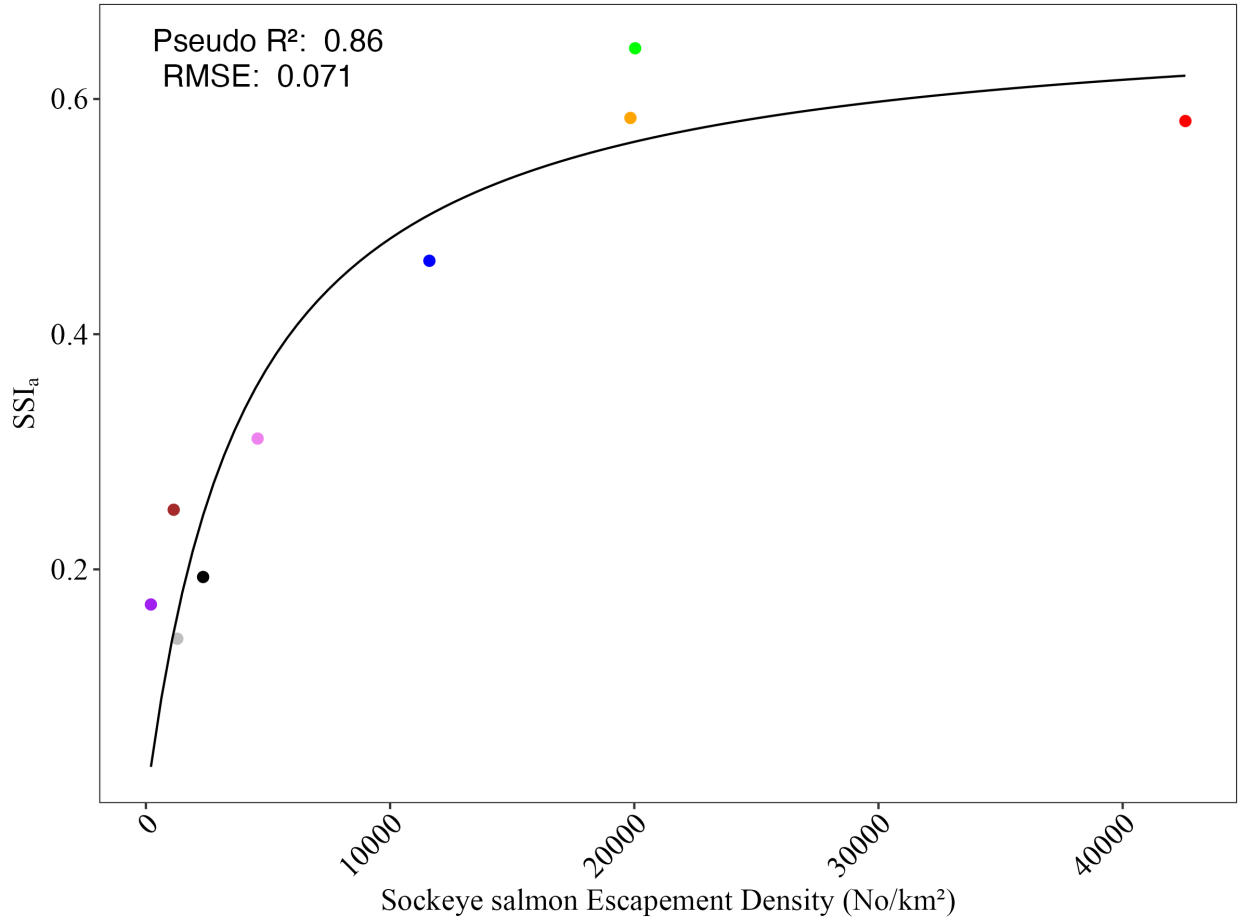


Figure 3.5 Relationship between salmon sterol index (SSI_a) [$(SSI_a = \text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol}) / (\text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol} + \text{fucosterol} + \text{sitosterol} + \text{stigmastanol})$] in lake surface sediments and sockeye salmon escapement density. The model was fitted using the non-linear function presented in Eq. 1. Statistical significance is indicated at $p < 0.05$. The fitted model demonstrated an R^2 of 0.86, and RMSE of 0.071 with the fitted constants $a = 0.68$ ($p < 0.001$) and $b = 4128$ ($p = 0.037$), reflecting a strong relationship between escapement density and the sterol index.

3.5. Reconstructing Sockeye salmon production dynamics using $\delta^{15}\text{N}$, sterols and stanols, SSIs, and the PDO

We assessed the potential of sterols, stanols, and their indices (SSIs) as proxies for sockeye salmon escapement by integrating their profiles with $\delta^{15}\text{N}$ in ^{210}Pb -dated sediment cores from Kinaskan, Frazer, Karluk, and Red lakes. Kinaskan Lake, a non-Sockeye salmon lake, served as a reference site in this study. Between 1920 and 1985, $\delta^{15}\text{N}$ values in Kinaskan Lake

increased from 0 to 3 ‰. These $\delta^{15}\text{N}$ values aligned with those recorded in pre-sockeye periods at Frazer Lake, confirming Kinaskan Lake's "pristine status" (Figure 3.6, Figure S3.8). Additionally, the absence of elevated cholesterol, epicoprostanol, and coprostanone in the sediments of Kinaskan Lake demonstrates that these sterols display low concentrations in lake sediments unaffected by sockeye salmon. All other sterols and stanols were below detection limits throughout the entire sediment core. In contrast, Frazer Lake, where sockeye salmon were introduced in 1951, exhibited a corresponding shift in sterols, SSIs and $\delta^{15}\text{N}$. Prior to 1951, both cholesterol and cholestanol remained stable at low concentrations. However, following the introduction of sockeye salmon, particularly after the construction of the diversion weir in 1971, there was a sharp increase in cholesterol, $\delta^{15}\text{N}$, SSIs, and other sterols, coinciding with the return of sockeye salmon to spawn (Figure 3.7, Figure S3.5 and Figure S3.9). This marked increase in sediment sterols, SSIs, and $\delta^{15}\text{N}$ indicates a concordance between salmon escapement density and the sediment records. Karluk Lake, a historically important sockeye salmon nursery, also showed strong relationships between $\delta^{15}\text{N}$ values, the SSIs, and sockeye salmon escapement density. Dynamics in this lake were notably influenced by the Pacific Decadal Oscillation (PDO), with the warm phases of the PDO corresponding to higher sockeye salmon escapement densities, elevated $\delta^{15}\text{N}$ values, and higher SSIs (Figure 3.8, Figure S3.6 and Figure S3.10). Although the increased salmon escapement during the 1925-1947 warm phase of the PDO was not captured by SSI_a (Figure 8), the SSI_c , SSI_e , and SSI_g indices appear to have captured this period of higher salmon escapement densities (Figure S3.6). Conversely, the cooler phases of the PDO were associated with reduced values of these variables. In Red Lake, another major sockeye salmon producer on Kodiak Island (KI), we observed increased SSI_a coeval with salmon escapement during the 1976-2006 warm phase of the PDO (Figure 3.9), whereas $\delta^{15}\text{N}$ showed an opposite (declining) pattern during that time. Unlike $\delta^{15}\text{N}$, coprostanone, epicoprostanol, desmosterol and SSIs aligned with increased sockeye salmon return density during the warm phase of the PDO (Figure 9, Figure S7 and Figure S11). These sterol markers displayed lower and relatively constant concentrations during the cooler PDO phase.

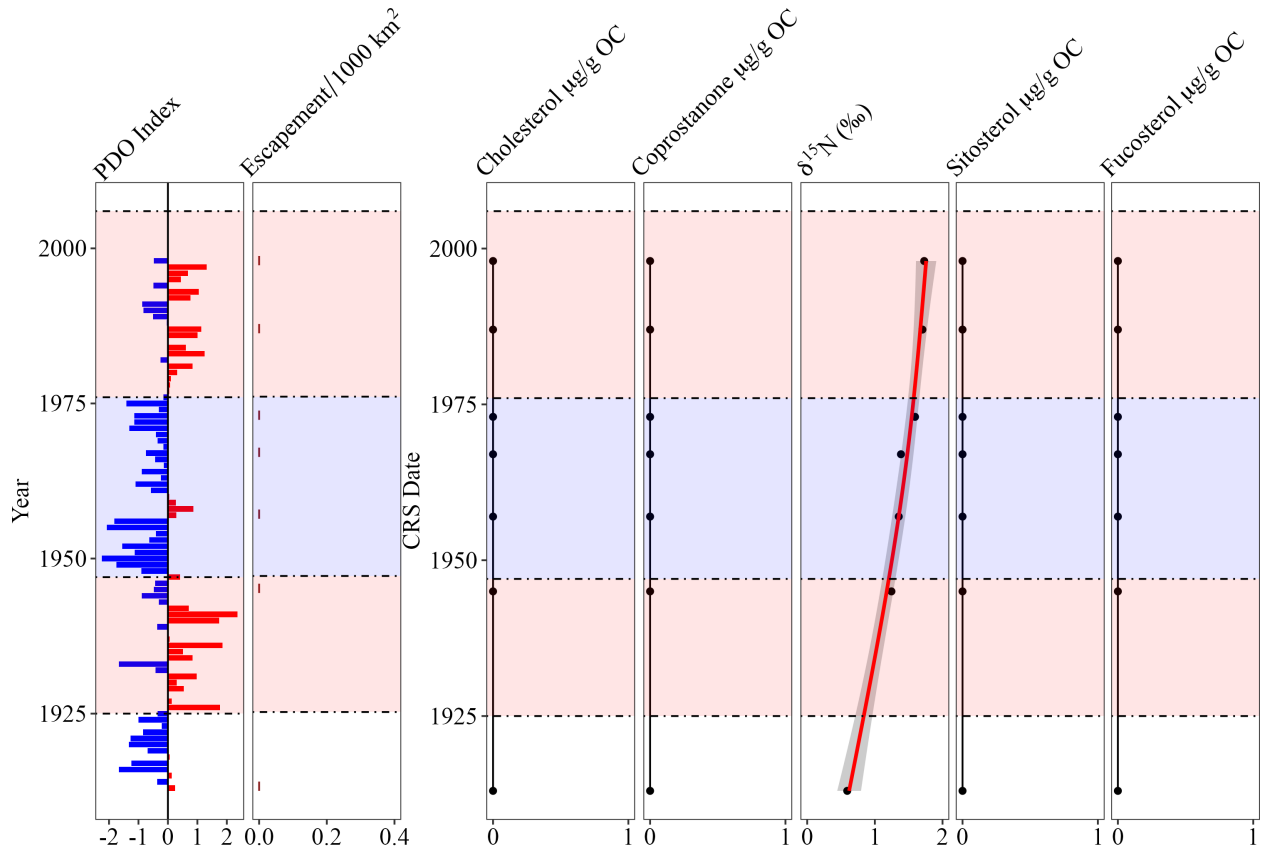


Figure 3.6 The warm (1925 – 1947 and 1976 – 2006) (shaded in red) and cool (1947 – 1976) (shaded in blue) phases of the Pacific Decadal Oscillation (PDO), and sockeye salmon escapement density versus downcore profiles of cholesterol, coprostanone, $\delta^{15}\text{N}$, sitosterol, and fucosterol in Kinaskan Lake sediments, modelled using generalized additive models (GAMs). The profile for $\delta^{15}\text{N}$ was reconstructed using square-root-transformed values, and the shaded area around $\delta^{15}\text{N}$ represents the 95% confidence interval of our smoothed estimate. Kinaskan Lake (the reference lake) has no salmon run and exhibited a relatively low $\delta^{15}\text{N}$ (0 - 3 ‰) and sterols were below detection limits. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model. Note: Salmon sterol index_a (SSI_a) was not shown because of non-detectable sterol values.

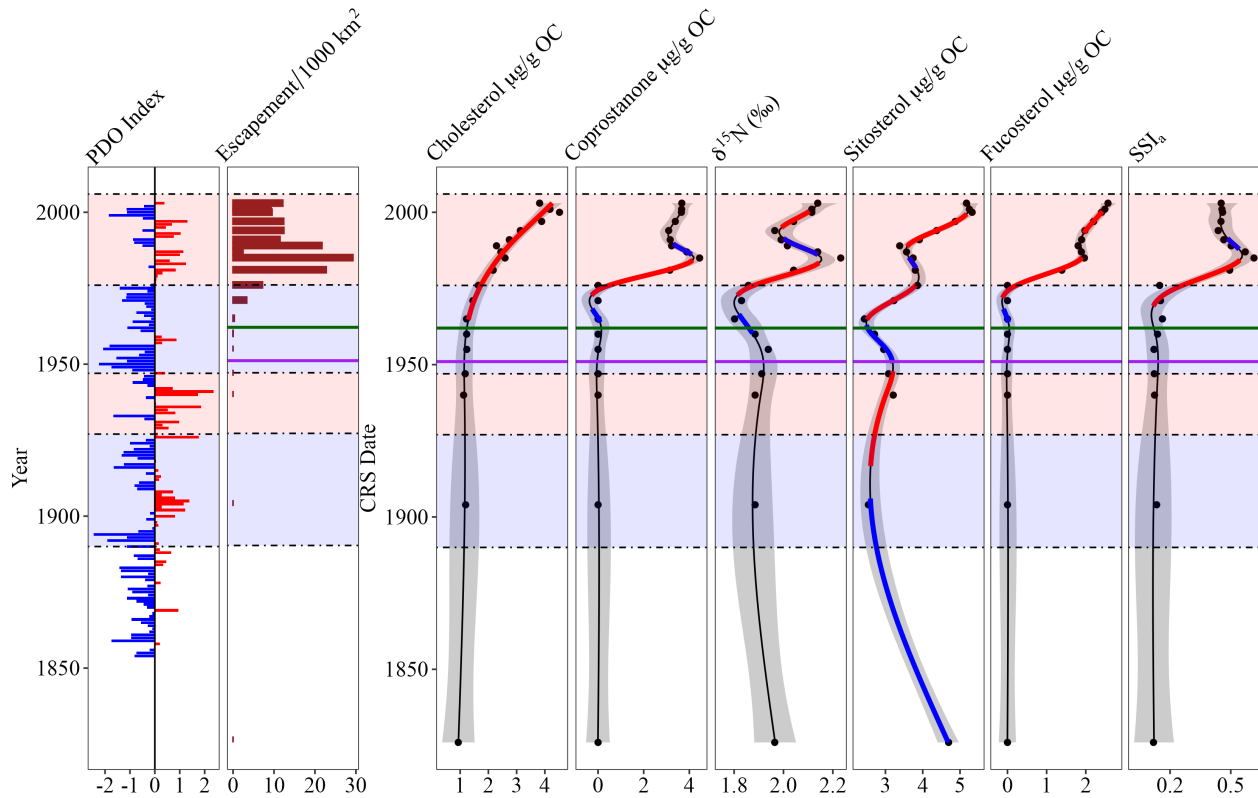


Figure 3.7 The warm (1925 – 1947 CE and 1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific Decadal Oscillation (PDO), and sockeye salmon escapement versus downcore profiles of square-root-transformed cholesterol, coprostanone, $\delta^{15}\text{N}$, sitosterol, fucosterol, and salmon sterol index_a (SSI_a) [$(\text{SSI}_a = \text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol}) / (\text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol} + \text{fucosterol} + \text{sitosterol} + \text{stigmastanol})$] in Frazer Lake sediments, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. The horizontal solid purple and green lines represent 1951, when the lake was fertilized, and 1962, when a fish ladder was constructed, respectively. Core dates were assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

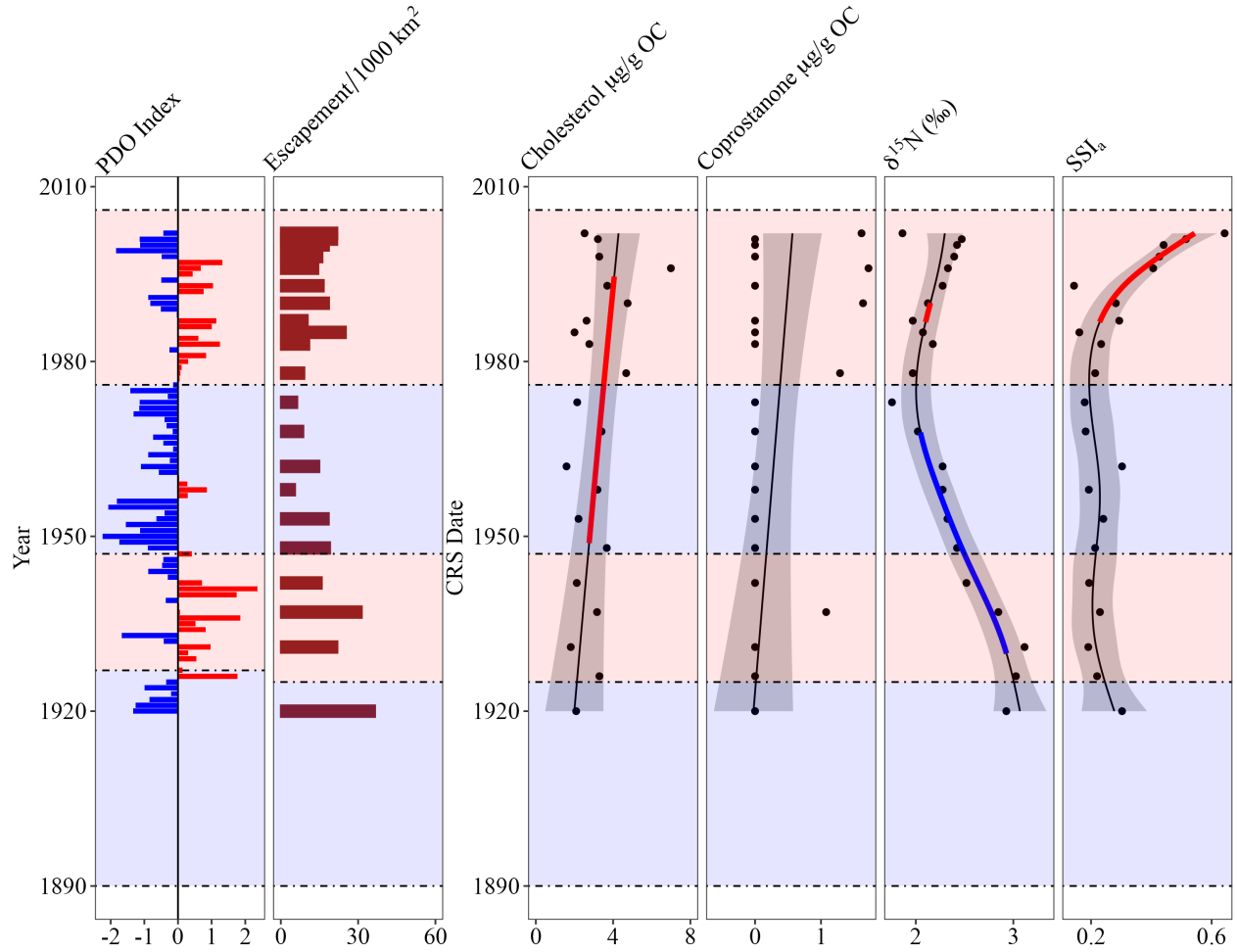


Figure 3.8 The warm (1925 – 1947 CE and 1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific Decadal Oscillation (PDO), and sockeye salmon escapement versus the downcore profiles of cholesterol, coprostanone, $\delta^{15}\text{N}$, and salmon sterol index_a (SSI_a) [$(\text{SSI}_a = \text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol})/(\text{cholesterol} + \text{coprostanone} + \text{epicoprostanol} + \text{desmosterol} + \text{fucosterol} + \text{sitosterol} + \text{stigmastanol})$] dynamics in Karluk Lake sediments, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. Core dates were assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

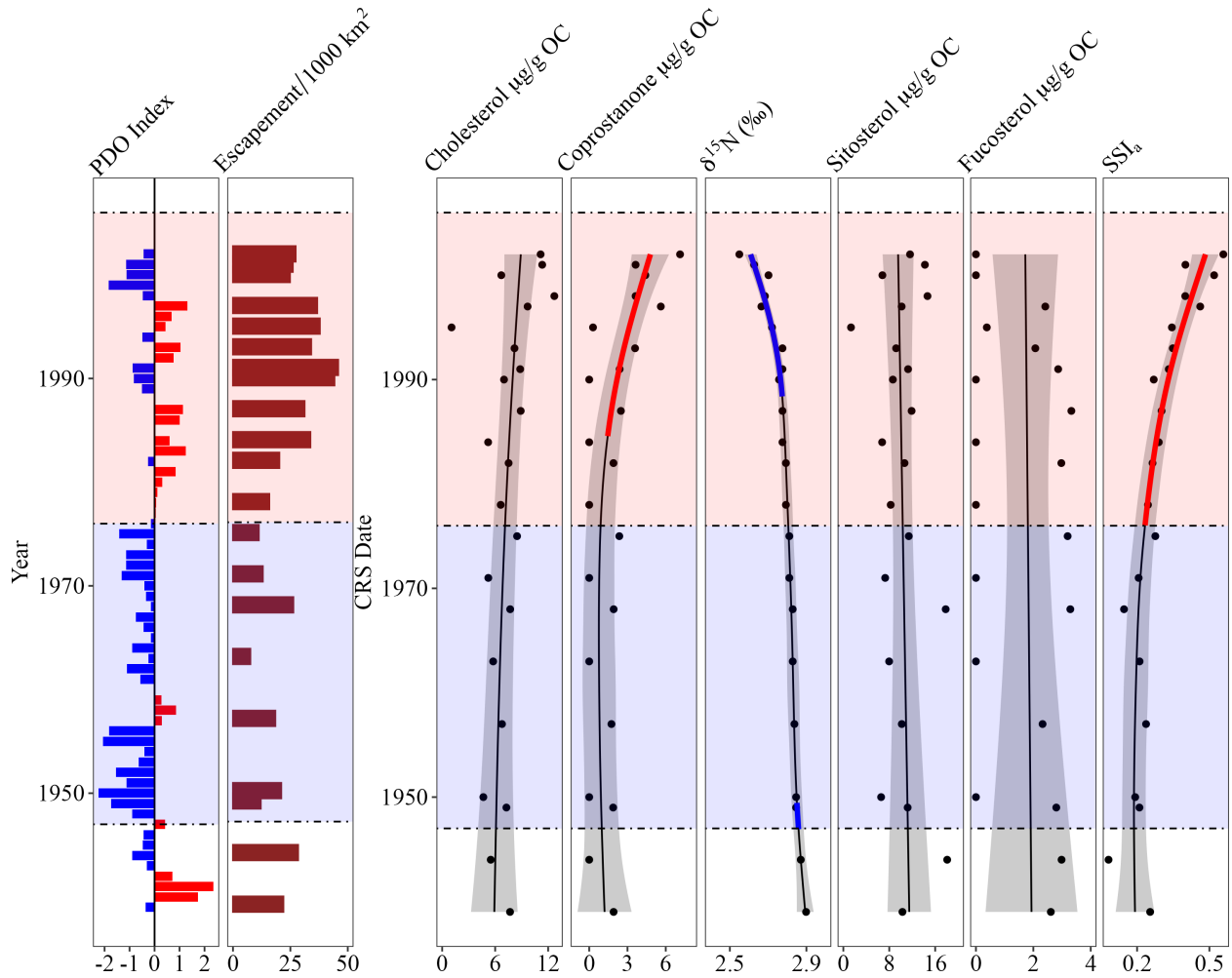


Figure 3.9 The warm (1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific Decadal Oscillation (PDO), and sockeye salmon escapement versus the downcore profiles of square-root-transformed cholesterol, coprostanone, $\delta^{15}\text{N}$, sitosterol, fucosterol, and salmon sterol index_a (SSI_a) [(SSI_a = cholesterol + coprostanone + epicoprostanol + desmosterol)/(cholesterol + coprostanone + epicoprostanol + desmosterol + fucosterol + sitosterol + stigmastanol)] in Red Lake sediments, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. Core dates were assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

4.0 Discussion

4.1. Sterols in Adult Sockeye Salmon Tissue and Their Importance

High cholesterol concentrations in sockeye salmon muscle tissue corroborate earlier research that reported similar values ($\sim 17.50 \pm 0.8$ g/g dry weight SD) in sockeye salmon fillets (Colombo & Mazal, 2020). High cholesterol may relate partly to the marine carnivorous diet of the fish, which consists mainly of cholesterol-rich zooplankton and micronekton (Fergusson et al., 2020; Serrazanetti et al., 1994). Elevated cholesterol content in sockeye salmon muscle tissue plays multiple vital roles beyond cell membrane maintenance. During their challenging spawning migration, sockeye salmon undergo physiological changes that require substantial energy reserves (Hinch et al., 2005). Cholesterol acts as a hormone precursor and crucial energy substrate, enabling salmon to meet the intense metabolic demands of their long upstream journey (Morash et al., 2013).

The importance of cholesterol oxidation for energy production in sockeye salmon muscle tissue during spawning migration highlights the adaptive significance of their pre-migration feeding behavior. Before embarking on their spawning journey, sockeye salmon feed extensively in the Pacific Ocean, where they accumulate substantial biomass (McDaniels et al., 2010; Radchenko & Mathisen, 2004). This strategic buildup of energy reserves, including cholesterol, enables the salmon to undertake their arduous upstream migration without additional feeding. The persistence of high cholesterol concentrations throughout the migration suggests a finely tuned metabolic strategy that enables sockeye salmon to balance energy expenditure with available reserves, ensuring the successful completion of their reproductive cycle.

Other cholesterol-related sterols, such as cholestanol, coprostanone, and desmosterol, were detected at lower concentrations in sockeye salmon muscle tissue. Cholestanol, a 5α -dihydro derivative of cholesterol, can be attributed to both dietary sources and endogenous biosynthesis. Its presence indicates that sockeye salmon may absorb this compound from their diet and incorporate it into their tissues, similar to the mechanism observed in mammals (Shefer et al., 1984). Furthermore, cholestanol is a steroid precursor in the cholesterol biosynthesis pathway, suggesting it can also be produced internally. Coprostanone, a hydrogenated derivative of cholesterol formed through microbial processes in the gut, was also detected. Its presence in sockeye salmon muscle tissue implies that microbial activity in the fish's gut may influence the

sterol composition of its muscle tissue (Bull et al., 2002). The detection of desmosterol, an intermediate in cholesterol biosynthesis, further supports active cholesterol production in sockeye salmon muscle tissues (Waterham & Vaz, 2024).

4.2. Relating Sterol and Stanol Concentrations in Lake Surface Sediments to Sockeye Salmon Escapement/Lake Area

Surface sediments of nine sockeye salmon nursery lakes contained a wide variety of sterols and stanols, emphasizing the importance of these compounds in the biogeochemical cycles of these aquatic environments (Martin-Creuzburg & Von Elert, 2009). Sitosterol and its microbial reduction product, stigmastanol, were prevalent among the sterols, indicating that sockeye salmon decomposition is a key driver of sitosterol production in these lakes (James & Naiman, 2001). Therefore, transformation of sitosterol to stigmastanol by sediment microbes represents an active biogeochemical process linked to salmon carcass decomposition in salmon nursery lake sediments (Hanisch et al., 2009). Although sitosterol and stigmastanol are often associated with terrestrial organic matter input, their concentrations in sediments of sockeye salmon nursery lakes primarily reflect primary production in those waterbodies that benefited from the nutrients released from decomposing salmon, rather than terrestrial sources. In lakes such as Spiridon, Upper Ugashik, and Becharof, where large numbers of salmon die annually, the high sitosterol levels strongly suggest that salmon carcasses serve as a major organic matter and nutrient source. This observation highlights the significant impact of salmon decomposition on lake sediment sterol concentrations, likely surpassing the contribution from terrestrial plant material. Although other sources of nutrients such as terrestrial runoff, atmospheric deposition, or other animals may occur, several of the lakes included in our study with the highest salmon escapement densities are known to receive >50% of their total P and N from sockeye salmon carcasses (Finney et al., 2000; 2002). Abundance of cholesterol and its derivatives in the lake sediments is consistent with previous studies that demonstrated that decomposing sockeye salmon carcasses are crucial contributors to the lake's organic matter pool (Finney et al., 2000; Gregory-Eaves et al., 2003). High cholesterol concentrations in surface sediments, comparable to those found in sockeye salmon muscle tissue, suggest that cholesterol released during salmon decomposition is incorporated into the lake sediments. As a fundamental component of animal cell membranes and an energy substrate for various biological processes, cholesterol is likely

deposited in the lake as spawning salmon carcasses decompose. This provides additional evidence that salmon contribute to lake nutrient dynamics, not only through their presence while alive but also through their decay following death.

Sockeye salmon escapement/return density is a measure of the number of salmon that evade capture and swim back to their natal lake to spawn, calculated per unit area of the lake. This indicator is crucial for assessing the well-being and longevity of salmon populations. Higher escapement densities typically suggest a healthy and productive salmon run, whereas lower densities may indicate possible ecosystem problems or overfishing (Price et al., 2008; Schindler et al., 2005). By tracking escapement density, fisheries managers can make informed decisions about harvest restrictions and develop conservation strategies to ensure sustainable salmon populations for the future (Parsons & Skalski, 2010; Price et al., 2008).

Our study revealed a strong relationship between certain sterols and stanols (including cholesterol, epicoprostanol, and coprostanone) and sockeye salmon escapement density, suggesting that the decomposition of salmon after spawning significantly increases the concentration of these compounds in lake sediments. The presence of cholesterol, epicoprostanol, desmosterol, and coprostanone in the sediments of salmon nursery lakes, as shown in our PLS model, establishes them as candidate indicators of sockeye salmon escapement. Additionally, sitosterol (a plant sterol) and fucosterol (an algal sterol) in lake sediments, which were not found in sockeye salmon muscle tissue, also showed positive correlations with salmon return densities. This result suggests that nutrients from decaying salmon may promote the production of plant-based and algae-based sterols in these lakes. Our SSIs show an asymptotic relationship with salmon escapement densities. In contrast, individual sterol concentrations show no such correlation, suggesting that nutrient enrichment of plant-based or algal-based sterols, like sitosterol and fucosterol, becomes more significant at higher salmon densities. Earlier studies demonstrated that $\delta^{15}\text{N}$ in the surface sediments of salmon nursery lakes correlated with salmon return densities, indicating that Pacific salmon transport this potentially limiting nutrient to their nursery lakes in quantities sufficient to stimulate aquatic macrophyte and algae growth (Finney et al., 2002; Gregory-Eaves et al., 2003). Whereas elevated plant sterols in salmon lake sediments reflect enhanced primary production stimulated by salmon-derived nutrients, we acknowledge that other environmental factors such as light availability, thermal regimes, and grazing pressure also influence primary production

(Matsumoto et al., 2014). These variables were not directly measured in this study and represent important directions for future research aimed at disentangling the complex drivers of lake productivity. Furthermore, our study's inclusion of SSIs, which encompassed a wide range of zoosterols and phytosterols, provided a more comprehensive and robust method for tracking sockeye salmon escapement density in surface sediments than $\delta^{15}\text{N}$ alone. Whereas both sterols and $\delta^{15}\text{N}$ may respond to salmon-derived nutrient inputs, they are independent in mechanism. Whereas $\delta^{15}\text{N}$ reflects the source and cycling of nitrogen within the ecosystem, sterols represent biologically synthesized lipids that reflect animal, algal and plant productivity as well as community composition.

4.3. Reconstructing Sockeye Salmon Production Dynamics Using $\delta^{15}\text{N}$, Sterols and Stanols, SSIs, and the PDO

Our multi-proxy paleolimnological study examined sterols, stanols, SSIs, and $\delta^{15}\text{N}$ in sediment cores to track sockeye salmon escapement density. This approach was applied across multiple waterbodies, including Kinaskan, Frazer, Karluk, and Red lakes, while integrating climate data (warm and cool phases of the PDO) to investigate the relationship between large-scale climate cycles and Sockeye return densities. The low $\delta^{15}\text{N}$ values observed in Kinaskan Lake sediments generally fall within the range observed in 'background' sediments of high-latitude lakes, which are often characterized by lower $\delta^{15}\text{N}$ because of high input of terrestrial organic matter (Das, 2002). Furthermore, the background values of $\delta^{15}\text{N}$ in Kinaskan Lake are consistent with those observed in the pre-sockeye salmon production periods of Frazer Lake. The observed pattern is notable as it contrasts markedly with lakes that support sockeye salmon production and lakes subject to anthropogenic influence, which typically have elevated $\delta^{15}\text{N}$ values that normally range from +10 to +20 ‰ (Finney et al., 2002; Karr et al., 2003; Kissinger et al., 2023; Xia et al., 2018). Absence of elevated $\delta^{15}\text{N}$ values in Kinaskan Lake is a key indicator of its “pristine” condition. Further corroborating this conclusion are the results of sterol and stanol analyses conducted on the Lake Kinaskan sediment core. Coprostanol, a biomarker utilized as an indicator of human fecal contamination (Duda et al., 2021; Hargan et al., 2018, 2019; Kissinger et al., 2023; Stewart et al., 2018), remained consistently below detection limits throughout the entire sediment core. In contrast, lakes affected by wastewater disposal, often exhibit much higher coprostanol levels (Stewart et al., 2018). Moreover, the Kinaskan Lake core

displayed no substantial increases in algal or plant sterols, which would typically indicate an influx of nutrients. Absence of detectable concentrations across all 11 sterols and stanols measured throughout the ^{210}Pb -dated sediment core further reinforces the lake's status as a “pristine” ecosystem and shows that these sterols do not show a significant presence in lake sediments unaffected by sockeye salmon or other sources of nutrient inflow.

Inclusion of Frazer Lake in the study presented a unique opportunity to investigate the effects of sockeye salmon introduction on $\delta^{15}\text{N}$, along with sterols and stanol dynamics in a single lake. Prior to 1951, the lake was devoid of anadromous sockeye salmon, rendering it an ideal natural experiment for comparing pre- and post-salmon lake sediment conditions. The stocking project, initiated in 1951 and continuing until 1971, involved the introduction of sockeye salmon at various life stages, including eggs, fry, and adults, sourced from Karluk and Red Lakes (Blackett, 1979; Fuerst, 2017; Thomsen et al., 2013). Construction of the fish pass in 1962 marked a milestone in the conservation efforts for the sockeye salmon population in Frazer Lake. Prior to this intervention, the 10-meter-high waterfall presented an insurmountable obstacle, impeding the return of adult sockeye salmon to their spawning grounds in the lake and its tributaries. Implementation of the fish pass not only facilitated the salmon's ability to overcome this barrier but also contributed to the establishment of a self-sustaining population within this Kodiak Island ecosystem. This engineering solution, in conjunction with the addition of a diversion weir across the lake, effectively expanded the available habitat for the sockeye salmon, enabling them to utilize previously inaccessible spawning areas (Finney et al., 2002; Thomsen et al., 2013). The Frazer Lake system supports all five salmon species, with sockeye salmon being the predominant species (Gregory-Eaves et al., 2003; Thomsen et al., 2013). The predominance of sockeye salmon in this lake suggests that a substantial amount of the $\delta^{15}\text{N}$ and cholesterol found in the lake sediment likely originated from the massive annual sockeye salmon die-off. In general, our sediment data were consistent with the sockeye salmon production history of Frazer Lake. We found that prior to 1951, the barren period of Frazer Lake, concentrations of cholesterol and its stanol derivative (cholestanol) were low and stable until 1962, when there was a slight increase, followed by a sharp increase after 1971. Thus, both cholesterol and $\delta^{15}\text{N}$ increased sharply around the time of large-scale sockeye salmon returns after 1971 when the diversion weir directed sockeye salmon to Frazer Lake. A subsequent decrease in sockeye salmon returns in the 1980s was more apparent in the shift to lower $\delta^{15}\text{N}$ and

corroborated by reductions in sterols, including coprostanone, fucosterol, sitosterol, and the salmon sterol ratio, than by reductions in cholesterol. Differences among sediment proxies in their ability to track sockeye salmon return densities underscore the importance of employing multi-proxy methods in such studies. The differences between cholesterol and cholestanol as indicators of measured sockeye salmon escapement may be attributed to sediment mixing or conversion of cholesterol to stanols in lake sediments. Sterol diagenesis is a complex process that may be affected by microbial activity in sediments, resulting in changes in sterol structure through processes such as depolymerization, decarboxylation, hydrogenation, and methylation under aerobic or anaerobic conditions (Bartlett, 1987; Bull et al., 2002; Cranwell et al., 1987). In anaerobic conditions, cholesterol is first converted to 4-cholesten-3-one, which is then reduced to coprostanone (Ren et al., 1996). This process is mediated by bacteria such as *Eubacterium coprostanoligenes*, which can efficiently convert cholesterol, 4-cholesten-3-one, and coprostanone to coprostanol (Ren et al., 1996). These processes can impact the absolute concentrations of sterols and potentially obscure original ecological signals. To mitigate this concern, we employed sterol ratio indices such as the SSIs, which normalize sterol abundances and reduce the influence of differential degradation. Ratio-based approaches have been shown to minimize diagenetic effects by focusing on relative rather than absolute changes in biomarker composition (Cheng et al., 2016; Hargan et al., 2018). Moreover, the strong and consistent statistical relationships observed between sockeye salmon escapement data and sterol indices across multiple lakes suggest that the preserved biomarker signals retain ecological integrity over time. Whereas diagenetic alteration cannot be entirely ruled out, the coherence of biomarker trends and the use of compositional indices support the reliability of our sterol-based reconstructions. Following the construction of the fish pass in 1962, coprostanone, sitosterol, fucosterol, the salmon sterol index, and $\delta^{15}\text{N}$ simultaneously dipped slightly, followed by a sharp increase, coincident with the increase in sockeye salmon escapement to this lake. Our sediment core data indicate that Frazer Lake had substantial salmon production only in the warm phase of the PDO, whereas during the cool phase, the lake did not receive sockeye salmon because of waterfall obstruction.

Karluk Lake, an important sockeye salmon nursery in Alaska, has a well-documented history of fishing activities dating back to the late 19th century. The lake's sockeye salmon population experienced a substantial decline between 1880 and 1960, decreasing to

approximately 10% of its pre-commercial-fishing levels (Gregory-Eaves et al., 2003). This decline was influenced by a complex interplay between human- and climate-related factors, including commercial fishing practices and the PDO. The PDO index exhibited alternating warm and cool phases (warm phases from 1925 to 1945 and 1976 to 2006, and a cool phase from 1946 to 1976). Warm phases generally corresponded to higher Sockeye salmon returns to Karluk Lake, whereas cool phases were associated with lower returns. The impact of commercial fishing on the sockeye salmon population was considerable, with cannery operations commencing in 1882 and rapidly escalating to harvest demands of several million fish annually (Gregory-Eaves et al., 2003). From the early 1900s, Sockeye salmon escapement continued to decline until 1990, when the population began to rebound (Gregory-Eaves et al., 2003). Among all the sediment variables investigated, only $\delta^{15}\text{N}$ and the salmon sterol ratio strongly correlated with sockeye salmon escapement density and the PDO cycle. During the warm phase of the PDO, Sockeye salmon escapement density, $\delta^{15}\text{N}$, and the salmon sterol ratio all remained high, whereas the situation was reversed during the cool phase. In contrast, Red Lake (Ayakulik Lake), another major sockeye salmon producer on Kodiak Island, exhibited some discrepancies between $\delta^{15}\text{N}$ and sterols and stanols. Whereas $\delta^{15}\text{N}$ tracked the decrease in sockeye salmon return from 1990 to 2002, coprostanone, epicoprostanol, and desmosterol aligned more with increased sockeye salmon escapement density during the warm phase of the PDO and remained relatively constant during the cool phase. Discrepancies between $\delta^{15}\text{N}$ and sterol biomarkers in this lake suggest that additional environmental or ecological factors may have influenced salmon abundance and nutrient cycling in this aquatic ecosystem. These findings emphasize the necessity for a comprehensive approach when investigating historical salmon populations, considering both large-scale climate patterns and local environmental conditions to enhance understanding and better predict future changes in salmon abundance and their ecological impacts. Nonetheless, the interpretation of our results should be viewed within the context of certain limitations. Whereas our approach is well-suited for sockeye salmon nursery lakes, where salmon are the dominant and traceable source of lipid and nutrient input, its broader applicability depends on the ecological context. This method may be applied to other systems where a single species contributes substantially to sediment lipid profiles. However, in ecosystems with significant overlapping lipid contributions from multiple species, disentangling sterol biomarkers may be challenging and require other independent biomarkers.

5.0 Conclusions

We measured the sterol composition of adult sockeye salmon muscle tissue, which revealed the presence of cholesterol, cholestanol, coprostanone, and desmosterol in different concentrations. Detection of these compounds elucidated the balance between dietary intake and endogenous biosynthesis in shaping the sterol profile of sockeye salmon. Analysis of adult sockeye salmon muscle tissue revealed a sterol profile dominated by cholesterol (98% of total sterol content), which is crucial for their spawning migration. Strong correlations between various sterols and stanols, particularly metabolites of cholesterol in lake surface sediments, and sockeye salmon escapement density, provided evidence for the potential utility of these compounds as proxies for salmon densities. Cholesterol displayed the most robust relationship with escapement density, likely because of its prevalence in salmon tissue. Significant correlations between both plant and algal sterols with salmon return densities, despite absence of those sterols in sockeye salmon muscle tissue, suggest a potential nutrient-driven increase in primary production in these lakes. This finding aligns with previous research on $\delta^{15}\text{N}$, which revealed the role of salmon in nutrient transport to their nursery lakes. SSIs expanded our ability to track salmon return density using both surface sediments and ^{210}Pb -dated sediment cores, along with individual sterol concentrations and $\delta^{15}\text{N}$ values. These results underscore the potential of sterol and stanol analysis in lake sediments as a tool for inferring historical salmon population fluctuations and elucidating ecosystem dynamics in salmon-bearing lakes. Analysis of sterols, stanols, and $\delta^{15}\text{N}$ in sediment cores, and the development of SSIs, in conjunction with documented histories of sockeye salmon escapement, demonstrated the potential for tracking salmon populations through time using lake sediments. Inclusion of Kinaskan Lake as a reference site and the unique case of Frazer Lake's salmon introduction, substantiated the validity of these biomarkers as indicators of salmon presence and abundance. The study also helped to elucidate the complex interplay between human factors such as commercial fishing, and natural climate oscillations like the Pacific Decadal Oscillation, in influencing sockeye salmon populations. Although some discrepancies were observed between individual proxies, particularly in Red Lake, the overall consistency of results across multiple lakes, particularly with respect to the SSIs, underscores the importance of employing multi-proxy methods in paleolimnological studies. These findings contribute substantially to our

understanding of long-term salmon population dynamics and highlight valuable methods that can be applied to future research and conservation efforts in salmon-bearing ecosystems.

Inclusion in Global Research Statement

This study was conceptualized and carried out in the Blais Lab at the University of Ottawa, Canada, in collaboration with research colleagues from the PEARL Lab at Queen's University in Kingston, Canada, and the Department of Fisheries and Oceans Canada. Funding for this research was provided by the Natural Sciences and Engineering Research Council of Canada Discovery Program (RGPIN-2018-04248) awarded to JMB, as well as an Environmental Sciences Strategic Research Fund grant to JMB and Robie W. Macdonald. We gratefully acknowledge Hugo Crites for producing the map for this study. We also thank Adriana Raats for reviewing the manuscript.

Open Research

The dataset supporting this study, including sterol concentrations, $\delta^{15}\text{N}$ values, Sockeye salmon escapement records, and the Pacific Decadal Oscillation (PDO) Index, is publicly available in Zenodo at [<https://doi.org/10.5281/zenodo.14984126>].

Supporting Information

Tracking long-term trends in Sockeye salmon (*Oncorhynchus nerka*) population dynamics using sterol and stanol biomarkers in lake sediments

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Introduction

This section presents tables and figures detailing the models, formulas, and computational methods used in our analysis. Additionally, we provide visual representations that illustrate the performance of these models in tracking sockeye salmon population dynamics.

Supplementary Tables

Table S3.1 Detection limits for the eleven sterols and stanols measured in the study.

DW stands for dry weight. The method detection limit is based on an assumed sample mass of 0.1 g. The method detection limits (MDLs) were calculated based on the procedure described by Loconto (2005) using the formula: $MDL = \frac{(IDL \times VE \times 100)}{(VS \times \% \text{ recovery})}$. In this equation, IDL refers to the instrument detection limit, VE represents the volume of the final extract, VS is the sample volume, and % recovery denotes the analyte recovery percentage. The IDL was derived by taking the average of the minimum detectable signal, adding three times the standard deviation above the instrument background, and dividing this sum by the calibration curve slope.

Sterols and stanols	Average blank (ng mL ⁻¹)	Standard deviation	Minimal detectable signal (ng mL ⁻¹)	Slope of calibration curve	Instrument detection limit (ng mL ⁻¹)	Method detection limit (µg g ⁻¹ DW)
Coprostanol	0.02	0.02	0.07	0.36	0.14	0.001
Epicoprostanol	0.02	0.01	0.06	0.35	0.10	0.001
Coprostanone	1.08	0.36	2.15	0.05	21.9	0.167
Cholesterol	0.43	0.21	1.06	0.09	6.76	0.051
Cholestanol	0.31	0.10	0.62	0.11	2.83	0.022
Cholestanone	0.91	0.04	1.03	0.06	2.18	0.017
Desmosterol	5.64	0.33	6.61	0.06	15.9	0.121
Campesterol	1.12	0.13	1.50	0.15	2.50	0.019
Fucosterol	0.73	0.14	1.15	0.09	4.64	0.035
β-Sitosterol	6.52	0.40	7.72	0.03	44.5	0.338
Stigmastanol	3.43	0.45	4.77	0.02	57.1	0.434

Table S3.2 Summary statistics from the linear regression model, showing the relationship between $\log(x+1)$ transformed sterols and stanols in lake surface sediments and sockeye salmon return density across the nine Alaskan salmon nursery lakes. Reported metrics include adjusted R^2 , p-values, and F-statistics. Statistically significant relationships are denoted by $p < 0.05$.

Sterols and stanols	Adjusted R^2	p value	$F_{1,8}$
Coprostanol	0.6	0.006	15.84
Coprostanone	0.7	0.004	17.15
Cholestanol	0.4	0.050	6.60
Cholestanone	0.4	0.050	5.40
Desmosterol	0.7	0.004	17.26
Campesterol	0.5	0.020	8.49
Fucosterol	0.7	0.005	16.73
Stigmastanol	0.2	0.100	3.47

Table S3.3 Summary statistics for the seven salmon sterol indices (SSIs) in lake surface sediments and their relationship with sockeye salmon escapement density. The model was fitted using the function presented in Eq. 1. Statistical significance is indicated at $p < 0.05$. * Denotes non-significant values ($p \geq 0.05$).

Salmon sterol indices (SSIs)	a	p-value	b	p-value	Pseudo R ²	RMSE
$SSI_a = \frac{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \text{desmosterol} \end{array} \right)}{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \\ \text{desmosterol} + \text{fucosterol} + \\ \text{sitosterol} + \text{stigmastanol} \end{array} \right)} \dots \text{Eq. 2}$	0.68	< 0.001	4128	0.037	0.86	0.071
$SSI_b = \frac{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \text{desmosterol} \end{array} \right)}{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \text{desmosterol} + \\ \text{sitosterol} + \text{stigmastanol} \end{array} \right)} \dots \text{Eq. 3}$	0.71	< 0.001	4399	0.036	0.86	0.072
$SSI_c = \frac{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \text{desmosterol} \end{array} \right)}{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \\ \text{desmosterol} + \text{sitosterol} \end{array} \right)} \dots \text{Eq. 4}$	0.81	< 0.001	3333	0.058*	0.79	0.101
$SSI_d = \frac{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} \end{array} \right)}{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \text{fucosterol} + \\ \text{sitosterol} + \text{stigmastanol} \end{array} \right)} \dots \text{Eq. 5}$	0.54	< 0.001	2950	0.033	0.82	0.059
$SSI_e = \frac{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} \end{array} \right)}{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \\ \text{fucosterol} + \text{sitosterol} \end{array} \right)} \dots \text{Eq. 6}$	0.64	< 0.001	2284	0.040	0.79	0.077
$SSI_f = \frac{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} \end{array} \right)}{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \\ \text{sitosterol} + \text{stigmastanol} \end{array} \right)} \dots \text{Eq. 7}$	0.57	< 0.001	3256	0.032	0.84	0.06
$SSI_g = \frac{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} \\ + \text{epicoprostanol} \end{array} \right)}{\left(\begin{array}{c} \text{cholesterol} + \text{coprostanone} + \\ \text{epicoprostanol} + \\ \text{sitosterol} \end{array} \right)} \dots \text{Eq. 8}$	0.69	< 0.001	2620	0.043	0.80	0.08

Supplementary Figures

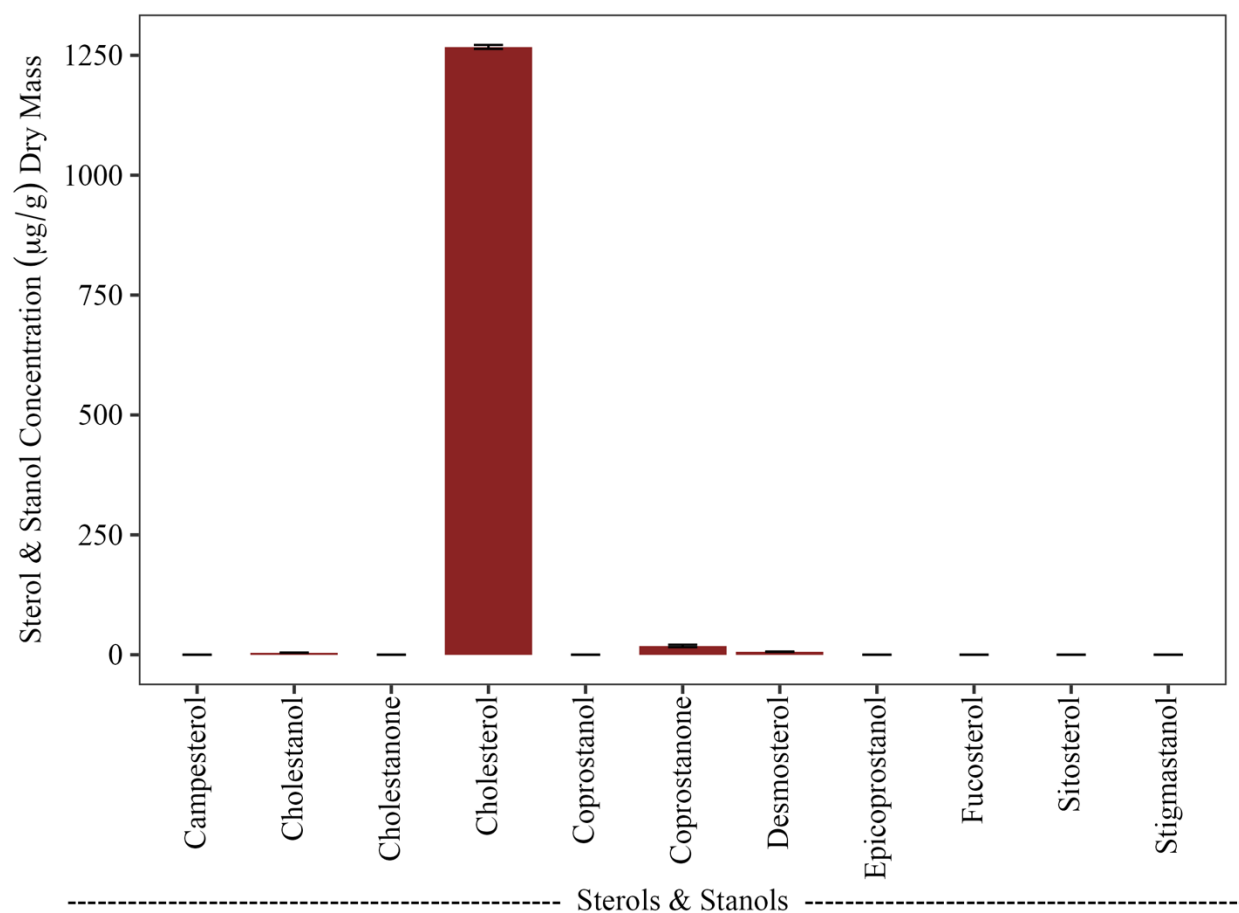


Figure S3.1 Sterol and stanol concentrations in muscle tissue of adult sockeye salmon (*Oncorhynchus nerka*). Concentrations in adult Sockeye salmon muscle tissue (n = 3) are predominantly composed of cholesterol, with minor concentrations of cholestanol, coprostanone, and desmosterol. Error bars represent standard deviation.

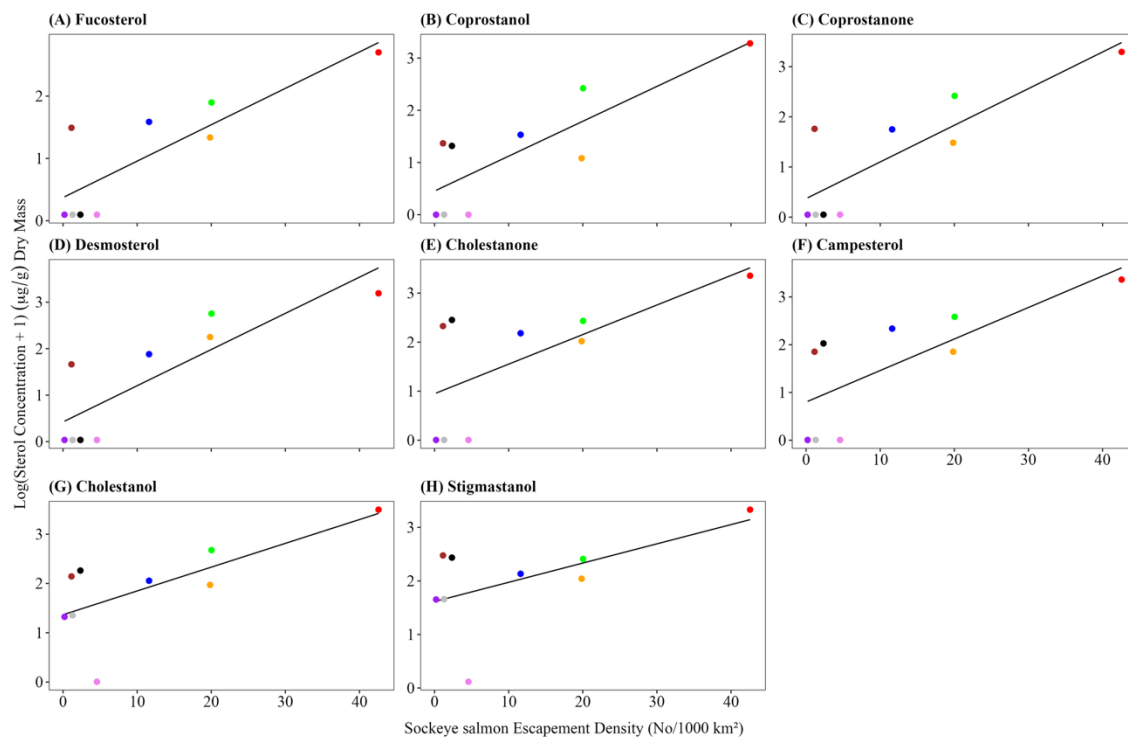


Figure S3.2 Linear regression models illustrating the relationship between $\log(x+1)$ transformed sterol and stanol concentrations in surface lake sediments versus sockeye salmon return density. Each data point represents one of nine Alaskan lakes: Spiridon (purple), Frazer (blue), Karluk (green), Red (red), Upper Olga (orange), Iliamna (black), Upper Ugashik (violet), Kijik (brown), and Becharof (gray).

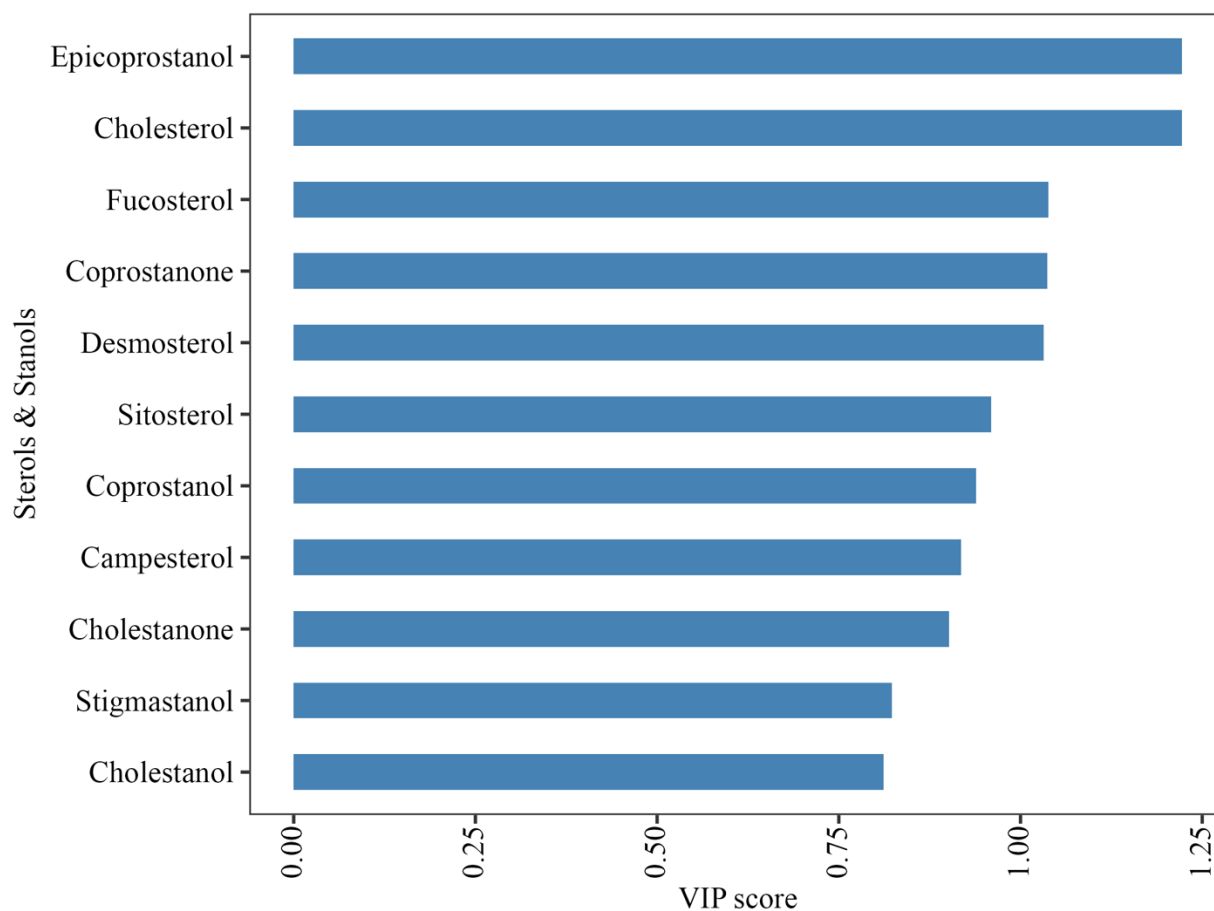


Figure S3.3 Bar plot showing the contributions of sterols and stanols to predicting sockeye escapement density. Five sterols and stanols, including cholesterol and epicoprostanol, showed a Variable Importance for the Projection (VIP) score > 1 , indicating that these sterols contributed significantly to predicting Sockeye escapement density.

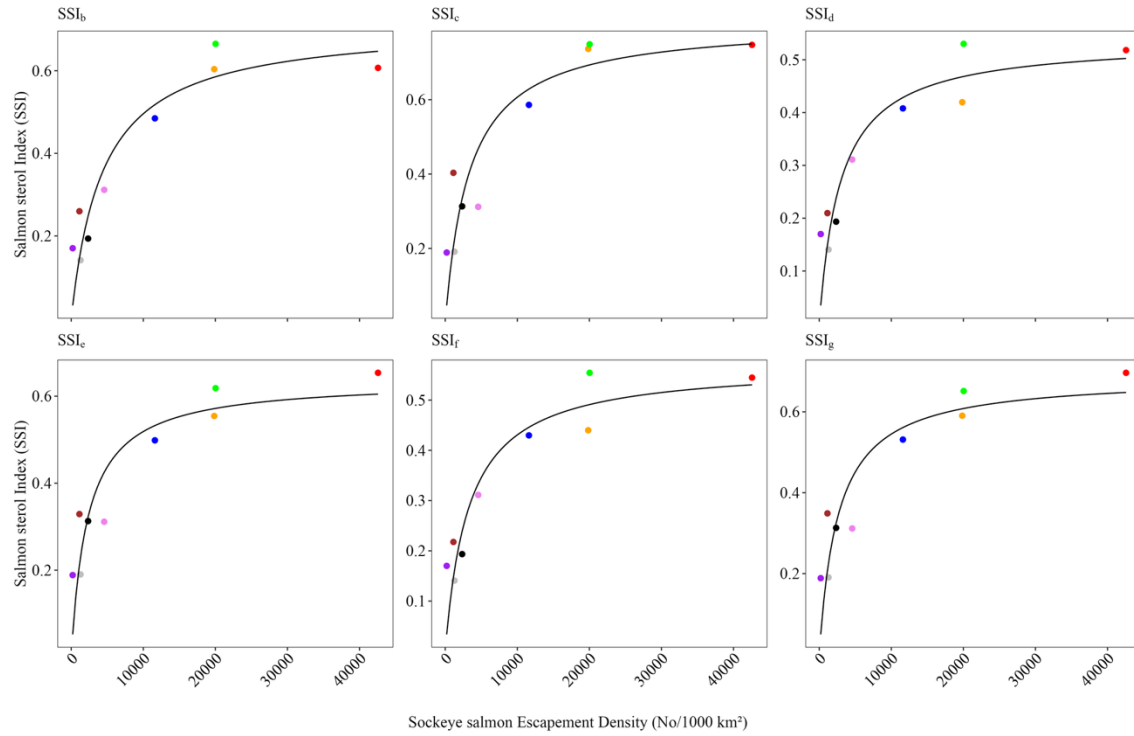


Figure S3.4 Curves showing the relationship between SSIs in lake surface sediments and sockeye salmon escapement density. The model was fitted using the function presented in Eq. 1. Refer to Table S3 for model summary statistics.

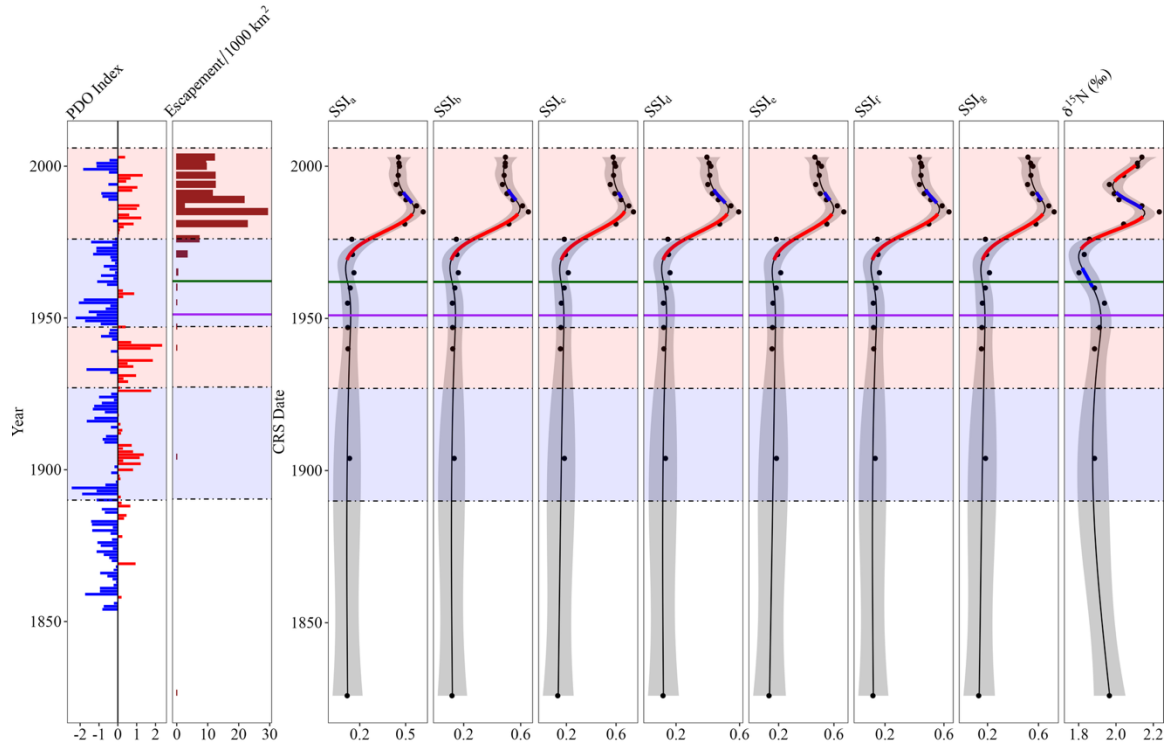


Figure S3.5 The warm (1925 – 1947 CE and 1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific decadal oscillation (PDO), and sockeye salmon escapement versus the downcore profiles of SSIs and $\delta^{15}\text{N}$ in Frazer Lake, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. The horizontal solid purple and green lines represent 1951, when the lake was fertilized, and 1962, when a fish ladder was constructed, respectively. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

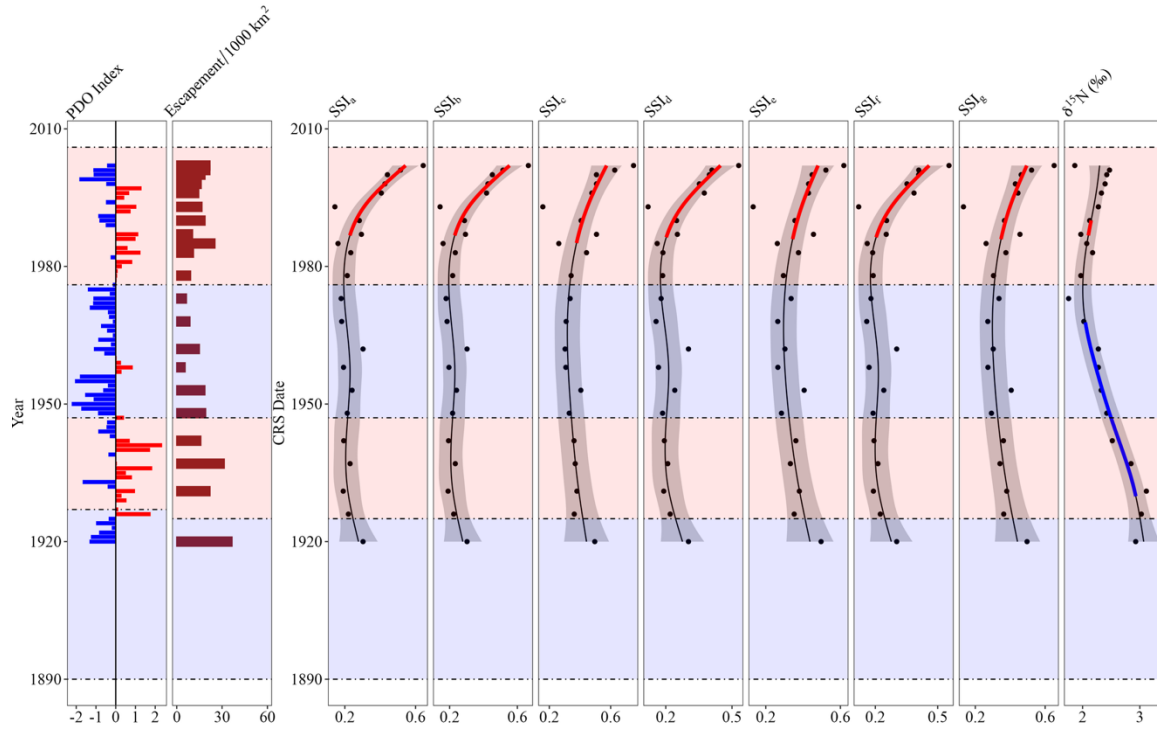


Figure S3.6 The warm (1925 – 1947 CE and 1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific decadal oscillation (PDO), and sockeye salmon escapement versus the downcore profiles of SSTs and $\delta^{15}\text{N}$ in Karluk Lake, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

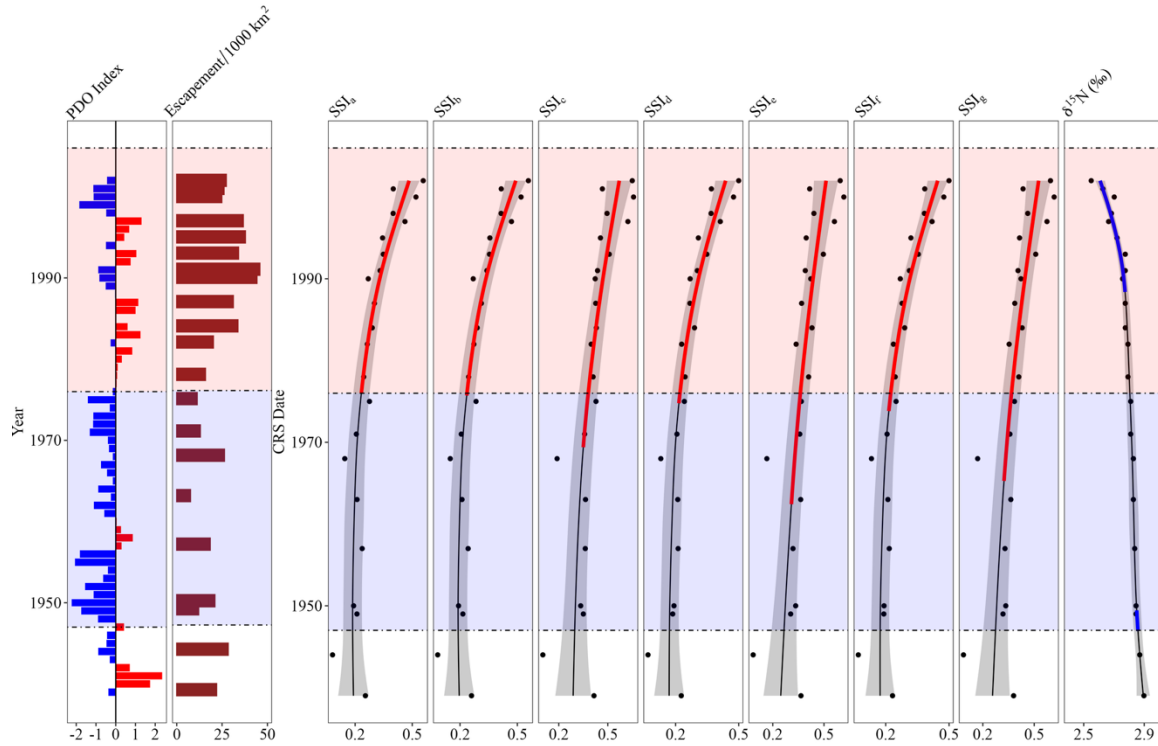


Figure S3.7 The warm (1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific decadal oscillation (PDO), and sockeye salmon escapement versus the downcore profiles of SSIs and $\delta^{15}\text{N}$ in Red Lake modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

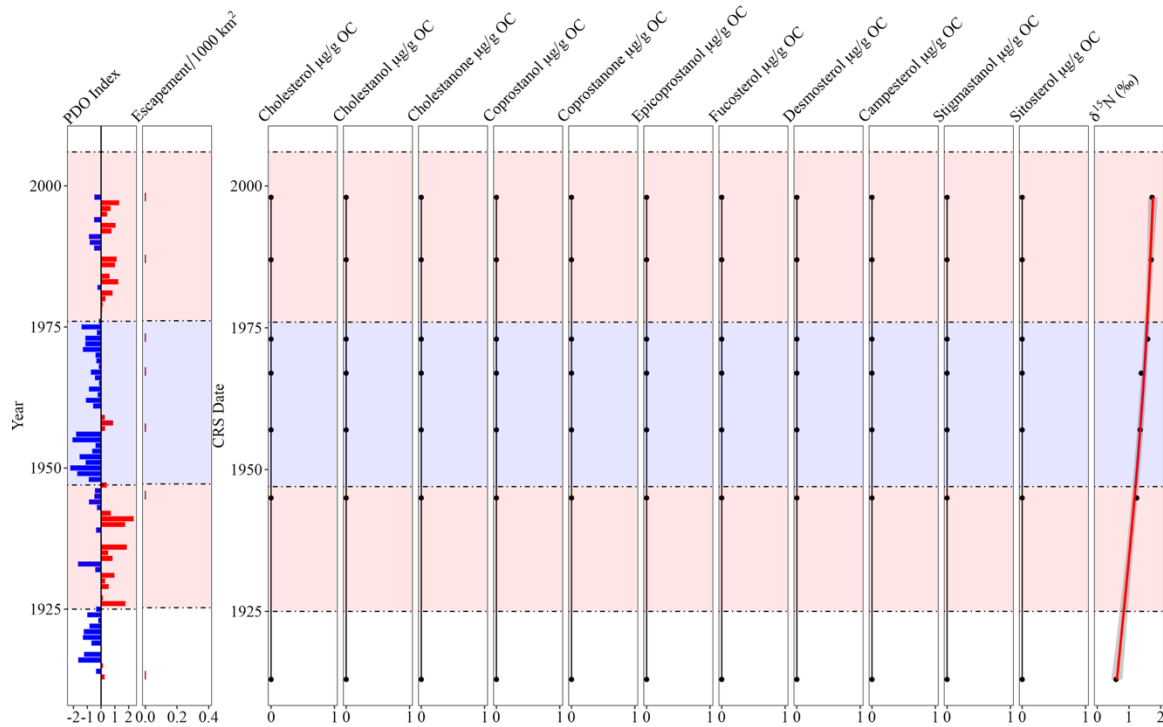


Figure S3.8 The warm (1925 – 1947 CE and 1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific decadal oscillation (PDO), and sockeye salmon escapement density versus profiles of sterols, stanols, and $\delta^{15}\text{N}$ in Kinaskan Lake sediments modelled using generalized additive models (GAMs). The profile for $\delta^{15}\text{N}$ was reconstructed using square-root-transformed values, and the shaded area around $\delta^{15}\text{N}$ represents the 95% confidence interval of our smoothed estimate. Kinaskan Lake, the reference lake, has no salmon run and exhibited a relatively low $\delta^{15}\text{N}$ (0 - 3 ‰), and sterols were below detection. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

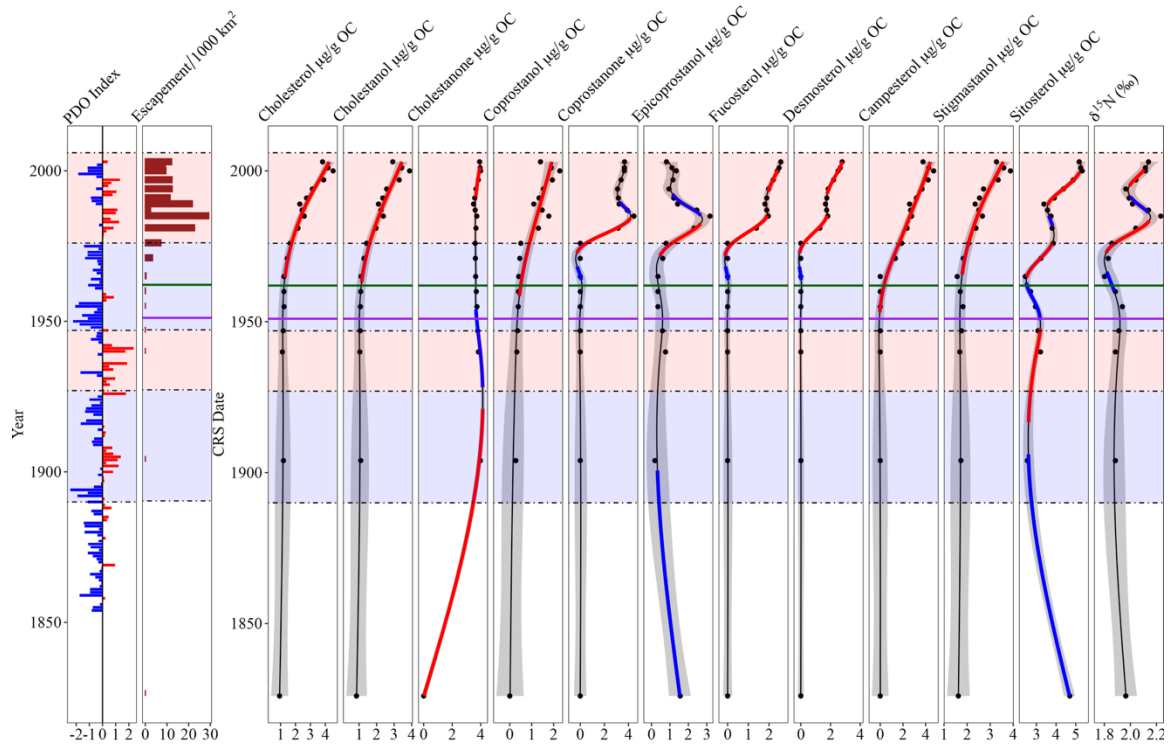


Figure S3.9 The warm (1925 – 1947 CE and 1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific decadal oscillation (PDO), and sockeye salmon escapement density versus profiles of sterols, stanols, and $\delta^{15}\text{N}$ in Frazer Lake sediments, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. The horizontal solid purple and green lines represent 1951, when the lake was fertilized, and 1962, when a fish ladder was constructed, respectively. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

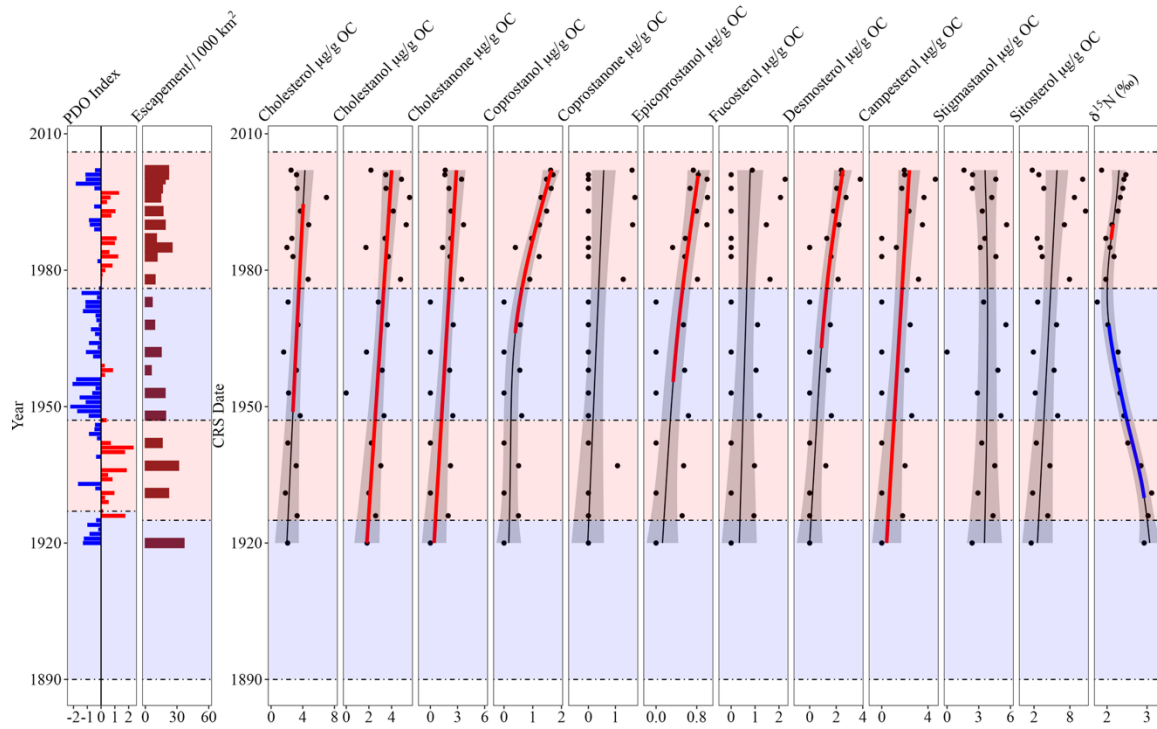


Figure S3.10 The warm (1925 – 1947 CE and 1976 – 2006 CE) (shade in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific decadal oscillation (PDO), and sockeye salmon escapement density versus profiles of sterols, stanols, and $\delta^{15}\text{N}$ in Karluk Lake sediments, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

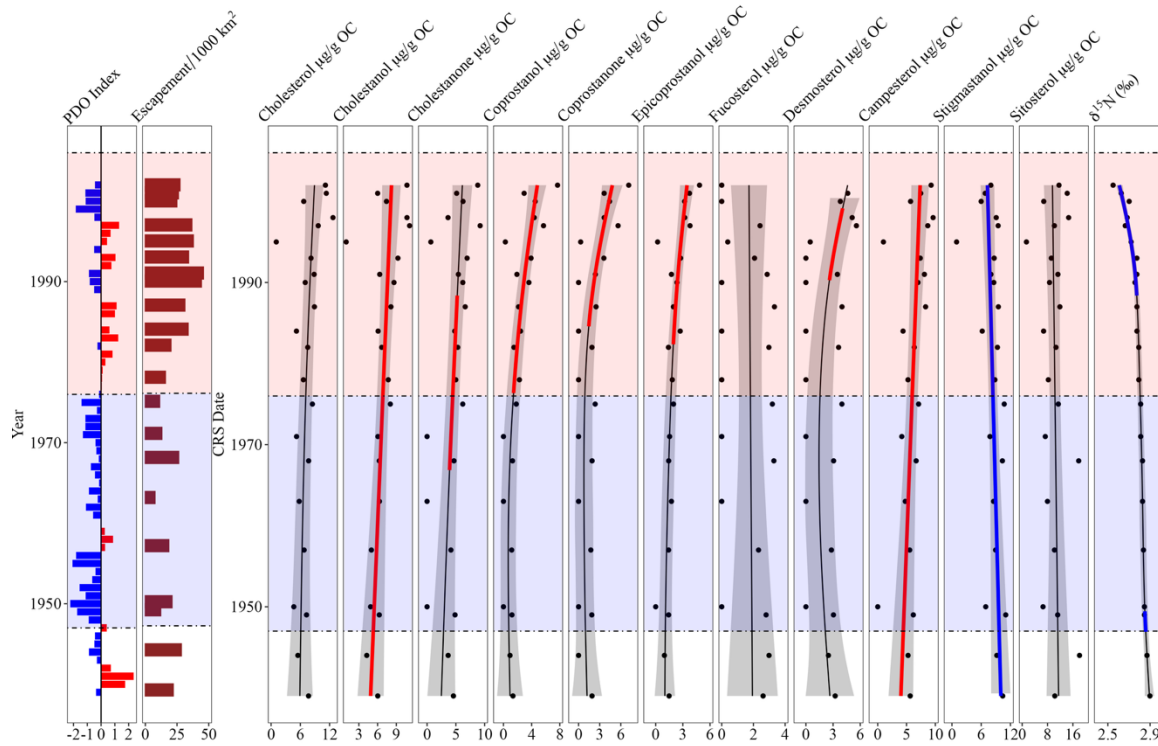


Figure S3.11 The warm (1976 – 2006 CE) (shaded in red) and cool (1947 – 1976 CE) (shaded in blue) phases of the Pacific decadal oscillation (PDO), and sockeye salmon escapement density versus profiles of sterols, stanols, and $\delta^{15}\text{N}$ in Red Lake sediments, modelled using generalized additive models (GAMs). The shaded area around each proxy represents the 95% confidence interval of our smoothed estimate. Core age was assigned using the constant rate of supply (CRS) ^{210}Pb dating model.

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4 CHAPTER 4: Branched Glycerol Dialkyl Glycerol Tetraether (brGDGT) - Temperature Calibrations for Cultus Lake, British Columbia, Canada

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Abstract

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are bacterial membrane lipids widely used as paleotemperature proxies. In this study, we developed a lake-specific brGDGT calibration for Cultus Lake, British Columbia, using seasonally resolved suspended particulate

matter (SPM) and *in situ* temperature measurements. In Cultus Lake's SPM, we identified brGDGT-Ib as the most sensitive brGDGT isomer, and multivariate modelling showed a two-predictor regression [(Calibration₁, which included brGDGT-Ib and Ila') ($R^2 = 0.87$, RMSE = 2.07 °C, SEE = 2.32 °C)] that markedly outperformed both MBT'_{5ME} ($R^2 = 0.66$, RMSE = 3.38 °C, SEE = 3.78 °C) and published regional/global calibrations. These findings demonstrated that lake-specific calibrations substantially improved the accuracy of temperature estimates, providing a more robust framework for generating hydroclimate histories in the North Pacific and advancing our capacity to resolve climate-ecosystem linkages across salmon-bearing watersheds.

1.0 Introduction

Climate change remains one of the most pressing global challenges of the 21st century, necessitating robust tools for tracking historical temperature trends to inform future climate projections (Bracegirdle et al., 2019; Tierney et al., 2020). Since instrumental climate records extend only a few centuries at most, paleoclimate proxies are indispensable for exploring Earth's long-term climate variability and understanding the full range of natural and anthropogenic climate dynamics (Castañeda & Schouten, 2011; Liang et al., 2022; Wu et al., 2023). Among these, lake water, soils, and sediments offer a valuable and widely distributed natural archive, preserving high-resolution, continuous records of local and regional environmental conditions across centennial to millennial timescales (Miller et al., 2018; Raberg et al., 2022; Russel et al., 2018; Zhao et al., 2021).

One promising suite of biomarkers in lakes is the glycerol dialkyl glycerol tetraethers (GDGTs), membrane-spanning lipids produced by archaea and bacteria (Damsté et al., 2000; Pearson et al., 2025; Raberg et al., 2021; Weijers et al., 2006; Zhao et al., 2021). GDGTs occur in two primary structural classes: isoprenoid GDGTs (isoGDGTs), produced mainly by archaea in marine and some freshwater environments (Bauersachs et al., 2024; De Jong et al., 2014, 2024; Li et al., 2023); and branched GDGTs (brGDGTs), which are synthesized predominantly by bacteria, particularly members of Acidobacteria, in soils, peats, and freshwater systems (Damsté et al., 2000; De Jong et al., 2014; Peterse et al., 2010; Raberg et al., 2024; Weijers et al., 2006; Yang et al., 2013). The brGDGTs are characterized by two alkyl chains ether bound to a glycerol backbone, with variability arising from the number and position of methyl branches

and the presence of zero, one, or two cyclopentane moieties (De Jonge et al., 2014; Russell et al., 2018; Weijers et al., 2006). These structural modifications yield a suite of tetra, penta, and hexamethylated brGDGTs, with methyl groups commonly positioned at the 5' and 6' carbon atoms of the alkyl chains (Damsté et al., 2000; De Jonge et al., 2014; Russell et al., 2018). Recent evidence has revealed the presence of novel 7'-methyl brGDGT isomers, distinguishable from conventional 5- and 6-methyl variants (Ding et al., 2016). These compounds exhibit distinct chromatographic behaviour, with defined peak shoulders indicative of methyl substitution at the C-7 position of the alkyl chain, providing further structural diversity among brGDGTs (Ding et al., 2016).

The environmental sensitivity of brGDGTs, particularly to temperature and pH, has enabled their use as quantitative proxies through the development of empirical indices: the Methylation of Branched Tetraethers (MBT) and the Cyclization of Branched Tetraethers (CBT) indices (Damsté et al., 2008; De Jonge et al., 2014; Weijers et al., 2007a; Weijers et al., 2007b). Studies have shown that the MBT index is positively correlated with ambient temperature, whereas cyclization is primarily modulated by pH (Damsté et al., 2008; Martínez-Sosa et al., 2020; Peterse et al., 2012; Raberg 2022; Yang et al., 2015). These indices have been applied across a wide array of terrestrial and lacustrine settings from tropical to polar latitudes and over timeframes ranging from the late Holocene to the Cretaceous (Weijers et al., 2007b; Woltering et al., 2014; Zheng et al., 2015). Advances in high-performance liquid chromatography (HPLC) have enabled more precise separation of 5- and 6-methyl brGDGT isomers, refining index calculations and markedly improving their predictive accuracy (De Jong et al., 2014; Hopmans et al., 2016). Specifically, the MBT'_{5ME} index, focuses exclusively on 5-methyl brGDGT isomers, excluding the 6-methyl homologues that can be influenced by non-thermal factors, thereby yielding stronger and more consistent correlations with measured temperature (De Jonge et al., 2014; Hopmans et al., 2016; Naafs et al., 2017). Similarly, CBT_{5ME} improves pH prediction by focusing on a more constrained set of brGDGTs linked to cyclization (De Jonge et al., 2014).

Global brGDGT calibrations are derived from large composite datasets that pool samples from geographically and climatically diverse environments, primarily soils and surface sediments, to generate universal temperature proxy relationships (Russell et al., 2018; Zhao et al., 2023). While these calibrations have been instrumental in establishing broad paleotemperature frameworks, they are not without limitations when applied at the site-specific

scale (Zhao et al., 2021). Increasing evidence shows that both global and non-local calibrations may obscure local climate signals or introduce systematic biases in environmentally complex or dynamic systems, particularly lakes (Cao et al., 2020; Dang et al., 2016; Raberg et al., 2022; Zhao et al., 2021; Zhao et al., 2023). A key issue is that global datasets often amalgamate samples collected across seasons, depths, and depositional settings, thereby conflating signals influenced by stratification, salinity, redox gradients, and microbial community heterogeneity (Russell et al., 2018; Zhao et al., 2023). These confounding effects demonstrate the need for lake-specific or regional calibrations that explicitly account for local microbial composition and environmental variability. Otiniano et al. (2023) conducted a comprehensive study investigating the variability of lacustrine brGDGTs across 67 lakes in Alaska and Northwest Canada. They found that brGDGT distributions are strongly influenced by seasonal and local environmental conditions and highlighted that the commonly used MBT'_{SME} temperature proxy may be biased by regional factors such as lake-specific microbial communities, salinity, and hydrological regimes. Most lake-specific brGDGT calibrations to date have relied on soils or surface sediments (Bauersachs et al., 2024; De Jonge et al., 2014; Otiniano et al., 2023; Raberg et al., 2021; Russell et al., 2018), with only a few studies employing water-based approaches (Zhao et al., 2021).

In this study, we examine Cultus Lake, a temperate, seasonally stratified lake in British Columbia, Canada, to refine brGDGT-based temperature proxies through the development of a lake-specific calibration. We systematically assessed the spatial and temporal distribution of brGDGTs across the lake's catchment soils, waters via suspended particulate matter (SPM), and surface sediments. Using brGDGT abundances from seasonally resolved water column, inflow water samples and concurrent *in situ* temperature measurements, we constructed an empirical temperature calibration tailored to Cultus Lake. This site-specific model provides a robust framework for evaluating the performance of global brGDGT-based temperature and other site-specific temperature proxies at the local scale (Bauersachs et al., 2024; De Jonge et al., 2014; Otiniano et al., 2023; Raberg et al., 2021; Russell et al., 2018; Zhao et al., 2021), revealing the advantages of customized calibrations for improving the accuracy of paleotemperature estimates. By advancing the precision of molecular proxies in lacustrine environments, the Cultus Lake calibration contributes to a broader effort to enhance local paleoclimate reconstructions.

2.0 Materials and Methods

2.1 Field Sampling and Study Site

Cultus Lake is a thermally stratified, warm monomictic lake situated in the Cascade Mountains of southwestern British Columbia, Canada. It resides within a bi-national watershed spanning 69 km², of which 82% lies in Canada and 18% in the United States (Figure 4.1). The lake occupies a glacially carved, steep-sided basin at an elevation of 150 m above sea level, encompassing a surface area of 6.3 km², with a mean depth of 31 m and a maximum depth of 44 m (Shortreed 2007). From late spring through early autumn, the lake undergoes stable stratification followed by complete winter overturn, forming well-defined seasonal thermal gradients. These characteristics make Cultus Lake a compelling site for investigating the production and distribution of temperature-sensitive biomarkers, such as brGDGTs.

The watershed is located in the Coastal Western Hemlock Biogeoclimatic Zone, marked by mild, rainy winters and warm, dry summers. From the Cultus Lake weather station, located about 45 m from the lake, the mean annual temperature is approximately 10 °C, with seasonal values ranging from mean winter lows of 1.9 °C to mean summer highs of 20.01 °C (Environment Canada 2025). Hydrologically, the lake is sustained by 11 primary tributaries, which together drain ~60% of the catchment area, while the remaining input comes from diffuse, unchanneled surface runoff. These tributaries define five sub-catchments: Vedder Mountain, International Ridge, Columbia Valley, Smith Falls Creek, and Sweltzer Creek, each differing in geomorphology, vegetation, and land use (Putt et al., 2019). Vedder Mountain is characterized by regenerating broadleaf forests dominated by *Acer macrophyllum* and *Alnus rubra*, whereas International Ridge, which falls largely within Cultus Lake Provincial Park, supports dense coniferous stands of *Thuja plicata* and *Pseudotsuga menziesii* (Shortreed 2007). The Columbia Valley sub-watershed spans both forested uplands and agricultural lowlands and includes Frosst Creek, the lake's largest tributary, and Spring Creek, which emerges from a regional aquifer (Shortreed 2007). The Sweltzer Creek sub-watershed encompasses the northern outlet and adjacent residential areas, introducing additional variability to the lake's hydrology (Shortreed 2007). The littoral zone of Cultus Lake, representing roughly 12% of its surface area, is extensively colonized by invasive Eurasian watermilfoil (*Myriophyllum spicatum*), a species known to alter benthic habitat complexity and biogeochemical cycling (Chiang et al., 2015). The

bathymetric structure of Cultus Lake, coupled with its diverse watershed, generates distinct environmental gradients that likely influence microbial community composition and the production of GDGTs. These features offer a robust framework for developing a lake-specific brGDGT-temperature calibration informed by *in situ* observations from this dynamic freshwater environment.

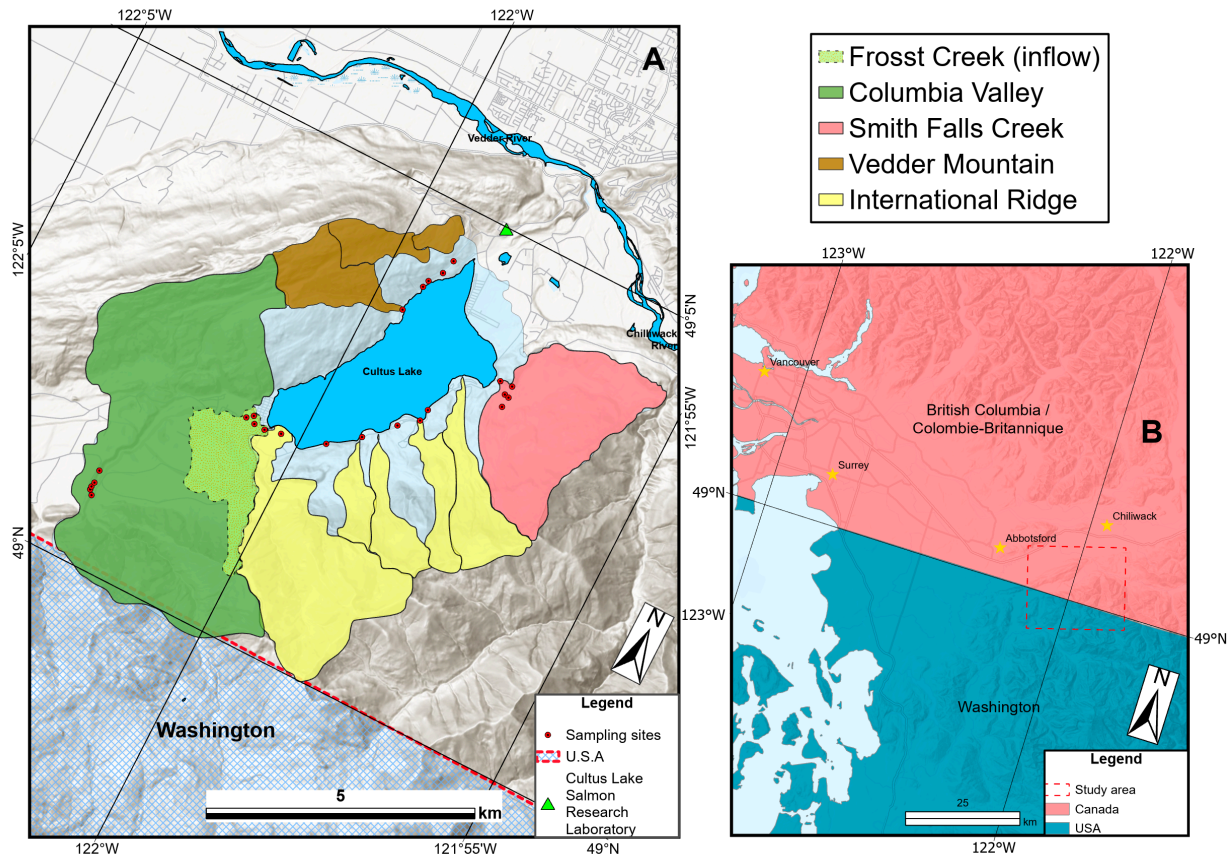


Figure 4.1 Map of Cultus Lake, BC, showing sampling location across its sub-watersheds. Map modified from Putt et al. (2019).

2.2 Soil Sampling

To assess spatial and temporal variability in GDGT distributions across the Cultus Lake watershed, soil samples were collected from five randomly selected locations within each of the lake's five principal sub-watersheds: Vedder Mountain, International Ridge, Columbia Valley, Smith Falls and Frosst Creek (Inflow). Soil sampling campaigns were conducted during three

distinct seasonal intervals: July (warm season), October (mid-season), and December (cold season) in 2022. This temporal resolution was designed to capture seasonal fluctuations in GDGT composition under varying environmental conditions. At each site, surface soil samples were taken from the upper 0-5 cm of the soil profile using a handheld stainless-steel shovel to target the biologically active zone. A total of five samples were collected from each watershed across the three seasons, yielding 75 soil samples. Sampling locations were georeferenced using a handheld GPS device to ensure spatial consistency across all time points. Immediately after collection, samples were sealed in sterile Whirl-Pak® bags, placed on ice in the field, and subsequently stored at -20 °C at the Fisheries and Oceans Canada (DFO) Cultus Lake Salmon Research Laboratory (CLSRL) until transfer to the University of Ottawa (uOttawa) for further processing.

In the laboratory, samples were freeze-dried to eliminate residual moisture and then dry-sieved to isolate the fine fraction suitable for GDGT analysis. A nested stainless-steel sieve stack (Fieldmaster™; 5 mesh [4000 µm], 10 mesh [2000 µm], and 35 mesh [500 µm]) was used in conjunction with a Thermo Scientific™ MaxQ 2508 orbital shaker to separate coarse organic debris from finer particles. Samples were shaken continuously for 15 minutes, and the < 500 µm fraction was retained for downstream lipid extraction and quantification.

2.3 Water Sampling

To characterize seasonal variability in GDGT composition in water and develop a lake-specific temperature and pH calibration, water samples were collected from both the main water column of Cultus Lake and its principal inflowing tributary. At Frosst Creek (the inflow), one water sample was collected during each seasonal window: July, October, and December. In the main water column, seven samples were collected: one each in August and September, and two each in November and December. The water-column and inflow sampling represent ten sampling events spanning three seasonal periods in 2022: July and August (warm season), September and October (mid-season), and November and December (cool season). At the pelagic site, integrated water column samples were obtained from the upper 10 m using a 5 mL Van Dorn sampler. Approximately 20 L of water per event was collected and immediately transferred into sterile polycarbonate bottles. In parallel, 20 L of inflow water was directly collected at Frosst Creek using sterile polycarbonate bottles. In all, 10 water samples were

obtained from July to December (JASOND). All water samples were processed on-site at DFO (CLSRL) using a gravity-fed vacuum filtration system equipped with Whatman GF/F glass microfiber filters (47 mm diameter, 0.7 μm pore size; GE Healthcare Life Sciences, CAT No. 1825-047). Filtration assemblies were pre-cleaned and assembled in advance to minimize the risk of contamination. Filters retaining SPM were transferred into small petri dishes, frozen at -20°C at the DFO (CLSRL) and subsequently shipped to uOttawa for further processing. Upon arrival, filters were stored at -20 °C and later transferred into sterile 60 mL polypropylene tubes and then freeze-dried before lipid extraction and GDGT analysis.

During each water column sampling event, vertical profiles of key limnological variables, including temperature, pH, dissolved oxygen (DO), and specific conductivity, were measured *in situ* at 0.5 m intervals from the surface to 10 m depth using a YSI ProDSS multiparameter probe. These profiles provided high-resolution data on thermal stratification and redox gradients, including the depth of the oxycline, critical for interpreting microbial lipid production dynamics. In November, water profiles were not measured; instead, water profile data available in Arbarzadeh et al. (2021) were used. At Frosst Creek, surface water temperature and pH were measured using a Hanna HI-98129 Combo pH/Conductivity/TDS Tester to characterize the biogeochemical properties of inflowing waters and assess their potential contribution to the GDGT source.

2.4 Surface Sediment Sampling

A 44 cm sediment core was retrieved on October 5, 2022, from the deepest depositional basin of Cultus Lake (41 m water depth) using a Universal Percussion Corer, equipped with a 6.67 cm internal diameter barrel. The core was immediately sectioned at 0.5 cm intervals from top to bottom using a calibrated vertical extruder. Sediment from the surface of each interval was collected using a spatula, cleaned between each use, and transferred into Whirl-Pak bags and subsequently preserved at -20 °C at the DFO (CLSRL). Samples were later transported to uOttawa. Upon arrival, all the sediment samples were freeze-dried to remove residual moisture prior to lipid extraction. For this study, the uppermost 5 cm of the core was used to represent recent depositional conditions.

2.5 Lipid Extraction and GDGT Analysis

Lipid extraction was conducted on three types of environmental matrices: soils, SPM retained on glass microfiber filters from the water column and inflow water samples, and surface sediments. For soil samples, 2 g of freeze-dried material was used, while 1 g was used for sediments. In the case of water samples, all available freeze-dried filters were included in the extraction. Blanks consisting solely of clean microfiber filters were processed. Prior to extraction, samples were homogenized with diatomaceous earth (Hydromatrix) at a 10:1 ratio (w/w) to facilitate solvent dispersion and improve extraction efficiency. The mixtures were loaded into 11 mL stainless steel extraction cells for an automated Dionex 200 Accelerated Solvent Extractor (ASE). Each cell was fitted at the base with a 20 mm cellulose ASE filter, followed by a layer of Hydromatrix, the sample mixed with Hydromatrix, and a final top layer of Hydromatrix to maintain uniform solvent flow and pressure throughout the extraction process. Lipid extraction was performed using a 9:1 (v/v) dichloromethane and methanol (DCM:MeOH) solvent mixture under the following ASE conditions: 100°C, 1450 psi, 5 min heat, 5 min static, 5% flush, 90 sec purge, and three extraction cycles. Approximately 33 mL of solvent was used per sample. The total lipid extracts (TLEs) were collected in pre-labelled glass vials and subsequently dried under a stream of high-purity nitrogen.

Following extraction, TLEs were subjected to column chromatography for the isolation of operationally defined lipid fractions. Extraction columns (5 mL) were dry packed with a quartz wool plug, followed by 2.0 g of activated aluminum oxide (activated, Brockmann I, standard grade, neutral (Sigma-Aldrich; Cat. No. 199974-1KG, Lot BCCN1509) and 1.8 g of anhydrous sodium sulphate (anhydrous, granular, Certified ACS (Fisher Chemical; Cat. No. S421-1, Lot 225879). Both compounds were pre-heated at 150 °C for at least two hours and allowed to cool before packing to remove residual moisture. Prior to the sample application, columns were preconditioned with 6mL 9:1 hexane:DCM. Dried TLEs were reconstituted in 9:1 hexane:DCM and fractionated into three components: 4.5 mL of 9:1 hexane:DCM for the apolar fraction, 4.5 mL of 1:1 hexane:DCM for the ketone fraction, and 4.5 mL of 1:1 DCM:MeOH for the polar fraction containing GDGTs. Each eluate was collected in 5 mL glass vials, and the polar fractions were subsequently dried under nitrogen.

The polar fractions were reconstituted in 99:1 (v/v) hexane:isopropanol (Hex:IPA) to prepare them for purification and instrumental analysis. Filtration was carried out using Costar

Spin-X 0.22 μm cellulose acetate centrifuge tube filters (Corning Inc., Cat. No. 8160) placed in sterile 2.0 mL polypropylene tubes. Each sample was vortexed for 20 seconds, sonicated for 10 minutes, and subsequently transferred to the centrifuge tube filter for centrifugation. This process was repeated three times to ensure maximum lipid recovery and remove particulate debris. The filtered extracts were dried under nitrogen and reconstituted in 500 μL of 99:1 Hex:IPA containing a 100 μL C46 internal GDGT standard (hydrogenated monogalactosyldiacylglyceride mixture; CAS 41670-62-6; Item 17886; $\geq 95\%$ purity; molecular formula $\text{C}_{45}\text{H}_{86}\text{O}_{10}$; stored at -20°C) at a final concentration of $1\ \mu\text{g mL}^{-1}$. 100 μL subsamples of the final preparations were vortexed, sonicated for 10 minutes, and transferred to autosampler vials fitted with glass inserts.

Soil and sediment extracts were analyzed using a Shimadzu LCMS-2020 liquid chromatography-mass spectrometer (LC-MS) equipped with atmospheric pressure chemical ionization (APCI). Chromatographic separation was achieved on Waters Acquity UPLC BEH HILIC columns ($1.7\ \mu\text{m}$, $2.1 \times 150\ \text{mm}$) connected in series, following the method of Hopmans et al. (2016), for the quantification of intact polar GDGTs. For enhanced detection sensitivity, GDGTs in SPM from water samples were analyzed using a Shimadzu LC-20 Nexera system coupled to a Sciex 4000 Q TRAP mass spectrometer operated in positive ion mode with atmospheric pressure chemical ionization (APCI+) and quadrupole 1 selected ion monitoring (Q1 SIM). Chromatographic separation was achieved using tandem Acquity UPLC BEH HILIC columns ($1.7\ \mu\text{m}$, $2.1 \times 150\ \text{mm}$).

2.6 Soil pH Analysis

Soil pH was determined following the protocol outlined in Carter and Gregorich (2007). For each analysis, 2 g of freeze-dried organic soil was weighed into clean polypropylene tubes and combined with 20 mL of deionized water to produce a 1:10 soil-to-water suspension. The mixture was thoroughly stirred to ensure uniform dispersion and then placed on a Thermo Scientific™ MaxQ 2508 orbital shaker for 30 minutes to facilitate equilibration. After shaking, samples were allowed to stand undisturbed at room temperature for 1 hour to permit the separation of particulates. The pH of the supernatant was then measured using high-resolution pH test strips (i.e. range 7-14), ensuring that the reading was taken from the clear aqueous phase to avoid interference from suspended solids.

2.7 Statistical Analysis

All statistical analyses were conducted in R (version 4.4.1) using the tidyverse, vegan, mgcv, pls, lmtest, car, and ggplot packages. Distributions of brGDGT fractional abundances in soil, water, and surface sediments were visualized using boxplots and scatterplots to assess distributional patterns, seasonal variability, and potential outliers. Because most brGDGT distributions deviated from normality, non-parametric methods were applied where appropriate. Differences in overall brGDGT community composition among soil, water, and surface sediment were tested using permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities with Bonferroni-adjusted pairwise comparisons. Homogeneity of variance was assessed using permutational analysis of multivariate dispersion (PERMDISP), and compound contributions to dissimilarities were identified by similarity percentage analysis (SIMPER). Ordination was performed using principal coordinates analysis (PCoA), and environmental variables (temperature, pH, MBT'_{5ME}, CBT_{5ME}) were projected passively into the ordination space. MBT'_{5ME} and CBT_{5ME} indices were calculated following De Jonge et al. (2014).

$$\text{MBT}'_{5\text{ME}} = \frac{\text{Ia} + \text{Ib} + \text{Ic}}{\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIb} + \text{IIc} + \text{IIIa}} \quad (1)$$

$$\text{CBT}_{5\text{ME}} = -\log_{10} \left(\frac{\text{Ic} + \text{IIb}}{\text{Ia} + \text{IIa}} \right) \quad (2)$$

For each brGDGT structure across soils and water samples, we examined whether their fractional abundances differed among warm, mid, and cool seasons using Kruskal-Wallis tests followed by Dunn's post-hoc comparisons with Benjamini-Hochberg correction. For the water column samples, mean values of temperature and pH of the top 10 m were calculated, while for inflow water, the mean of three temperature measurements taken during sampling was used.

We then assessed the relationships between brGDGTs fractional abundance and temperature and pH using ordinary least squares and robust regressions, with R², p-values, and 95% confidence intervals reported. Both linear and quadratic models were fitted, and model selection used Akaike's Information Criterion (AIC); a $\Delta\text{AIC} > 2$ was taken as evidence of superior fit except in cases where the dataset comprises too many zeros (Burnham and Anderson, 2004). Relationships were considered significant at $p < 0.05$. We regressed the MBT'_{5ME} in SPM against observed lake water temperature to develop an MBT'_{5ME} derived temperature calibration for Cultus Lake BC.

We used stepwise forward selection to identify the best model that tracks Cultus Lake water temperature. We also performed Partial Least Squares (PLS) regression to identify the brGDGTs most predictive of lake water temperature and to develop alternative novel lake-specific calibration indices. Prior to PLS analyses, all brGDGT variables were screened for near-zero variance to ensure model stability and reduce noise. Variables with a standard deviation ≤ 0.001 were considered low-variance and excluded from further analysis. This procedure identified brGDGT-IIIc' as a low-variance compound, and it was subsequently removed from the dataset before PLS regression and multiple linear regression modelling. The `pls()` function from the *pls* package was used to model the relationship between the suite of 14 brGDGTs (predictors) and measured lake water temperature (response). Variable Importance in Projection (VIP) scores were computed to identify key compounds contributing to model performance. Based on PLS results, brGDGTs with high VIP scores (≥ 1) and whose datasets do not include many zeros were considered most predictive of temperature and were selected for MLR analysis. Ten MLR models (Cal₁ - Cal₁₀) were constructed using two brGDGT predictors per model combination to evaluate compound-specific calibration performance. We reported adjusted R², Root Mean Square Error (RMSE), and Standard Error of Estimate (SEE) values and considered relationships statistically significant at $p < 0.05$.

We evaluated the performance of our MBT'_{5ME}-derived calibrations against previously published MBT'_{5ME} models (Table S4.4) and further compared our Cal₁ - Cal₁₀ formulations with established fractional-abundance-based calibrations (Table S4.5). Model stability and predictive skill were assessed using leave-one-out cross-validation (LOOCV), which provided an unbiased estimate of calibration performance by iteratively withholding each observation and predicting it using a model trained on the remaining dataset.

3.0 Results

3.1 Cultus Lake Water Profile

Seasonal water column profiles of Cultus Lake (Figure 4.2) illustrate its warm monomictic character, with strong stratification in late summer followed by full mixing by winter. In August, surface waters warmed above 20 °C, with a well-defined thermocline at 8-12 m. Stratification persisted into September but weakened as surface cooling progressed, and by

early to mid-October, the thermocline had deepened and attenuated. Water column profiles were not collected in November; however, Arbarzadeh et al. (2021) provide a full vertical profile for this period. Their observations indicated that stratification persists into November, with cooler surface waters ($\sim 11\text{ }^{\circ}\text{C}$) and a thermocline located at approximately 12 m depth. By December, the lake was isothermal at $\sim 1\text{--}3\text{ }^{\circ}\text{C}$, confirming turnover. These transitions were most pronounced in the upper 10 m, the zone from which all water column samples were collected for brGDGT analysis. Across August to December, this surface layer cooled from $> 20\text{ }^{\circ}\text{C}$ to $\sim 10\text{ }^{\circ}\text{C}$, while dissolved oxygen remained high ($9\text{--}11\text{ mg L}^{-1}$). In summer, elevated surface conductivity and high epilimnetic pH ($8.5\text{--}9.0$) showed stratification, whereas after turnover these parameters converged with depth, producing uniform vertical profiles. Below 10 m, stratified months (August - October) showed higher conductivity, lower pH, and oxygen depletion beneath 12 m, whereas winter mixing (December) erased these gradients. The dashed line at 10 m (Figure 4.2) showed the dual role of this depth in structuring our calibration dataset. During stratified months, it intersected the thermocline and captured the transition between a warm, oxygen-rich epilimnion and a cooler, oxygen-reduced hypolimnion, whereas in December it lay within a well-mixed column. Thus, brGDGT assemblages sampled above 10 m represent surface-dominated microbial production during stratification but an integrated whole-lake signal during turnover. In Cultus Lake, epilimnetic waters remain well oxygenated throughout the period from July to December, with little seasonal variability in oxygen compared to the pronounced shifts in temperature.

Mean inflow water pH and temperature in July were 8.26 and $24.5\text{ }^{\circ}\text{C}$, respectively. By October, mean inflow pH and temperature were 8.25 and $10.1\text{ }^{\circ}\text{C}$, and by early December, they had decreased to 7.38 and $8.23\text{ }^{\circ}\text{C}$, respectively. Across these sampling periods, neither pH nor temperature differed significantly between the inflow and the main water column. Paired t-tests indicated no significant difference in water temperature between sites ($t(2) = -0.31$, $p = 0.79$, mean difference = $-0.79\text{ }^{\circ}\text{C}$, 95% CI: -11.80 to 10.22) and similarly no significant difference in pH ($t(2) = -2.33$, $p = 0.15$, mean difference = -0.11 , 95% CI: -0.31 to 0.09). Given the lack of statistically meaningful differences in these key environmental parameters, inflow and main water column samples are pooled and collectively referred to as “water (SPM)” throughout this study.

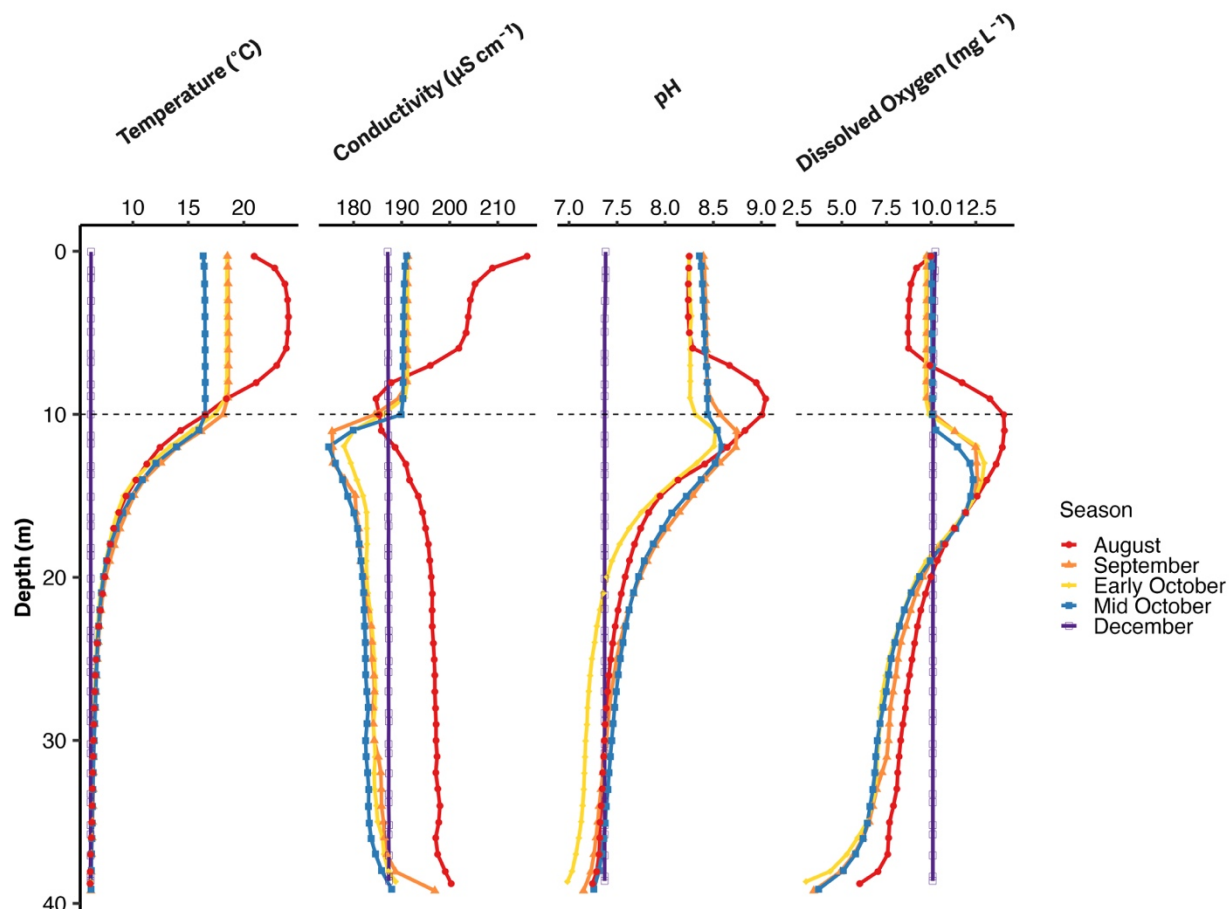


Figure 4.2 Depth profiles (0-40 m) of temperature ($^{\circ}\text{C}$), specific conductivity ($\mu\text{S cm}^{-1}$), pH, and dissolved oxygen (mg L^{-1}) in Cultus Lake at five sampling dates (August, September, early October, mid-October, and December). Curves are 1 m binned means with depth increasing downward. The horizontal dashed line marks 10 m, the upper limit of the water-column integration (i.e. epilimnion) sampled for brGDGT analysis.

3.2 BrGDGTs Abundance in Cultus Lake Catchment Soils, Water Samples, and Surface Sediment

BrGDGTs were consistently detected in soils, water, and surface sediments of Cultus Lake, but their relative distributions varied markedly in soil, water, and surface sediment (Figure 4.3A). Soils were dominated by brGDGT-Ia and IIa, with mean fractional abundances exceeding 0.25, alongside moderate contributions from brGDGT-IIa'. In contrast, soils showed a comparatively low abundance of highly methylated brGDGT compounds, including brGDGT-IIIa and IIIa' with a mean fractional abundance of approximately 0.04. Water samples exhibited

a more diffuse profile, with brGDGT-Ia, IIa, and IIa' remaining prominent, but also displaying higher proportions of brGDGT-Ib, IIb, IIb', IIIa and IIIa'. In contrast to soils and water, surface sediments were strongly enriched in brGDGT-IIIa (~0.42) and IIIa' (~0.22), while exhibiting lower abundances of brGDGT-Ia, IIa, IIa', IIb and IIb'. Surface sediment also contained the lowest proportions of brGDGT-Ib and Ic. For the remaining brGDGTs, including IIc, IIc', IIb, IIb', IIc, and IIc', fractional abundances were uniformly low and often nondetectable across soil, water and surface sediment.

Statistical comparisons using Kruskal-Wallis tests followed by Dunn's post-hoc contrasts (BH-adjusted; $p < 0.05$) revealed significant differences among soil, water, and surface sediment for nearly all brGDGT compounds, as indicated by distinct letter groupings above boxplots (Figure 4.3A). BrGDGT-IIb was the only compound that did not differ significantly among soil, water, and surface sediment.

PCoA of brGDGT fractional abundances based on Bray-Curtis dissimilarities revealed clear and compartment-specific clustering of soil, water, and surface sediment samples (Figure 4.3B). Soil formed a tight and coherent cluster along the positive PCoA1 axis, indicating high within-group similarity and a distinct brGDGT signature. Surface sediments clustered strongly along the negative PCoA1 axis, reflecting substantial compositional divergence from soils. Water samples occupied an intermediate and more dispersed region, positioned between soils and sediments along PCoA1. These patterns were statistically supported by PERMANOVA, which showed significant global differences in brGDGT composition among compartments ($p < 0.001$). Pairwise comparisons indicated the strongest separation between water and surface sediments ($F = 22.87$, $R^2 = 0.560$, $p \text{ adj.} = 0.001$), followed by soil and surface sediment ($F = 62.63$, $R^2 = 0.436$, $p \text{ adj.} = 0.001$), while soil and water showed more moderate but still significant differentiation ($F = 12.01$, $R^2 = 0.129$, $p \text{ adj.} = 0.001$) (Table S4.1). PERMDISP results indicated no significant dispersion differences for water and surface sediment ($p \text{ adj.} = 0.393$) or soil and water ($p \text{ adj.} = 0.418$), confirming true centroid differences, although some heterogeneity in dispersion was detected for soil and surface sediment ($p \text{ adj.} = 0.042$). SIMPER analysis (Table S4.2) showed that the water-surface sediment contrast was primarily driven by a small set of brGDGTs. The surface sediment-enriched compounds, brGDGT-IIIa (34.7%) and IIIa' (9.4%), were the strongest contributors to dissimilarity, while Ia and IIa', each contributing 10.7% and elevated in water, were the primary opposing contributors. Together, these four

brGDGTs accounted for approximately 66% of the total compositional separation between water and surface sediments.

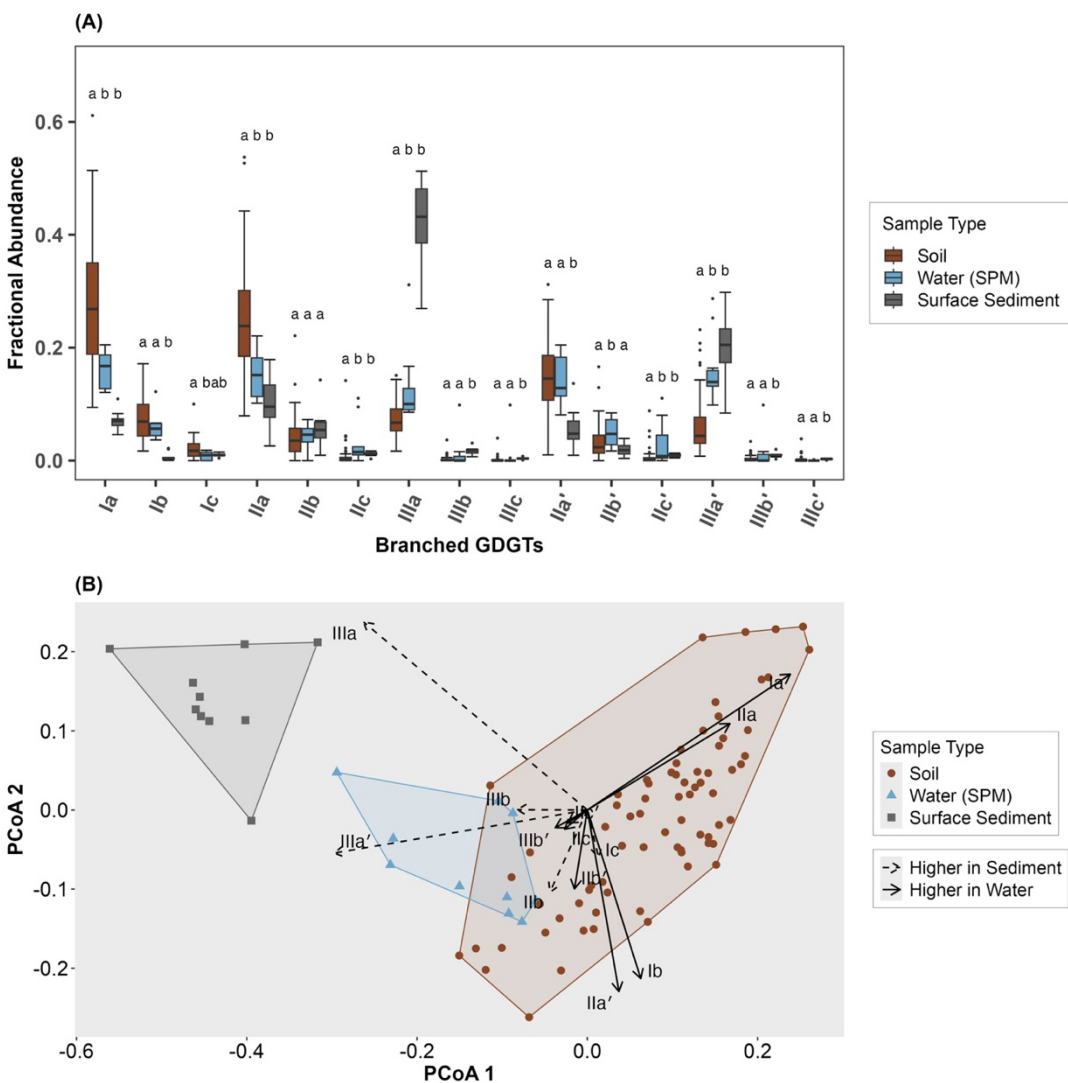


Figure 4.3 (A) Boxplots showing fractional abundances of brGDGTs in soil ($n = 75$), water ($n = 10$), and surface sediment ($n = 10$). Different letters above boxes indicate significant differences among sample types based on Kruskal-Wallis tests followed by Dunn's post-hoc comparisons with Benjamini-Hochberg correction ($p < 0.05$). (B) Principal coordinates analysis (PCoA) based on Bray-Curtis's dissimilarities illustrates compositional differences among sample types. Polygons outline 95% sample-type groupings. Arrows represent compounds with the greatest loadings along the first two PCoA axes, indicating which brGDGTs are enriched in water and surface sediments.

3.3 BrGDGTs Abundance and MBT'_{5ME} in Cultus Lake soil, Water, Surface Sediment in Warm, Mid, and Cool Periods

Soil samples from the five sub-watersheds (Smith Falls, Columbia Valley, Vedder Mountain, International Ridge, and Frosst Creek) were first analyzed separately across the warm, mid-season, and cool sampling periods. Across all sites, none of the 15 brGDGT compounds exhibited significant seasonal differences (Kruskal-Wallis tests, $df = 2$, $p > 0.05$), with the exception of brGDGT-IIIc' at International Ridge, which approached marginal significance ($p \approx 0.05$; Table S4.3; Figure S4.1C). To improve statistical power, all soil samples were pooled and re-evaluated (Figure 4.4A). Dunn's post-hoc comparisons showed that for all brGDGTs except IIIc and IIIc', seasonal groups did not differ across warm, mid, and cool periods. Only IIIc and IIIc' showed a significant pairwise difference between mid and cool-season soil samples (Figure 4.4A). However, brGDGT-IIIc and IIIc' consist of many non-detectable (i.e. zero) values, making their pairwise seasonal differences more driven by one or two extreme datapoints.

Water samples combining inflow and water-column samples, following preliminary confirmation of no difference in temperature or pH between locations, were similarly evaluated. Seasonal boxplots showed higher mean fractional abundances of brGDGT-Ib, IIa', IIb, and IIb' in the warm period, although none of these differences were statistically significant (Figure 4.4B). Only brGDGT-IIIa differed significantly between mid and cool-season water samples. MBT'_{5ME} indices were computed for all samples. Soils showed no discernible seasonal pattern in MBT'_{5ME}, whereas water samples displayed a gradual decline from warm to cool periods, although this trend was not statistically significant (Figure 4.5). Surface sediments consistently exhibited lower MBT'_{5ME} values than soils and water. A separate comparison among sample types demonstrated significant differences in MBT'_{5ME} across soil, water, and surface sediment (Kruskal-Wallis $\chi^2 = 33.70$, $df = 2$, $p = 4.8 \times 10^{-8}$). Dunn's post-hoc tests indicated significantly higher MBT'_{5ME} values in soils relative to water ($p \text{ adj.} = 0.0108$) and surface sediments ($p \text{ adj.} = 1.66 \times 10^{-7}$), and higher values in water relative to surface sediments ($p \text{ adj.} = 0.0383$).

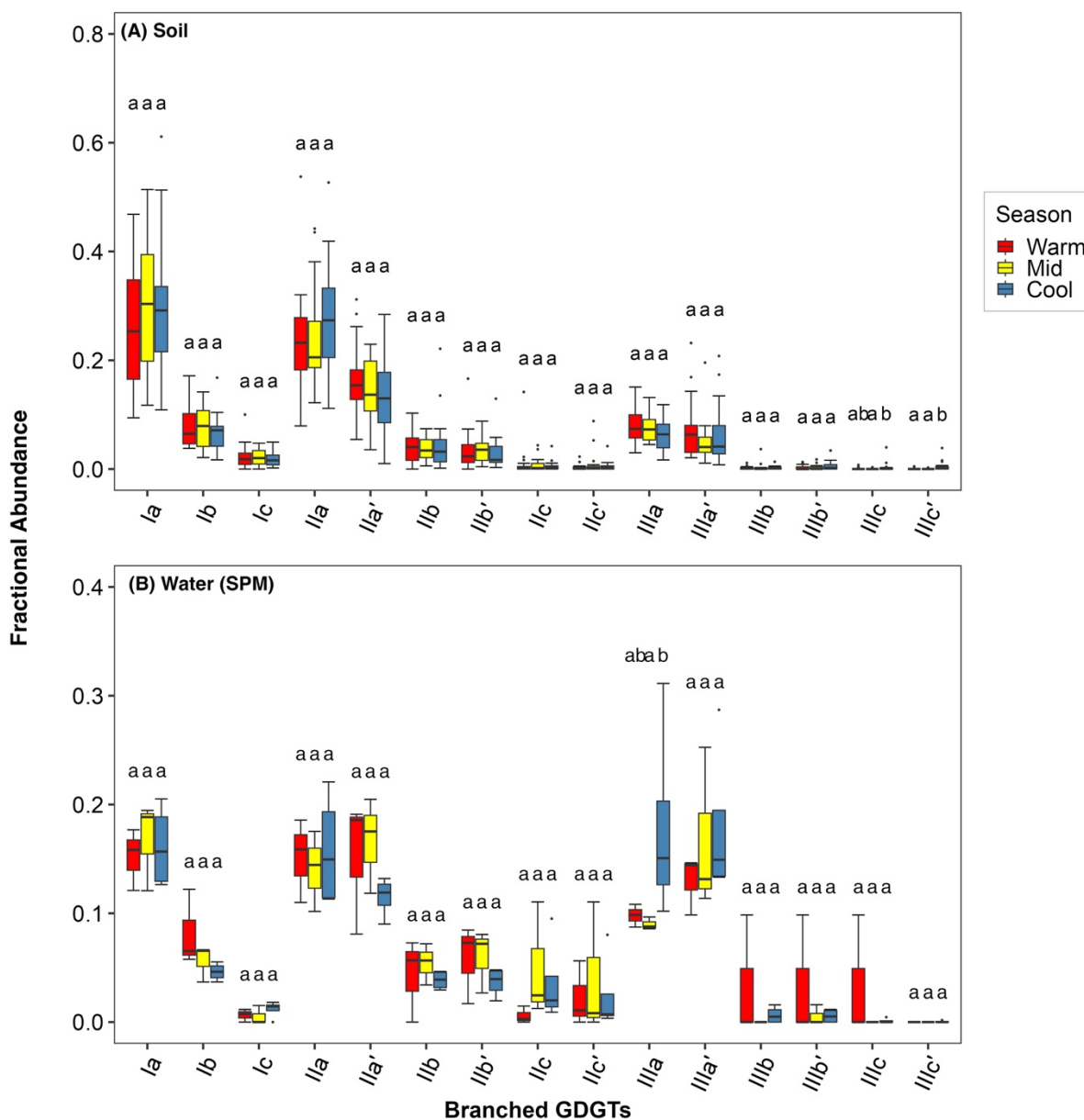


Figure 4.4 Seasonal distribution of the fractional abundances of branched GDGTs in (A) soils from all five sub-watersheds, and (B) water from the inflow tributary and water column of the Cultus Lake. Boxplots display the fractional abundances of individual brGDGT compounds across three seasonal categories: Warm, Mid, and Cool, corresponding to July, October, and December, sampling periods, respectively. Different letters above boxes indicate significant differences among sampling seasons based on Kruskal-Wallis tests followed by Dunn's post-hoc comparisons with Benjamini-Hochberg correction ($p < 0.05$).

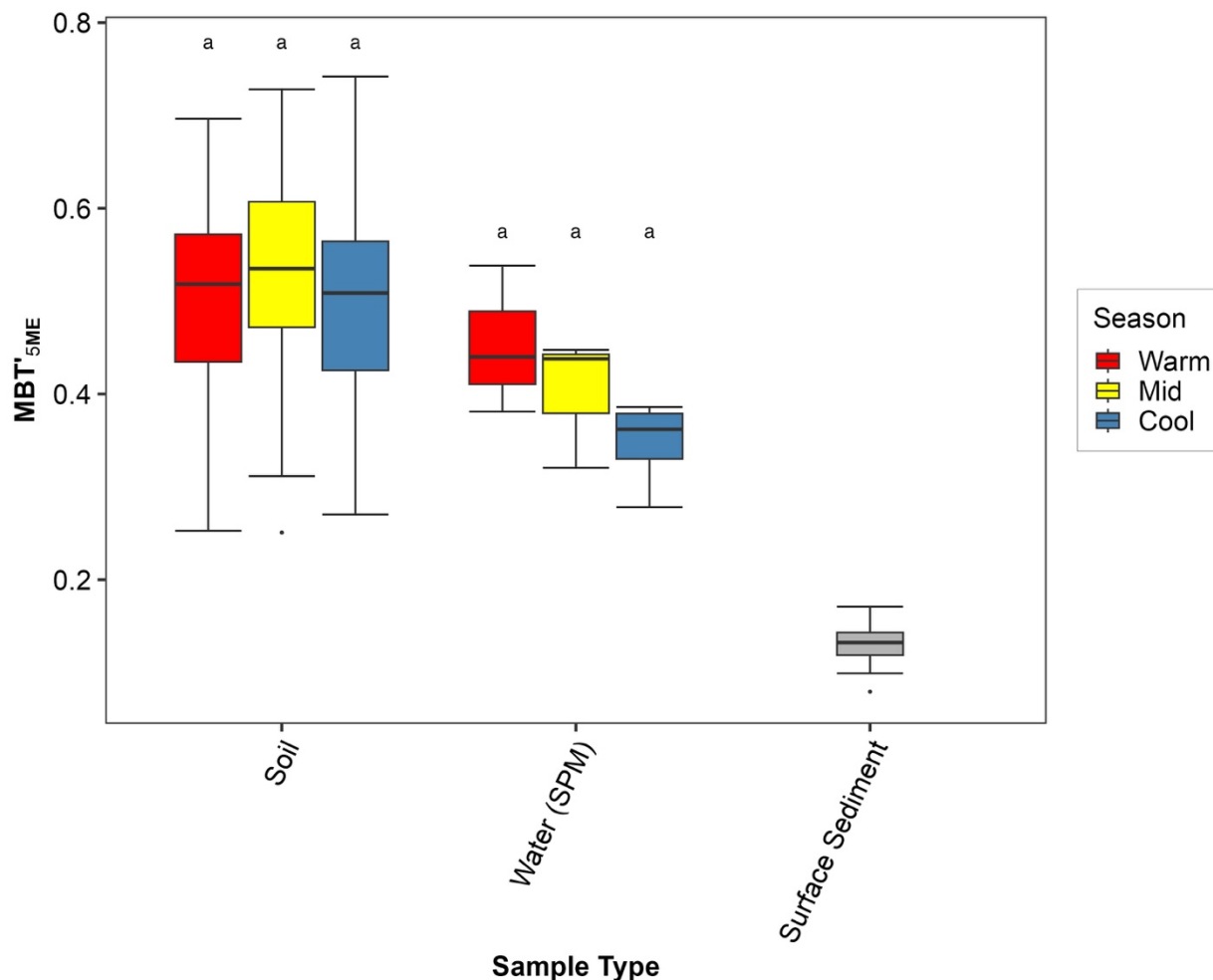


Figure 4.5 Boxplots showing 5-Methyl Methylation of Branched Tetraethers (MBT'_{5ME}) values for soil, water, and sediments of Cultus Lake. Seasonal groups were provided for soil and water and are colour-coded: blue (Cool) for December samples, yellow (Mid) for October samples, and red (Warm) for July samples.

3.4 Relationships between BrGDGT Fractional Abundance and Cultus Lake Water Temperature and pH

BrGDGT-water temperature relationships were examined for all 15 compounds using ordinary least-squares and quadratic regressions across seasonally resolved water samples (Figure S4.2). Several compounds exhibited significant temperature dependence. Quadratic models were significant for brGDGT-IIa' ($p = 0.021$, $R^2 = 0.57$), IIIa ($p = 0.049$, $R^2 = 0.46$), and IIb' ($p = 0.022$, $R^2 = 0.57$). A significant linear relationship was detected only for brGDGT-Ib ($p = 0.005$, $R^2 = 0.60$). In contrast, compounds such as brGDGT-Ia, IIa, IIIa', and IIb showed no

significant linear or quadratic fit. For the remaining compounds (IIIb, IIIb', Ic, IIc, IIIc, IIc', and IIIc'), no temperature dependence was observed, and fractional abundances exhibited no directional trends across the measured temperature range.

Across all brGDGT compounds, fractional abundances showed no clear or consistent relationship with lake water pH (Figure S4.3). For most compounds (Ia, Ib, Ic, IIa, IIa', IIb, IIb', IIc, IIc', IIIa, IIIa', IIIb, IIIb', IIIc, and IIIc'), data points were widely scattered across the observed pH range (7.5-8.5), with no visible monotonic trend.

3.5 Cultus Lake-specific BrGDGT Calibration Based on Lake Water Temperature and MBT'_{5ME}

The MBT'_{5ME} index showed a significant positive relationship with Cultus Lake water temperature. The Cultus Lake calibration yielded an R² of 0.66, with an RMSE of 3.38 °C and SEE of 3.78 °C (p = 0.003) (Figure 4.6; Equation 3). The regression line fell within narrow 95% confidence and wider 95% prediction intervals, indicating moderate explanatory power with expected natural variability in the temperature signal. When compared with previously published MBT'_{5ME} calibrations, the Cultus Lake relationship displayed a steeper slope and higher temperature sensitivity across the observed MBT'_{5ME} range.

$$Cultus_{JASOND} = -14.23 + 72.70 \times MBT'_{5ME} \quad (3)$$

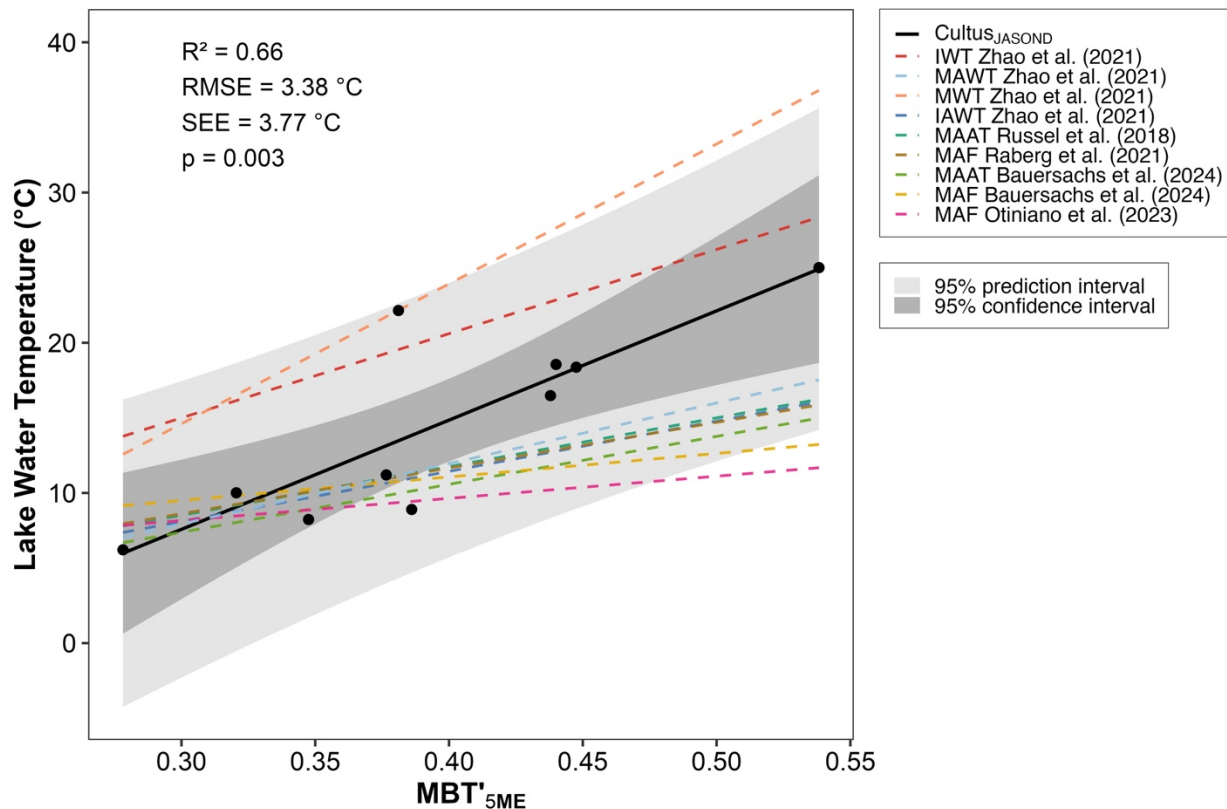


Figure 4.6 New Cultus Lake brGDGT calibration for water temperature using the MBT'_{5ME} index (Equation 2). The Cultus calibration is compared against previously published MBT'_{5ME} -based calibrations (Bauersachs et al., 2024; Otiniano et al., 2023; Raberg et al., 2021; Russell et al., 2018; Zhao et al., 2021) (Table S4.4).

3.6 Cultus Lake-specific BrGDGT Calibration Based on SFS and PLS

PLS regression identified brGDGTs that are most responsive to the changing water temperature of Cultus Lake. The PLS biplot showed that PLS1 explained 27.5% of the total variance and PLS2 explained 17.23%. Environmental vectors for temperature, pH, and MBT'_{5ME} , loaded strongly along PLS1, indicating a common underlying gradient, whereas CBT_{5ME} aligns with PLS2 (Figure 4.7). VIP analysis identified seven brGDGTs-Ib, IIIa, IIIa', IIIc, IIIb', IIc, and IIIb as important predictors of temperature ($VIP > 1$). However, brGDGT-IIc, IIIb, and IIIb' were not included in our calibration since they do not show a temperature relationship (i.e. linear or quadratic relationship (Figure S4.2)), and their high VIP score was due to the strong influence of two to three outliers. Instead, other brGDGTs with VIP almost equal to 1, including brGDGT-IIa' and IIb', showed good data distribution and temperature relationship as seen in Figure S4.2.

Our most temperature-sensitive brGDGT set from the Cultus Lake includes brGDGTs-Ib, IIIa, IIIa', IIa', and IIb'. Among these, brGDGT-Ib exhibited the strongest positive correlation with lake-water temperature.

Using the temperature-responsive compounds identified in the PLS analysis, ten two-predictor multiple linear regression (MLR) models (Cal₁-Cal₁₀) were constructed to estimate the lake water temperature of Cultus Lake. Stepwise forward selection indicated that two-predictor models provided the optimal balance between model complexity and predictive power; adding additional predictors did not improve performance. Across models, performance metrics (adjusted R², AIC, F-statistics, predictor significance, SEE, RMSE, and LOOCV RMSE) were summarized in Table 1. Cal₁, as confirmed by SFS, yielded the highest overall predictive skill (Adj. R² = 0.852; AIC = 51.0; F_{2,7} = 27.00, p < 0.001), with both Ib and IIa' contributing significantly (p < 0.001 and p = 0.006). Cal₂ performed similarly well (Adj. R² = 0.814; AIC = 53.3; F_{2,7} = 20.70, p = 0.001), with both predictors significant at p < 0.05. Cal₃ and Cal₄ showed moderate explanatory power (Adj. R² = 0.622 and 0.606), each with significant overall model fits (p < 0.05), although only Ib was significant in both models. Cal₆ also achieved moderate performance (Adj. R² = 0.589; AIC = 61.2; F_{2,7} = 7.44, p = 0.019), with both IIIa and IIIa' being significant predictors. The remaining models (Cal₅, Cal₇-Cal₁₀) showed weak explanatory power (Adj. R² ≤ 0.199), non-significant model fits (p > 0.05), and higher RMSE and LOOCV RMSE values, indicating limited predictive utility. Across all models, SEE ranged from 2.48 to 6.71 °C, RMSE from 2.07 to 5.61 °C, and LOOCV RMSE from 4.54 to 9.33 °C, with Cal₁ consistently producing the lowest error.

Model performance differed widely when Cultus Lake-specific MLR calibrations were compared with previously published brGDGT-temperature equations (Figure 4.8). Among local models, Cal₁ and Cal₂ most accurately reproduced observed lake water temperatures, yielding R² values of 0.87 and 0.84, RMSE values of 2.07 and 2.33 °C, and SEE values of 2.32 and 2.60 °C, respectively. Cal₃ and Cal₄ provided moderate predictive accuracy (R² = 0.67 - 0.66; RMSE = 3.30 - 3.39 °C), whereas models Cal₅-Cal₁₀ performed poorly, with R² ≤ 0.30 and RMSE values between 4.83 and 5.61 °C.

External calibrations generally showed lower predictive skill and higher error. The De Jonge et al. (2014) Index1 MAAT model showed moderate agreement (R² = 0.71; RMSE = 3.30 °C). In contrast, the Russell et al. (2018) calibration explained only 8% of temperature variance

(RMSE = 5.85 °C), and the Otiniano et al. (2023) model performed similarly weakly ($R^2 = 0.23$; RMSE = 7.51 °C). The Raberg et al. (2021) and Bauersachs et al. (2024) calibrations performed worst overall, with R^2 values ranging from -0.12 to 0.42 and RMSE values from 6.53 to 24.84 °C. Overall, the Cultus Lake-specific models, particularly Cal₁ and Cal₂, most accurately reproduced measured lake water temperatures, aligning most closely with the 1:1 line and yielding the lowest predictive errors among all evaluated calibrations.

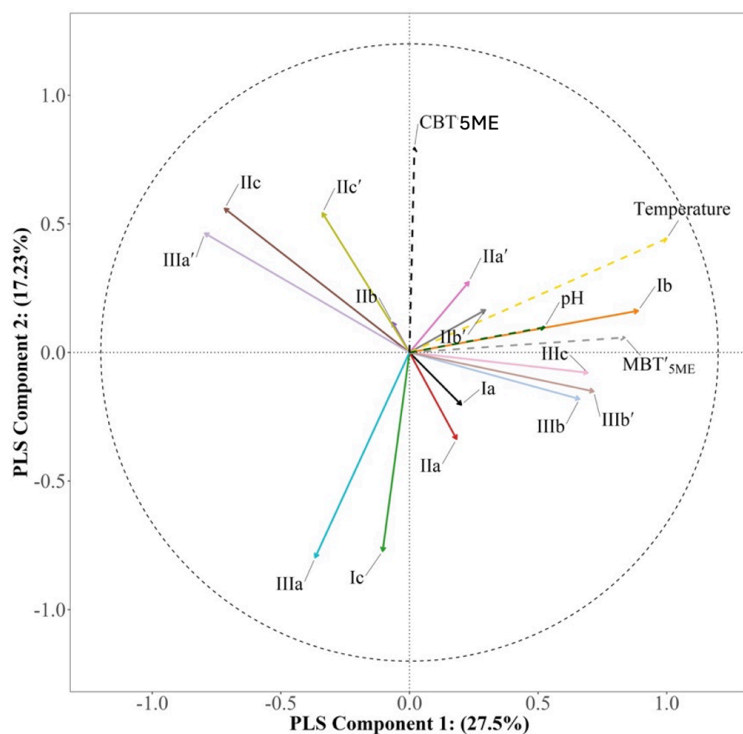


Figure 4.7 Partial Least Squares (PLS) biplot showing the relationship between 14 branched glycerol dialkyl glycerol tetraethers (brGDGT), environmental parameters (temperature, pH), and temperature indices (MBT'_{5ME}, CBT_{5ME}) in Cultus Lake. The first PLS component (PLS1) explains 27.5% of the variance, while the second component (PLS2) accounts for 17.23%. Temperature, pH, MBT'_{5ME}, and CBT_{5ME} align strongly along PLS1, with brGDGT Ib, IIa', IIIc, IIIb, and IIIb' showing the strongest positive relationship with temperature. In contrast, isomers like IIc, IIc', IIIa, and IIIa' display a negative relationship with temperature. BrGDGT-IIIc' not shown due to low variance.

Table 4.1 Multiple linear regression calibrations (Cal₁-Cal₁₀) for Cultus Lake water temperature using combinations of temperature-sensitive branched glycerol dialkyl glycerol tetraether (brGDGT) in lake water. For each calibration, the table reports the fitted equation, adjusted R², AIC, F statistic with degrees of freedom, and p values for each predictor and for the overall model; asterisks indicate p < 0.05. RMSE values are computed by nested leave-one-out cross-validation, i.e. the model is re-fit for each left-out observation.

Model Equation	Adjusted R ²	AIC	F-Statistic	DF	p-value (Predictor 1)	p-value (Predictor 2)	Overall (Model p-value)	SEE °C	RMSE °C	LOOC V RMSE °C
Cal ₁ -9.21 + 225.70 * Ib + 72.10 *IIa'	0.852	51.0	27.00	2, 7	< 0.001*	0.006*	< 0.001*	2.48	2.07	4.93
Cal ₂ -4.35 + 219.09 * Ib + 114.81 *IIb'	0.814	53.3	20.70	2, 7	< 0.001*	0.015*	0.001*	2.78	2.33	4.54
Cal ₃ 6.94 + 180.56 * Ib - 25.30 *IIIa	0.622	60.4	8.40	2, 7	0.018*	0.267	0.014*	3.97	3.32	5.24
Cal ₄ 8.09 + 178.87 * Ib - 26.68 *IIIa'	0.606	60.8	7.93	2, 7	0.024*	0.325	0.016*	4.05	3.39	5.85
Cal ₅ 15.21 - 45.24 * IIIa + 35.91 *IIa'	0.186	68.0	2.03	2, 7	0.167	0.460	0.202	5.82	4.87	7.79
Cal ₆ 31.90 - 55.04 * IIIa - 64.46 *IIIa'	0.589	61.2	7.44	2, 7	0.028*	0.025*	0.019*	4.14	3.46	6.85
Cal ₇ 16.70 - 45.29 * IIIa + 66.09 *IIb'	0.199	67.9	2.12	2, 7	0.161	0.421	0.191	5.77	4.83	7.62
Cal ₈ 18.87 + 29.37 * IIa' - 53.07 *IIIa'	0.186	68.0	2.03	2, 7	0.555	0.167	0.202	5.82	4.83	8.91
Cal ₉ 9.51 + 0.67 * IIa' + 98.07 *IIb'	-0.082	70.9	0.66	2, 7	0.998	0.828	0.548	6.71	5.61	7.97
Cal ₁₀ 20.99 - 53.0 * IIIa' + 40.67 *IIb'	0.168	68.3	1.91	2, 7	0.190	0.651	0.218	5.82	4.92	9.33

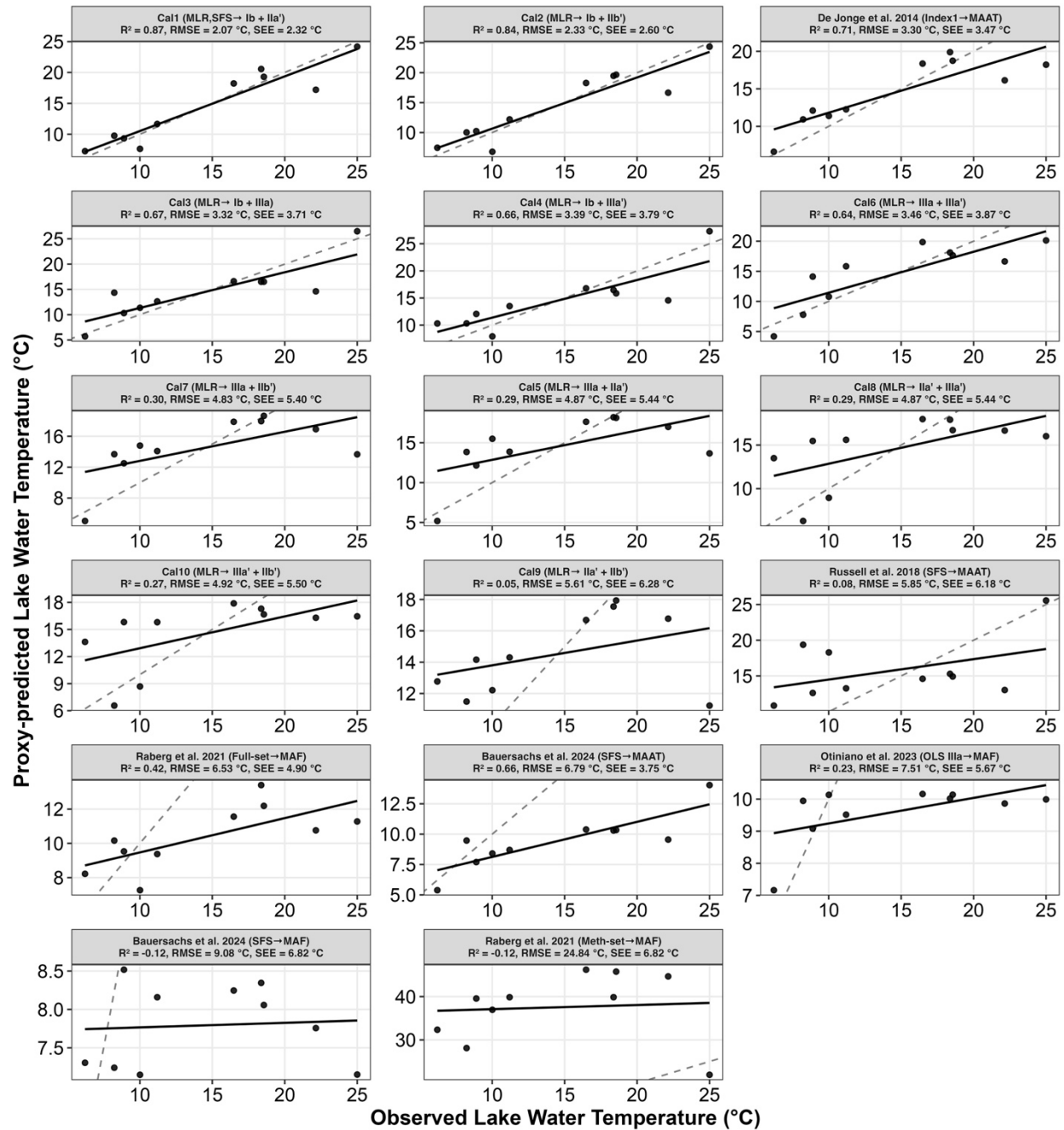


Figure 4.8 Observed versus proxy-predicted water temperatures from Cultus Lake multiple linear regression (MLR) calibrations compared with published models. Cultus calibrations Cal₁ and Cal₂ closely track the 1:1 line, with high correlations (R^2 up to 0.87) and low error (RMSE $\approx$ 2.07-2.33 °C). Published models show weaker fits and systematic biases, typically underpredicting or overpredicting local temperatures. Calibrations are ordered from best to the worst-performing model.

4.0 Discussion

4.1 BrGDGT Abundance and Temperature Controls in Cultus Lake

The abundance of brGDGTs in Cultus Lake soils, water (SPM), and surface sediments, and their seasonal and spatial patterns showed a coherent framework explaining both their distributions and their differing capacities to encode temperature. Soils from all five sub-watersheds showed no detectable seasonal variability in brGDGT fractional abundances (Figure S4.1, Table S4.3) even after pooling all samples together to increase statistical power (Figure 4.4A), indicating that terrestrial assemblages are largely insensitive to short-term climatic fluctuations (De Jonge et al., 2014; Geis et al., 2021; Martínez-Sosa et al., 2021; Weijers et al., 2007a). The absence of seasonal variation in soil brGDGT distributions across the five sub-watersheds further demonstrated the stability of terrestrial brGDGT pools. This stability reflects both the slow turnover of soil microbial communities and the strong physical protection of brGDGTs within mineral-organic matrices (Geis et al., 2021). Once sorbed onto mineral surfaces, these lipids become shielded from rapid microbial degradation and buffered against short-term environmental fluctuations (Geis et al., 2021). Radiocarbon (^{14}C) analyses by Geis et al. (2021) showed that brGDGTs preserved in mineral subsoils can persist for millennia, demonstrating extremely long residence times. Consequently, the composition of soil brGDGT assemblages integrates climate signals over extended periods rather than responding to seasonal temperature variability, explaining their insensitivity to short-term climatic fluctuations despite differences in geomorphology, vegetation, or land use across sub-watersheds of the Cultus Lake (Geis et al., 2021; Putt et al., 2019). The compositional dominance of soils by less-methylated brGDGTs (Ia, IIa, IIa') and high $\text{MBT}'_{5\text{ME}}$ values (Figure 4.3B; Figure 4.5) reflects temperature conditions typical of warm, oxic upland soils rather than colder autochthonous inputs near or below the thermocline (De Jonge et al., 2014; Martínez-Sosa et al., 2021). Because soil $\text{MBT}'_{5\text{ME}}$ values remain stable and do not covary with short-term temperature fluctuations, they cannot account for the temperature-dependent patterns observed in surface sediments (i.e. low $\text{MBT}'_{5\text{ME}}$) of the Cultus Lake, confirming that the sedimentary record is dominated by autochthonous production rather than allochthonous sources.

The lake-wide thermal gradients and seasonal shifts expressed in aquatic brGDGT assemblages within the SPM point to direct temperature influences on lake-water processes,

which in turn drive brGDGT membrane-lipid modification *in situ* in Cultus Lake. This allowed us to develop a water-based calibration that can be applied to sediments to reconstruct temperature, effectively creating an allochthonous domain-match calibration. BrGDGTs in the water column of Cultus Lake exhibited intermediate abundances characterized by a mixture of warm-associated (brGDGT-Ia, IIa, IIa') and cooler-associated (brGDGT-IIIa, IIIa') compounds and a comparatively intermediate MBT'_{5ME} values, reflecting the lake's moderate thermal regime (Figure 4.3A; Figure 4.5). The substantially lower abundance of warm-associated brGDGT-Ia, IIa, and IIa' in lake water relative to soils suggests that the water, particularly from July to December, remained significantly cooler than surrounding upland soils. This temperature difference likely suppresses the production of warm-associated brGDGTs in aquatic microbial communities, indicating that lake-water brGDGT production responds sensitively to short-term thermal conditions. In contrast to soils, where warm-associated brGDGTs dominate under humid, oxic, and thermally stable upland conditions, cooler-associated isomers such as IIIa and IIIa' were more characteristic of aquatic systems and showed elevated relative abundances in water and surface sediments. Notably, brGDGT-IIIa displayed clear seasonal variability aligned with measured temperature shifts, indicating its utility as a short-term thermal indicator in the water column. Although some brGDGTs (e.g., Ia, IIa) appear seasonally stable, several other isomers exhibit temperature-correlated patterns that may become statistically clearer with greater sampling resolution. Overall, the mixed brGDGT assemblage in Cultus Lake highlighted the strong influence of *in situ* aquatic production, with abundance ratios reflecting localized thermal conditions and stratification dynamics rather than terrestrial soil inputs.

Surface sediments showed a distinctly different brGDGT signature from both soils and the water column, characterized by markedly low abundances of warm-associated compounds (brGDGT-Ia, and IIa') and strong enrichment of cooler-associated isomers, particularly IIIa, IIIa', and several 6-methyl brGDGTs (Figure 4.3A; Figure 4.3B). The dominance of these cooler-associated compounds, together with substantially lower sediment MBT'_{5ME} values (Figure 5), indicated that surface sediments record thermal conditions that are colder than those of upland soils and even cooler than the seasonally chilled epilimnion. The pronounced enrichment of brGDGT-IIIa and IIIa', both of which were present in SPM but became disproportionately higher in sediments, points to significant benthic production (Xiao et al., 2016; Zhao et al., 2021). In contrast, the relatively low abundance of several brGDGTs in sediments compared with water

(e.g., Ia, Ib, IIa', IIIb') indicated that not all aquatic compounds are efficiently transferred to the benthos and suggests that *in situ* sedimentary production may also contribute to the sedimentary pool. These patterns suggest that sedimentary brGDGT assemblages reflect a mixture of settling particles and local benthic contributions, rather than a direct, unaltered archive of epilimnetic conditions (Loomis et al., 2014; Zhao et al., 2021). Consequently, elevated sedimentary brGDGT-IIIa or IIIa' values in the Cultus Lake should be interpreted with caution when applying water-based calibrations to sediments, as high proportions of these compounds may reflect colder benthic microenvironments where they are produced. Compositional difference analyses further confirmed that the pronounced contrast between water and sediment arises chiefly from strong enrichment of brGDGT-IIIa and IIIa' in surface sediments, underscoring the importance of distinguishing among production environments when developing and applying water-based paleotemperature calibrations.

4.2 Relationship between BrGDGT and Cultus Lake Water Temperature

Compound-specific analyses improved our understanding of the relationships between brGDGT fractional abundances and water temperature in Cultus Lake. Of the 15 isomers evaluated, brGDGT-Ib emerged as the most clearly temperature-responsive compound, displaying a strong positive linear relationship with water temperature ($R^2 = 0.60$, $p = 0.005$). This pattern aligns with findings from Bauersachs et al. (2024), who showed that brGDGT-Ib consistently exhibited one of the strongest temperature relationships across Central European lakes and frequently serves as a leading predictor in compound-specific calibrations. The agreement between our dataset and independent global observations underscores the robustness of brGDGT-Ib as a thermally sensitive biomarker and supports its focused inclusion in site-specific calibration models.

Several additional compounds brGDGT-IIa', IIb', and IIIa displayed significant quadratic relationships with temperature. The inverted parabolic patterns for brGDGT-IIa' and IIb' indicated that their abundances increase under cooler conditions, reach a maximum at intermediate temperatures (~15-18 °C), and then decline at the warmest intervals. Although the brGDGT-IIb isomer showed a similar pattern to IIb', the relationship was not statistically significant. BrGDGT-IIIa exhibited clear temperature sensitivity in Cultus Lake, with higher fractional abundances under cooler conditions and a decline across the mid-temperature range

(~14-16 °C). Although Otiniano et al. (2023) demonstrated that sedimentary brGDGT-IIIa provides a precise, linear predictor of months above freezing (MAF) in high-latitude lakes, supported by strong negative temperature dependence and low prediction error (RMSE $\approx$ 1 °C), their calibration reflects production environments of those high-latitude systems. In contrast, our SPM data from Cultus Lake, a mid-latitude system, showed that IIIa follows a non-linear (quadratic) relationship with lake water temperature, indicating that IIIa responded to a broader range of thermal variability than observed in high-latitude systems. This suggests that, in principle, the temperature sensitivity observed in SPM could be transmitted into sedimentary archives, provided it is not overwhelmed by the strong benthic enrichment of IIIa observed in Cultus Lake sediments. Under depositional conditions where sedimentary brGDGT-IIIa faithfully reflects water-column production, as appears to be the case in the high-latitude systems calibrated by Otiniano et al. (2023), IIIa may function as an effective sediment-based temperature proxy. However, in lakes where benthic overprinting is substantial, the primary water-column temperature signal may be dampened or lost, underscoring the importance of evaluating production environments before applying IIIa as a standalone paleotemperature indicator.

A large subset of compounds, including brGDGT-Ia, IIa, IIIa', IIIb, IIIb', Ic, IIc, IIc', IIIc, and IIIc', showed no significant linear or quadratic relationships with temperature and exhibited no discernible evidence of encoding a thermal signal. Many of these brGDGTs occurred at near-zero fractional abundances across most samples, with occasional isolated outliers at higher temperatures, resulting in patterns too sparse or inconsistent to support meaningful temperature correlations. Consequently, these compounds appear to contribute little to estimating thermal variability in Cultus Lake.

Similarly, relationships between individual brGDGTs' fractional abundances and pH showed no discernible patterns (Figure S4.4). The absence of clear pH effects is likely attributable to the extremely narrow pH range observed across samples, which limits the ability to detect meaningful correlations even if underlying sensitivities exist. In many lacustrine systems, brGDGTs often showed a weak relationship with pH (Russel et al., 2018; Raberg et al., 2021). The lack of a pH signal, combined with the strong and consistent temperature sensitivities observed for multiple compounds, suggests that temperature, not pH, is the dominant environmental control on brGDGT variability in this system.

4.3 MBT'_{5ME} as a Local Temperature Proxy for Cultus Lake

We quantified the relationship between MBT'_{5ME} values in epilimnetic SPM and *in situ* lake water temperatures. Linear regression yielded a statistically significant calibration (Adj. $R^2 = 0.66$, RMSE = 3.38 °C, $p = 0.003$), with temperature explaining approximately two-thirds of the variability in MBT'_{5ME}. This level of predictive skill is notable given the modest sample size ($n = 10$) and is consistent with global observations identifying MBT'_{5ME} as a robust paleotemperature indicator (De Jonge et al., 2014; Russell et al., 2018). Within Cultus Lake, this relationship reflects strong seasonal stratification, during which brGDGT-producing microbial communities in the epilimnion respond directly to water temperature. Because SPM settles efficiently on the lake floor, the temperature signal encoded in MBT'_{5ME} is expected to be transferred to the sediment record. The Cultus Lake MBT'_{5ME} calibration (Equation 3), therefore, provides an appropriate basis for downcore temperature reconstruction.

As this is the first brGDGT-based calibration developed specifically for Cultus Lake, no local models exist for direct comparison. To contextualize the Cultus MBT'_{5ME} calibration, we compared its performance with published regional and global MBT'_{5ME} temperature models. A wide range of empirical and mechanistic brGDGT temperature calibrations has been developed across lacustrine and terrestrial environments. Early global soil-based frameworks (Weijers et al., 2007a; De Jonge et al., 2014) established MBT'_{5ME} as a broadly consistent temperature proxy. Subsequent regional studies in Arctic and temperate lakes (Russell et al., 2018; Weijers et al., 2007b) demonstrated that in-lake production can generate strong site- or region-specific relationships, while also highlighting the influence of stratification and microbial ecology on calibration performance. Surface-sediment studies across altitudinal gradients in Central Europe further illustrated the sensitivity of brGDGT distributions to local hydroclimatic regimes (Bauersachs et al., 2024). More recently, direct water-column calibrations (Zhao et al., 2021) have improved mechanistic understanding by explicitly linking brGDGT production to seasonal water temperatures. These previous studies show that while MBT'_{5ME} performs consistently as a global paleotemperature indicator, lake-specific calibrations are often needed to capture the nuances of local production dynamics (Zhao et al., 2021). Our comparison with external models was therefore intended to assess whether published equations adequately characterize Cultus Lake conditions or whether lake-specific approaches provide superior performance.

The Cultus calibration (Equation 3) closely tracked observed lake-water temperatures, yielding both a strong fit and relatively low predictive error. In contrast, global and regional calibrations systematically misfit the Cultus Lake signal. Soil-based and mixed-archive models (e.g., De Jonge et al., 2014; Russell et al., 2018; Bauersachs et al., 2024; Raberg et al., 2021; Otiniano et al., 2023) consistently underpredicted Cultus Lake water temperatures, whereas the Integrated Water Temperature (IWT) and Mean Water Temperature (MWT) calibrations of Zhao et al. (2021) tended to overpredict. Model parameters are provided in Table S4.4. These divergences are expected because published models rely on different temperature metrics, including MAAT, IWT, or MWT, which are not directly equivalent to the JASON lake water temperatures used in this study. Differences in the underlying temperature variable may strongly influence calibration slopes, intercepts, and apparent predictive skill. Even so, when each equation is applied to Cultus Lake, the site-specific MBT'_{5ME} model consistently yields the lowest prediction errors, demonstrating a substantially better match to local thermal conditions. Overall, the strong performance of the Cultus calibration confirms that MBT'_{5ME} variability in this system is tightly linked to local water temperature dynamics. External models, developed from soils, sediments, or mixed lacustrine sources, do not capture this behaviour and would misrepresent Cultus Lake temperatures if applied directly. These findings emphasize that robust paleotemperature reconstructions in stratified lakes require calibrations that reflect the lake-specific thermal regime governing brGDGT production.

We also assessed whether the CBT_{5ME} index tracked lake water pH, but no significant relationship was detected (Figure S4.3). These findings are consistent with previous studies showing that the narrow pH range in freshwater systems (< 1 unit) constrains the diagnostic value of CBT_{5ME} (Raberg et al., 2021). Building on this foundation, we next evaluated multivariate approaches using PLS regression to identify the most temperature-responsive brGDGTs and stepwise forward selection (SFS) to test whether incorporating additional brGDGT variables can improve brGDGT temperature estimates beyond those provided by MBT'_{5ME} -based calibration.

4.4 Fractional Abundance-Based Multiple Linear Regression Calibrations

Our linear, quadratic, and PLS analyses revealed that temperature sensitivity in Cultus Lake brGDGTs is highly selective and compound-specific. Only a subset of brGDGTs,

particularly Ib, IIIa, IIIa', Ila', and IIb', displayed consistent and reproducible thermal responses. Focusing calibrations on this subset avoids the heterogeneity embedded in community-level indices and improves the reliability of lacustrine brGDGT-based temperature estimates. Fractional-abundance MLR models built from these temperature-responsive compounds substantially outperformed the MBT'_{5ME} index and all external calibrations when applied to Cultus Lake. The best-performing models (Cal₁ and Cal₂) demonstrated that locally responsive compounds, especially Ib and Ila', captured lake water thermal variability with considerably greater fidelity than global multiparameter equations (Figure 4.8). This supports a process-oriented calibration strategy in which predictor selection is guided by demonstrated local temperature sensitivity rather than by reapplying broad global indices developed from environmentally heterogeneous datasets (Bauersachs et al., 2024; Raberg et al., 2021; Russell et al., 2018; Zhao et al., 2021). The large prediction errors and systematic underestimation of lake temperatures by most external calibrations underscore the limited transferability of globally derived models to thermally stratified freshwater systems. Many global equations incorporate brGDGTs sourced from soils, mixed sediment archives, or depth-integrated water-column production domains that differ markedly from the predominantly water production regime characterizing Cultus Lake (Raberg et al., 2021; Bauersachs et al., 2024).

The only external calibration that approximated local temperatures with reasonable accuracy was the MAAT Index 1 equation of De Jong et al. (2014). Importantly, this appears to reflect its mathematical formulation rather than true cross-system applicability. Index 1 explicitly includes brGDGT-Ib and Ila' in its numerator, the same compounds we identified as the strongest and most consistent temperature predictors in Cultus Lake. Its partial success, therefore, arises because its structure unintentionally aligns with the locally responsive brGDGT subset rather than because the calibration is broadly transferable across systems.

Our MLR lake-specific calibrations further demonstrated that not all brGDGTs contribute meaningfully to thermal reconstruction. For example, PLS analyses consistently highlighted Ib and Ila' as high-importance predictors, whereas others (e.g., IIIa', IIIc, IIb') showed weak, inconsistent, or non-monotonic temperature relationships. This observation revealed a key limitation of MBT'_{5ME}: the index aggregates compounds with heterogeneous behaviours, thereby diluting temperature sensitivity. In contrast, fractional-abundance MLR models that isolate only the strongest predictors achieved far higher precision and stability. The superior performance of

Cal₁, which uses only brGDGT-Ib and IIa', demonstrated that a minimal, mechanistically grounded predictor set can accurately reproduce observed lake water temperatures and outperform index-based approaches.

Conversely, models that incorporated compounds with weak or inconsistent temperature relationships (Cal₅-Cal₁₀) performed poorly under cross-validation, illustrating the pitfalls of purely statistical or combinatorial predictor selection. Without ecological interpretability, such models are prone to overfitting and yield unstable calibrations. The convergence between PLS results and the top MLR models confirms that brGDGT-temperature relationships in Cultus Lake are reproducible, compound-specific, and not driven by statistical artifacts.

Compared with both regional and global calibrations, the Cultus Lake MLR models, particularly Cal₁, exhibited superior predictive accuracy and clearer mechanistic grounding. External equations consistently underestimated water temperatures and produced greater residual variance, reflecting the well-documented challenges of transferring brGDGT calibrations across systems with contrasting thermal regimes, redox structures, production depths, and microbial communities. Such environmental differences reshape brGDGT assemblages and weaken the performance of models derived from mixed or non-analog ecosystems.

5.0 Conclusion

This study demonstrated that lake-specific, process-informed calibrations based on brGDGT fractional abundances substantially improved the accuracy of temperature estimates in stratified freshwater systems. By integrating seasonal water samples, compound-level lipid analyses, and multiple calibration frameworks, we showed that temperature-sensitive brGDGT subsets, including brGDGT-Ib and IIa' (Cal₁), yield far more reliable estimates than globally derived or index-based calibrations. The calibration framework established here provided a reproducible approach for developing robust paleotemperature estimates and highlighted the importance of regional validation for strengthening the use of brGDGTs as temperature quantitative tools in freshwater paleoclimate research.

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Conflicts of Interest

The authors declare no conflict of interest.

Open Research/Data Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Information

Branched Glycerol Dialkyl Glycerol Tetraether (brGDGT) - Temperature Calibrations for Cultus Lake, British Columbia, Canada

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Table S4.1 Pairwise differences in brGDGT composition among compartments. Entries report PERMANOVA results on Bray-Curtis dissimilarities computed from brGDGT fractional abundances (999 permutations): test statistic (F), effect size (R^2), raw p and Benjamini-Hochberg-adjusted p (p adj.). To verify the assumption of equal within-group dispersion, matching PERMDISP (betadisper + permutest) F, p, and adjusted p values are shown for the same pairwise subsets. Higher PERMANOVA R^2 denotes stronger separation in brGDGT composition relative to within-group variation.

Pair	PERMANOV A F	PERMANOVA R^2	PERMANOVA p	PERMDISP F	PERMDISP p	PERM ANOVA p adj.	PERMDISP p adj.
Water vs Sediment	22.8726	0.560	0.001	1.509	0.262	0.001	0.393
Soil vs Sediment	62.6343	0.436	0.001	7.368	0.014	0.001	0.042
Soil vs Water	12.0055	0.129	0.001	0.594	0.418	0.001	0.418

Table S4.2 SIMPER (Similarity Percentages) for Water vs. Sediment, using Bray-Curtis dissimilarities of brGDGT fractional abundances (999 permutations). “Average contribution” is the mean per brGDGT contribution to the between-group dissimilarity; “Contribution (%)” is its percentage share, with “Cumulative (%)” summing in descending order. “Direction” indicates which compartment has the higher group mean (computed on original fractional abundances). p-values are Benjamini-Hochberg-adjusted (p adj.). The separation is dominated by IIIa (~35% of the dissimilarity), with additional contributions from Ia and IIa’ (higher in water) and IIIa’ (higher in sediments), together explaining ~66% of the Water-Sediment difference.

BrGDGT	Direction	Average contribution†	Contribution (%)	Cumulative (%)	p adj.
IIIa	Higher in Sediment	0.145	34.7	34.7	0.003
Ia	Higher in Water	0.045	10.7	45.5	0.003
IIa’	Higher in Water	0.045	10.7	56.2	0.003
IIIa’	Higher in Sediment	0.039	9.4	65.5	0.062
IIa	Higher in Water	0.032	7.6	73.1	0.088
Ib	Higher in Water	0.026	6.4	79.5	0.003
IIb’	Higher in Water	0.016	3.9	83.4	0.015
IIb	Higher in Sediment	0.015	3.6	87.0	0.779
IIc’	Higher in Water	0.013	3.0	90.0	0.114
IIc	Higher in Water	0.012	2.9	92.9	0.082
IIIb	Higher in Sediment	0.011	2.6	95.4	0.010
IIIb’	Higher in Water	0.008	1.9	97.4	0.058
IIIc	Higher in Water	0.006	1.5	98.9	0.015
Ic	Higher in Sediment	0.003	0.8	99.7	0.092
IIIc’	Higher in Sediment	0.001	0.3	100.0	0.003

†Average contribution is in Bray-Curtis units.

Table S4.3 Results of Kruskal-Wallis tests (Chi-square (χ^2) statistic, and degrees of freedom (df) = 2) evaluating seasonal differences (warm, mid, cool) in fractional abundances of individual brGDGTs across five subwatersheds (Smith Falls, Columbia Valley, Vedder Mountain, International Ridge, and Frosst Creek). Except for IIIc', which is ~ 0.05 , no brGDGT exhibited significant seasonal variation in any subwatershed (all $p > 0.05$).

BrGDGT	Smith Falls		Columbia Valley		Vedder Mountain		International Ridge		Frosst Creek	
	χ^2 statistic	p-value	χ^2 statistic	p-value	χ^2 statistic	p-value	χ^2 statistic	p-value	χ^2 statistic	p-value
IIIa	1.48	0.476	0.111	0.946	0.560	0.756	0.560	0.756	4.02	0.134
IIIa'	1.29	0.526	3.38	0.185	6.18	0.0455	0.060	0.970	1.46	0.482
IIIb	0.696	0.706	1.29	0.526	1.09	0.581	2.68	0.262	3.72	0.156
IIIb'	1.30	0.522	0.360	0.835	2.00	0.368	1.83	0.400	3.72	0.156
IIIc	1.42	0.492	0.440	0.802	4.29	0.117	5.42	0.0666	2.00	0.368
IIIc'	3.24	0.198	2.22	0.330	4.29	0.117	6.18	0.0454	2.00	0.368
IIa	1.37	0.505	0.966	0.617	2.34	0.310	1.22	0.543	0.860	0.651
IIa'	2.47	0.291	2.28	0.320	2.16	0.340	3.50	0.174	0.740	0.691
IIb	0.386	0.825	0.111	0.946	1.52	0.468	0.420	0.811	4.50	0.105
IIb'	3.24	0.198	0.111	0.946	1.26	0.533	1.86	0.395	0.240	0.887
IIc	1.66	0.437	0.272	0.873	0.768	0.681	0.996	0.608	0.037	0.982
IIc'	3.01	0.222	2.47	0.291	0.156	0.925	1.51	0.469	1.25	0.536
Ia	0.591	0.744	2.44	0.295	2.58	0.275	0.780	0.677	0.080	0.961
Ib	0.0057	0.997	0.180	0.914	3.12	0.210	0.740	0.691	2.34	0.310
Ic	0.783	0.676	0.771	0.680	0.215	0.898	0.740	0.691	3.66	0.160

Table S4.4 Published MBT'_{5ME} temperature transfer functions used for comparison with Cultus Lake calibrations. MAWT, IAWT denote mean annual water temperature and integrated water column temperature, respectively, whereas MWT and IWT represent mean water temperature and integrated water temperature for June, July, and August, respectively. MAAT and MAF represent mean annual temperature and months above freezing, respectively.

Calibration	Source
$IWT = -1.82 + 56.06 \times MBT'_{5ME} \quad (S1)$	Zhao et al. (2021)
$IAWT = -1.92 + 33.40 \times MBT'_{5ME} \quad (S2)$	Zhao et al. (2021)
$MWT = -13.34 + 94.15 \times MBT'_{5ME} \quad (S3)$	Zhao et al. (2021)
$MAWT = -4.16 + 40.3 \times MBT'_{5ME} \quad (S4)$	Zhao et al. (2021)
$MAAT = -1.21 + 32.42 \times MBT'_{5ME} \quad (S5)$	Russel et al. (2018)
$MAF = -0.5 + 30.40 \times MBT'_{5ME} \quad (S6)$	Raberg et al. (2021)
$MAF = 3.74 + 14.75 \times MBT'_{5ME} \quad (S7)$	Otiniano et al. (2023)
$MAAT = -2.19 + 31.91 \times MBT'_{5ME} \quad (S8)$	Bauersachs et al. (2024)
$MAF = 4.81 + 15.64 \times MBT'_{5ME} \quad (S9)$	Bauersachs et al. (2024)

Table S4.5 Published brGDGT temperature calibrations based on fractional abundances used for comparison with Cultus Lake calibrations. For Raberg et al. (2021) calibrations, fractional abundances within each methylation set (a, b, c) were calculated as the ratio of a given compound to the sum of all isomers in that set, (e.g., $fIb_{Meth} = \frac{Ib}{Ib + IIb + IIIb + IIb' + IIIb'}$) (S10) whereas the fractional abundances bearing the subscript “Full” were those calculated as the ratio of a given compound to the sum of all the 15 brGDGT structural set.

Calibration	Source
$Index1 = \log[(Ia + Ib + Ic + IIa' + IIIa') / (Ic + IIa + IIc + IIIa + IIIa')] \quad (S11)$	De Jonge et al. (2014)
$MAAT = 12.22 + 18.79 \times Index1 \quad (S12)$	De Jonge et al. (2014)
$MAAT = 23.81 - 31.02 \times IIIa - 41.91 \times IIb - 51.59 \times IIb' - 24.70 \times IIa + 68.80 \times Ib \quad (S13)$	Russel et al. (2018)
$MAF = -13.29 \times IIIa + 11.30 \quad (S14)$	Otiniano et al. (2023)
$MAAT = 7.11 + 67.66 \times Ib - 13.54 \times IIIa \quad (S15)$	Bauersachs et al. (2024)
$MAF = 5.19 + 16.22 \times Ia \quad (S16)$	Bauersachs et al. (2024)
$MAF = 92.9 + 63.84 \times fIb_{Meth}^2 - 130.51 \times fIb_{Meth} - 28.77 \times fIIa_{Meth}^2 - 72.28 \times fIIb_{Meth}^2 - 5.88 \times fIIc_{Meth}^2 + 20.89 \times fIIIa_{Meth}^2 - 40.54 \times fIIIa_{Meth} - 80.47 \times fIIIb_{Meth} \quad (S17)$	Raberg et al. (2021)
$MAF = -8.06 + 37.52 \times fIa_{Full} - 266.83 \times fIb_{Full}^2 + 133.42 \times fIb_{Full} + 100.85 \times fIIa_{Full}^2 + 58.15 \times fIIIa_{Full}^2 + 12.79 \times fIIIa_{Full} \quad (S18)$	Raberg et al. (2021)

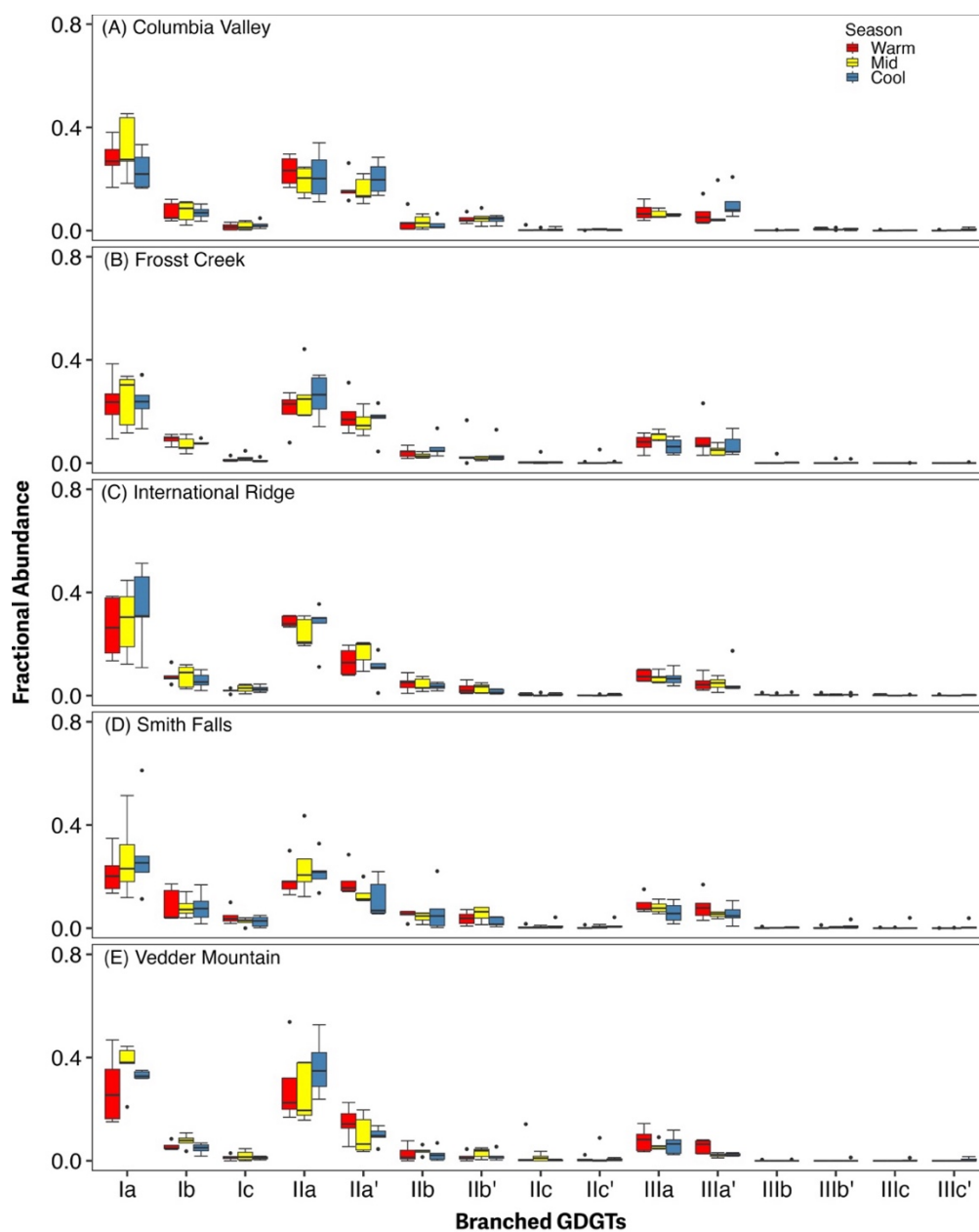


Figure S4.1 Seasonal variations in the fractional abundances of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in surface soils were examined across five sub-watersheds within the Cultus Lake watershed. Boxplots illustrate the abundance of 15 individual brGDGTs (Ia – IIIc') during the Warm (red), Mid (yellow), and Cool (blue) seasons, corresponding to sampling periods in July, October, and December, respectively. The subplots are organized by watershed: (A) Columbia Valley, (B) Frosst Creek, (C) International Ridge, (D) Smith Falls, and (E) Vedder Mountain. The overall distributions remained stable across seasons, suggesting compositional stability. Each seasonal category comprises $n = 5$ replicate soil samples.

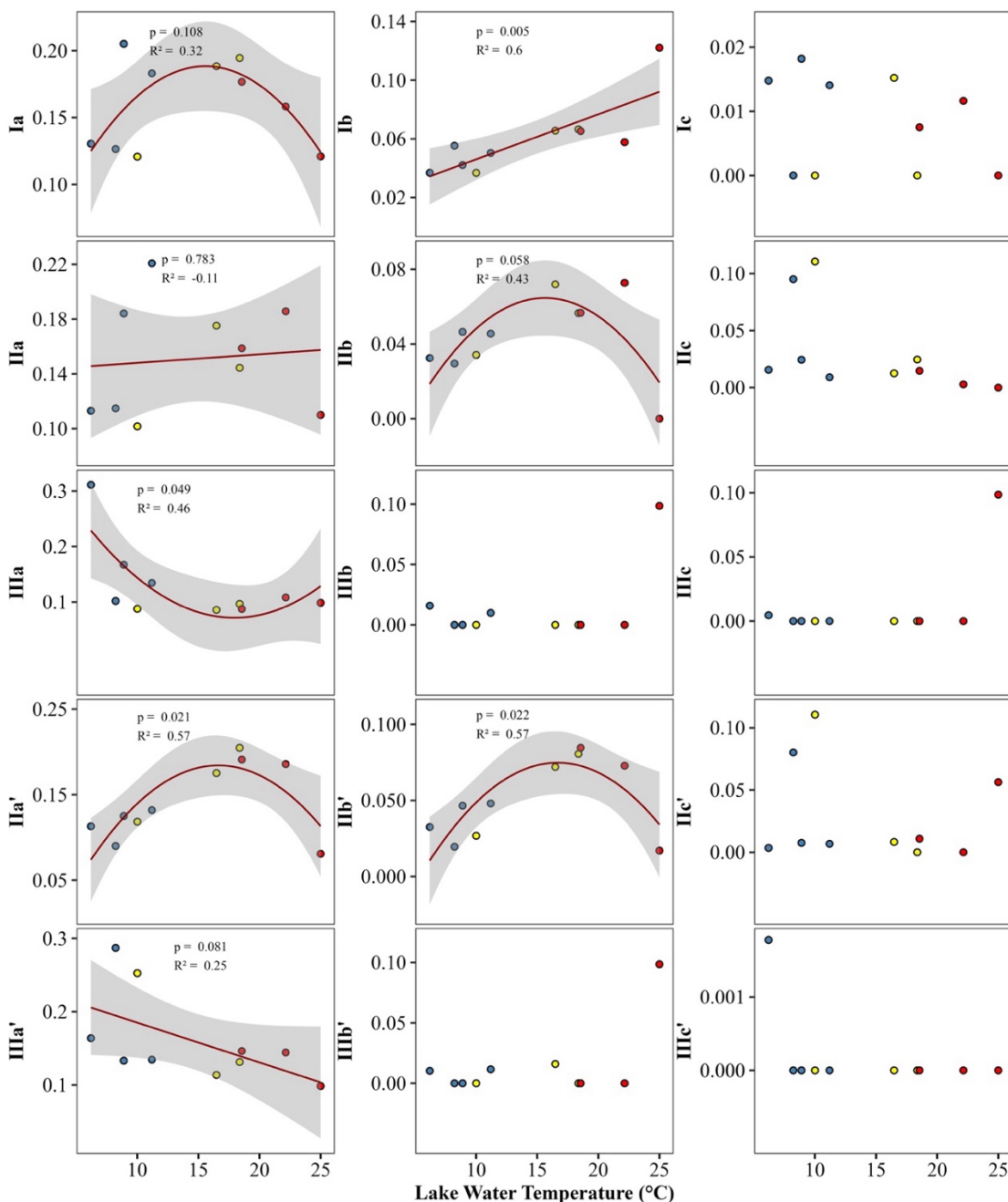


Figure S4.2 Relationships between mean lake water temperature (0 - 10 m depth) and the fractional abundances of individual branched GDGTs (brGDGTs) in Cultus Lake (n = 10). Each point represents an individual water sample, color-coded by season: warm (red) samples collected in July and August, mid (yellow) samples collected in September and October, and cool (blue) samples collected in November and December. Each panel displays a fitted linear regression (red line) with 95% confidence intervals (gray shading), including R² and p-values.

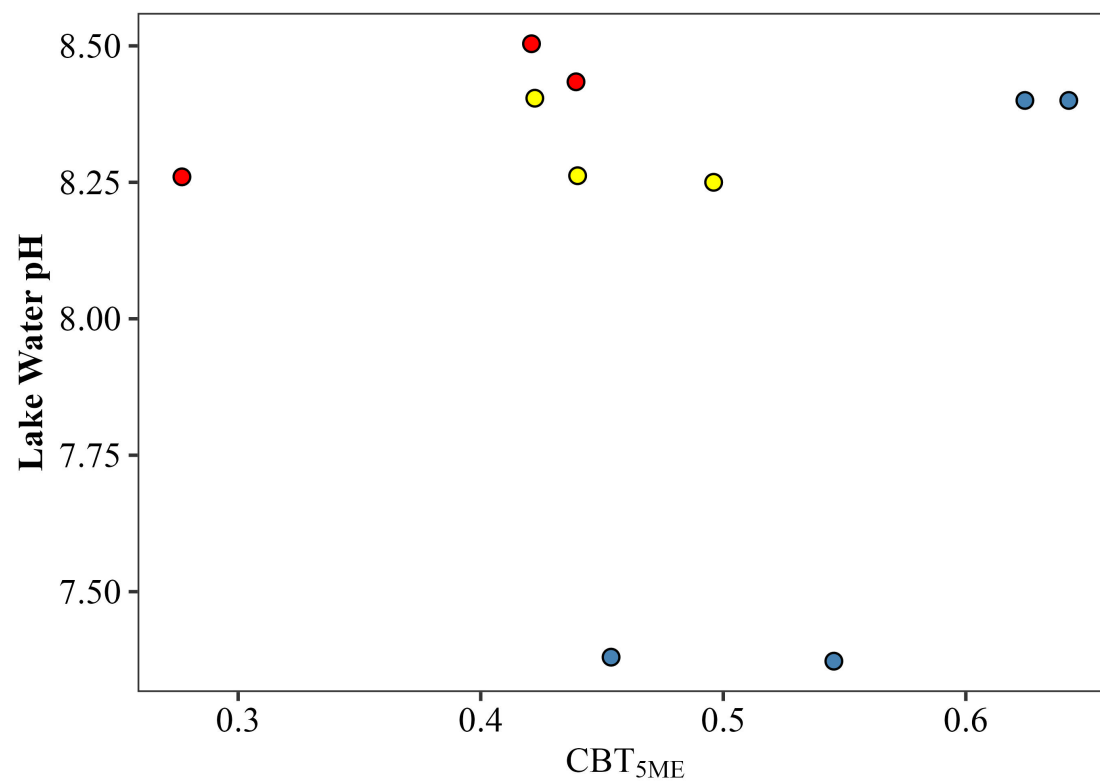


Figure S4.3 Cultus Lake brGDGT calibration for water pH using the CBT_{5ME} index (Equation 3). No clear relationship was observed between lake water pH and the CBT_{5ME} index.

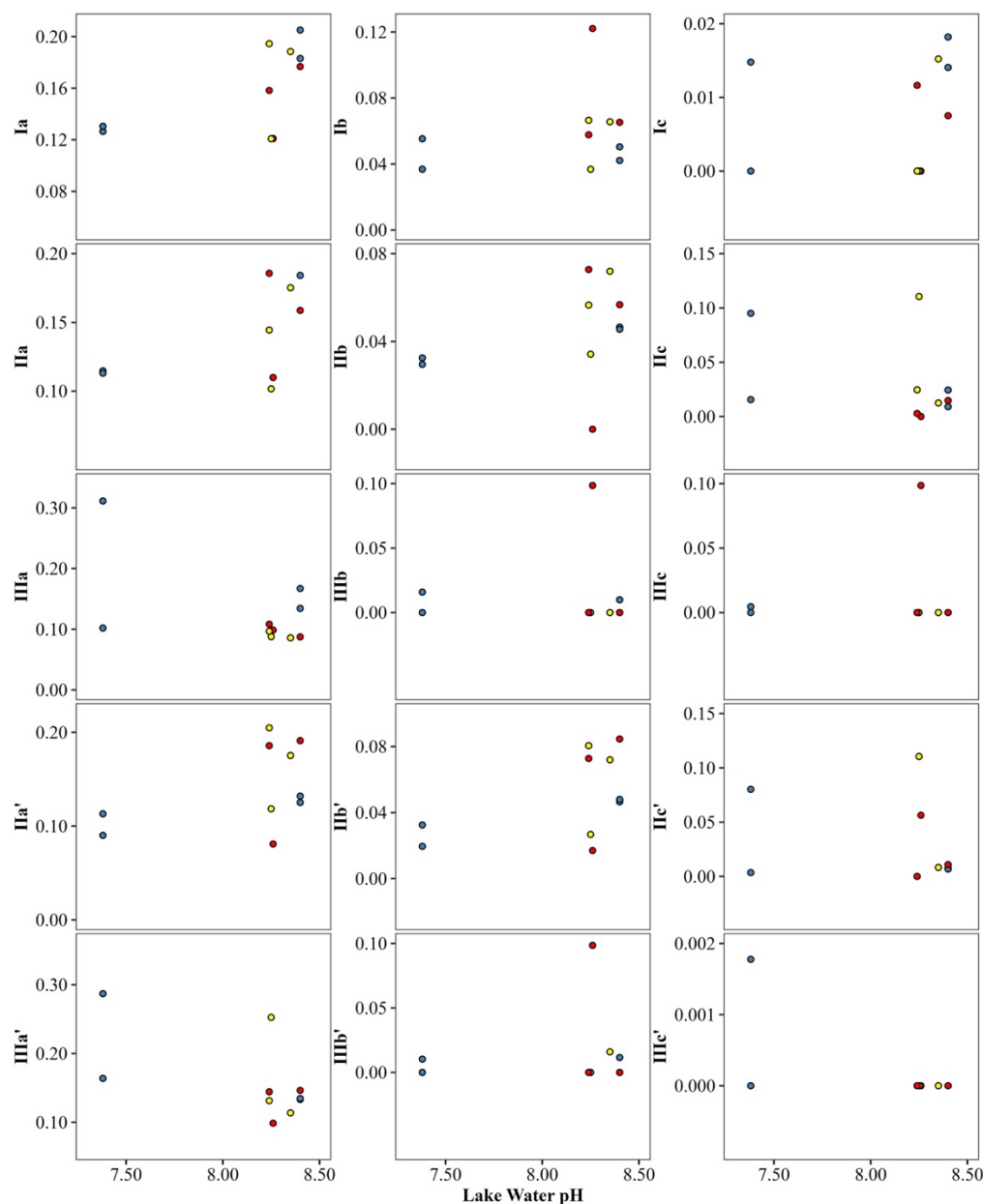


Figure S4.4 Relationships between JASOND lake water pH and the fractional abundances of individual branched glycerol dialkyl glycerol tetraether compounds in Cultus Lake, British Columbia. Each point represents an individual water sample, colour-coded by season: warm (red), mid (yellow), and cool (blue). No clear relationship was observed between lake water pH and the branch glycerol dialkyl glycerol tetraethers (brGDGTs).

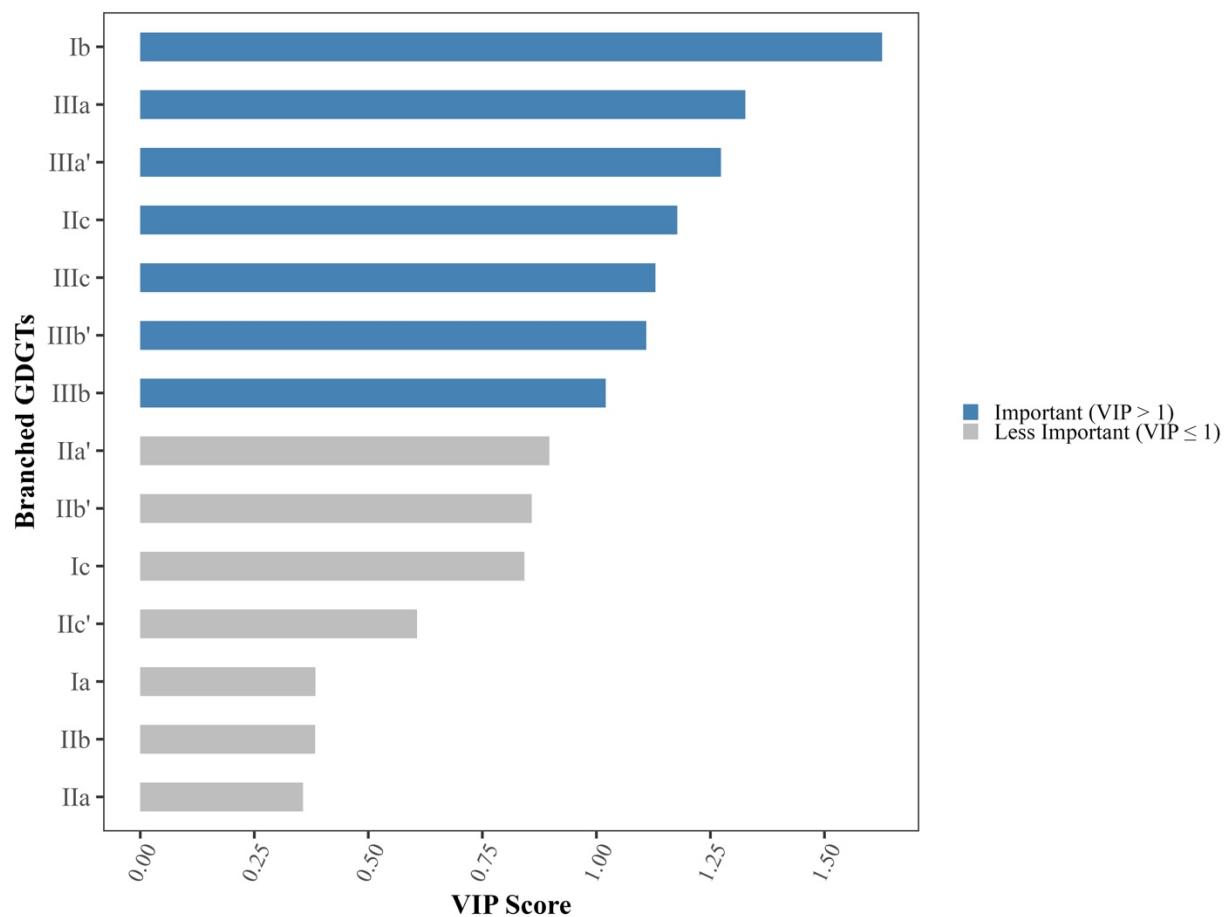


Figure S4.5 Variable Importance in Projection (VIP) scores for individual branched GDGTs in relation to lake water temperature. Compounds with $VIP > 1$ (blue bars) are considered important predictors, with GDGT Ib having the highest VIP score, followed by IIIa, IIIa', and IIIc. These brGDGTs contribute most to temperature calibration in the PLS model, while others (grey bars) are less influential ($VIP \leq 1$).

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5 CHAPTER 5: North Pacific Climate Variability Inferred from a 600-Year Sedimentary Lipid Biomarker Record and Ecosystem Responses in Cultus Lake, British Columbia, Canada

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Abstract

This study examined relationships among the Pacific Decadal Oscillation (PDO), the El Niño-Southern Oscillation (ENSO), mean annual air temperatures (MAAT), and Pacific salmon returns to Cultus Lake (British Columbia, Canada), and evaluated how these climate drivers and ecological dynamics are recorded in sedimentary branched glycerol dialkyl glycerol tetraethers (brGDGTs) and nitrogen stable isotopes ($\delta^{15}\text{N}$). Instrumental data showed that MAAT at Cultus Lake is strongly and positively correlated with both PDO ($r = 0.40$) and ENSO ($r = 0.50$), with the strongest coupling occurring during the cold season (November-April). Down-core analyses revealed compound-specific responses of individual brGDGTs to MAAT and PDO phases, with brGDGT-IIIa exhibiting the strongest inverse relationship with MAAT ($r = -0.57$, CI = -0.67 , -0.08). Lake-specific calibrations applied to an ~600-year sediment record (since ~1400 CE) reconstruct pronounced multi-centennial temperature variability, consistent with independent regional climate proxies (i.e. tree-ring indices; Na^+ from Mt. Logan ice core records). Selected temperature calibrations, particularly Cal₂ and Cal₄, showed higher temperatures during the warm PDO phases and lower temperatures during the cool phases throughout the 20th and early 21st century. The sedimentary $\delta^{15}\text{N}$ record tracked centennial-scale brGDGT, tree ring, and Na^+ variability until the late nineteenth century, after which sustained enrichment reflected increasing anthropogenic nitrogen loadings, a decoupling from recording Pacific salmon production dynamics. Our results demonstrated that lake-specific brGDGT calibrations capture both long-term regional climate variability, and decadal Pacific Ocean-atmospheric forcings, while highlighting the substantial and intensifying influences of anthropogenic and other nutrient loadings on modern lake biogeochemistry.

1.0 Introduction

North Pacific climate variability exerts a strong influence on the productivity, resilience, and long-term dynamics of Pacific salmon (*Oncorhynchus* spp.), species that are ecologically foundational and culturally and economically vital across the North Pacific basin (Beamish et al., 1997; Finney et al., 2000, 2002; Mantua & Hare, 2002; Pitman et al., 2020). Sockeye salmon (*O. nerka*) are highly sensitive to climatic forcings, as temperature-driven changes in freshwater and marine environments affect migration and ecological phenologies, juvenile survival, nutrient

cycling, and population persistence (Fergusson et al., 2020; Reed et al., 2011; Wilson et al., 2023). However, evaluating the long-term role of North Pacific climate variability in shaping salmon population trajectories remains challenging, because instrumental climate and fisheries monitoring records extend back only a few decades. This limited temporal scope complicates efforts to distinguish the relative contributions of climate forcings, habitat alterations, and other anthropogenic pressures as influential to observed population variability.

Paleolimnological approaches provide a powerful means of extending ecological and climatic baselines beyond the observational era (Finney et al. 2002; Dagodzo et al., 2025; 2026; Duda et al., 2026). Lake sediments preserve molecular and isotopic archives of past environmental conditions, allowing reconstructions of past climate variabilities, and ecosystem responses, over centennial timescales. In Pacific salmon nursery lakes, sedimentary lipids including sterols and stanols derived from decomposing carcasses have been shown to provide robust, independent proxies of salmon returns, closely tracking historical return records (Dagodzo et al., 2025). Complementarily, nitrogen stable isotopes ($\delta^{15}\text{N}$) record salmon-derived nutrient inputs to lake ecosystems, offering long-term insight into nutrient subsidies and biogeochemical change (Finney et al., 2002; Gregory-Eaves et al., 2003; Selbie et al., 2009). These proxies serve as a means of reconstructing salmon population histories, and ecosystem responses, beyond the period of direct observations.

In parallel, branched glycerol dialkyl glycerol tetraethers (brGDGTs), bacterial membrane lipids that respond to water and air temperature changes, have been widely applied as paleotemperature proxies in lakes and soils (De Jonge et al., 2014; Russell et al., 2018; Weijers et al., 2007a, b; Zhao et al., 2021). Despite their broad utility, the performance of brGDGT-based temperature reconstructions is known to vary substantially among sites due to differences in microbial community compositions, sedimentary processes, and environmental contexts (Miller et al., 2018; Raberg et al., 2024; Zander et al., 2024). Recent studies have highlighted the importance of developing regional or lake-specific calibrations, to improve the accuracy and ecological relevance of temperature estimates (Bauersachs et al., 2024; Foster et al., 2015; Martínez-Sosa et al., 2021). For example, regional brGDGT-temperature calibrations for Antarctica and sub-Antarctic lakes demonstrated significantly improved model performance compared to global calibrations. Foster et al. (2015) reported an increase in explained variance from 67% (global calibration) to 83% (regional calibration), accompanied by a marked reduction

in prediction errors for high-latitude lakes. Similar improvements were observed in Cultus Lake, where lake-specific multiple linear regression calibrations yielded strong predictive skill ($R^2 = 0.87$, RMSE = 2.07 °C, SEE = 2.32 °C), substantially outperforming both the MBT'_{5ME} ($R^2 = 0.66$, RMSE = 3.38 °C, SEE = 3.78 °C) and published regional/global calibrations (Dagodzo et al., in preparation). Despite these advances, no study has systematically evaluated the performance of site-specific brGDGT calibrations, relative to established models, in the context of large-scale climate variability, or in combination with independent ecological proxies.

Coastal British Columbia experiences relatively mild mean temperatures but pronounced interannual to multi-decadal climate variability, driven primarily by ocean-atmospheric processes yielding the Pacific Decadal Oscillation (PDO) and El Niño-Southern Oscillation (ENSO) index (Biondi et al., 2001; Stahl et al., 2005; Whitfield et al., 2010). ENSO operates on inter-annual timescales, altering sea surface temperatures and atmospheric circulation patterns, that propagate into western North America, while the PDO modulates ocean-atmosphere conditions over multi-decadal periods (Mantua et al., 1997; Mantua & Hare, 2002; Mills & Walsh, 2013; Whitfield et al., 2010). These oscillations exert strong controls over regional air temperatures, precipitation amounts and types, and lake thermal structures, and have been shown to influence salmon productivity throughout the North Pacific (Dagodzo et al., 2025; Mantua et al., 1997; Mantua & Hare, 2002). Importantly, PDO effects on salmon populations differ regionally, with contrasting responses observed between Alaska and British Columbia (Dagodzo et al., 2025; Mantua et al., 1997; Mantua & Hare, 2002), underscoring the need for site-specific reconstructions, beyond observational periods.

Cultus Lake, currently a warm monomictic lake in the lower Fraser Valley of southwestern British Columbia, is particularly sensitive to atmospheric forcing. Observational data and hydrodynamic modelling indicate that lake water surface temperatures closely track regional air temperature, with only a short seasonal lag, reflecting strong coupling between atmospheric conditions and lake heat content (Gauthier et al., 2021; Selbie et al., 2022). As a critical rearing habitat for Fraser River sockeye salmon, Cultus Lake sediments provide an ideal natural archive for investigation of the coupled influences of North Pacific climate variability, and salmon population dynamics. Historically supporting an abundant late-run Fraser River sockeye populations, the lake has experienced severe population declines, and is assessed by Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as endangered, and at

the time of writing, before the federal Minister, for consideration of listing under Canada's Species at Risk Act (Government of Canada, 2002).

Here, we develop a multi-proxy reconstruction of lake temperature responses to North Pacific climate variability, and linkages to salmon production in Cultus Lake, by integrating brGDGT-based paleotemperature calibrations with nitrogen isotope biomarkers of salmon returns. Specifically, we (1) apply Cultus Lake-specific brGDGT temperature calibrations, and evaluate their performance relative to other independent paleoclimate proxies (i.e. dendroclimatology, and soluble Na from the Mount Logan ice core) (Macdonald & Case, 2005; Osterberg et al., 2014); and other published brGDGT models, (2) assess reconstructed temperature variability alignment with PDO, ENSO, and MAAT dynamics, and (3) examine how salmon-derived nutrient proxies track salmon returns, and ecosystem responses over the past century. By combining independent paleoclimatic and paleoecological archives, within a unified framework, this study provides a new understanding of the long-term role of North Pacific climate variability in shaping salmon population dynamics, and demonstrates the value of site-specific biomarker calibrations for informing conservation and management in a rapidly changing climate.

2.0 Materials and Methods

2.1 Field Sampling and Study Site

We obtained a sediment core measuring 44 cm on October 5, 2022, from the main deep-water depositional area of Cultus Lake, at a depth of 41 m, using a Universal Percussion Corer, with an internal diameter barrel measuring 6.67 cm (Figure 5.1). Once retrieved, the core was sectioned at 0.5 cm intervals with a calibrated vertical extrusion system and retained in Whirl-Pak bags, stored at -20 °C, at the Fisheries and Oceans Canada Cultus Lake Salmon Research Laboratory. Samples were subsequently shipped frozen to the University of Ottawa (uOttawa), where they were freeze-dried before lipid extraction. BrGDGT extractions and analyses were done following the methods described in Chapter 3 (Dagodzo et al., in preparation).

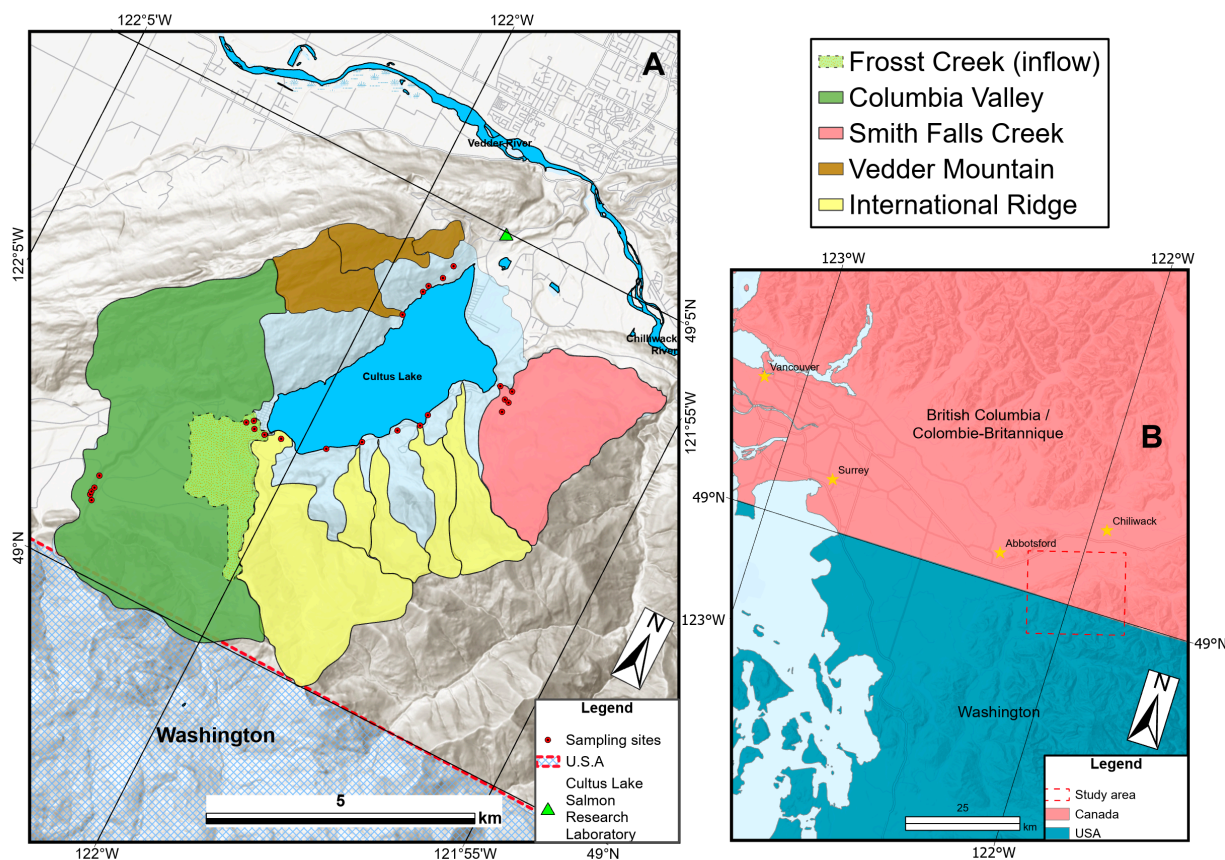


Figure 5.1 Map of Cultus Lake, BC, from which a sediment core was collected. Map modified from Putt et al. (2019).

2.2 Sediment Chronology, ^{210}Pb and ^{14}C Analysis

Downcore activities of ^{210}Pb , ^{226}Ra , and ^{137}Cs were determined by gamma spectrometry using a calibrated high-purity germanium well detector. Sediment cores were sectioned at 0.5cm intervals, dried to constant mass, homogenized, and analyzed with corrections applied for background radiation, detector efficiency, sample mass, and radioactive decay to a common reference date. Total ^{210}Pb activity was quantified from its low-energy gamma emission, while supported ^{210}Pb was estimated from ^{226}Ra -derived daughter activities assuming secular equilibrium within the sediment matrix. Unsupported (excess) ^{210}Pb was calculated as the difference between total and supported activities. Sediment ages and accumulation rates were derived from the downcore distribution of excess ^{210}Pb using cumulative dry mass as the depth scale. An exponential decline in excess ^{210}Pb activity with depth was fitted to the data, consistent with radioactive decay under steady-state depositional conditions. The Constant Rate of Supply

(CRS) model was applied to accommodate variable sedimentation through time while assuming a constant atmospheric flux of unsupported ^{210}Pb (Appleby, 2001). The vertical distribution of ^{137}Cs was used as an independent stratigraphic marker to assess the internal consistency of the ^{210}Pb -derived chronology. Sediment focusing factors were estimated by comparing the integrated excess ^{210}Pb inventory within the core to the expected regional atmospheric ^{210}Pb flux, providing an assessment of lateral sediment redistribution within the basin (Omelchenko et al., 2005).

To extend the chronology beyond the range of ^{210}Pb , accelerator mass spectrometry (AMS) radiocarbon dating was performed on terrestrial macrofossil plant remains (twig fragments). Samples were pretreated using an acid-base-acid protocol to remove secondary carbonates and humic contamination prior to combustion in an elemental analyzer. The resulting CO_2 was graphitized and analyzed on the MICADAS AMS system at the A.E. Lalonde AMS Laboratory (University of Ottawa), where $^{14}\text{C}/^{12}\text{C}$ and $^{13}\text{C}/^{12}\text{C}$ ratios were determined. $\delta^{13}\text{C}$ values were used to correct for isotopic fractionation. Radiocarbon ages were reported as conventional ^{14}C years BP (before present, AD 1950) and calibrated using the IntCal20 calibration curve (Reimer, 2020). The final age-depth model integrates the ^{210}Pb CRS chronology for the upper sediments with calibrated radiocarbon ages from macrofossils deeper in the core, using Bayesian age-depth modelling in rPlum (for ^{210}Pb) and rbacon (for radiocarbon ages). This provided a continuous temporal framework spanning the past century and extending further into the late-Holocene sediment record.

2.3 Sediment brGDGTs analysis

BrGDGT extraction and analysis were conducted following the procedure described in Chapter 4, subsection 2.5 (Dagodzo et. al., in preparation).

2.4 Stable Nitrogen (N) Isotope Analysis ($\delta^{15}\text{N}$ values)

For each sample interval, approximately 20 mg of freeze-dried sediment was weighed into tin capsules together with ~20 mg of tungsten trioxide (WO_3) to facilitate complete combustion (following Talbot, 2001). The encapsulated samples were analyzed for total nitrogen concentration and nitrogen isotopic composition using a continuous-flow elemental analyzer interfaced with an isotope ratio mass spectrometer (EA-IRMS) at the Jan Veizer isotope laboratory in the University of Ottawa. $\delta^{15}\text{N}$ values were expressed in per mil (‰) relative to

atmospheric nitrogen (air-N₂) and were calibrated against international reference materials, including C-51 nicotinamide and caffeine standards. We also made duplicate preparations to ensure reproducibility. Organic matter content was determined separately using the loss-on-ignition (LOI) method, following Heiri et al. (2001).

2.5 Compilation of Instrumental Temperature Data of Cultus Lake

Instrumental air temperature data for the Cultus Lake region (Environment and Climate Change Canada, 2025) were compiled to provide an independent reference for evaluating brGDGT-based paleotemperature reconstructions. Monthly air temperature records were obtained from seven meteorological stations located within ~25 km of Cultus Lake (Table S1; Table S2). Temperature records were screened for completeness and quality, and years with substantial data gaps were excluded. To maximize temporal coverage while preserving local climatic relevance, monthly temperatures were combined into a single composite time series using a distance-prioritized gap-filling approach. For each year-month, data from the station closest to Cultus Lake were preferentially-selected, with progressively more distant stations used only when nearer records were unavailable.

Pairwise correlation analysis among annual mean temperatures from the seven stations demonstrated strong regional coherence, with correlation coefficients commonly exceeding $r = 0.85$ among stations located within ~25 km of the lake (Table S2). The strongest correlations occurred among stations closest to Cultus Lake (0-3 km) and those with the longest periods of overlap (e.g., Cultus Lake A, Lindell, Mission West Abbey, and Agassiz CDA; $r = 0.87 - 0.99$). Weaker or unstable correlations were confined to station pairs with limited temporal overlap, indicating that reduced correlation strength reflects short record lengths rather than spatial decoupling. These results supported our use of a merged temperature record to represent an extended regional air temperature variability influencing Cultus Lake.

Monthly composite temperatures were aggregated to annual means when at least nine months of data were available per year. In addition to mean annual air temperature (MAAT), warm-season temperature metrics were calculated by averaging monthly temperatures over relevant intervals corresponding to periods of lake stratification. All temperature series were aligned temporally with the sediment core chronology and standardized to a common reference

period. The compiled MAAT record was smoothed using a centred 10-year moving average to highlight decadal-scale variability.

2.6 Compilation of PDO, ENSO, and Salmon Return Datasets

To evaluate the influence of large-scale climate changes on local climate variability and salmon populations, we compiled indices of the PDO and the ENSO. The PDO index was obtained from the National Oceanic and Atmospheric Administration-National Centers for Environmental Information (NOAA-NCEI, 2024), which provided monthly values spanning January 1854 to February 2024. Positive PDO phases were associated with below-average sea level pressures across the North Pacific, and sea surface temperature (SST) anomalies that were warm along the Pacific coast of North America and cool in the central basin, whereas negative phases exhibited the opposite pattern. The monthly PDO index was smoothed using a centred 10-year moving average, calculated with a 120-month rolling window to emphasize decadal-scale variability.

ENSO variability was characterized using the Niño 3.4 index from the NOAA Climate Prediction Center (2024). Positive anomalies (El Niño events) represent warming events linked to weakened trade winds, and altered atmospheric teleconnections, while negative anomalies (La Niña) reflect cooler SSTs, enhanced trade winds, and intensified upwelling. The annual ENSO index was also smoothed using a centred 10-year moving average to highlight decadal-scale variability.

Sockeye salmon annual escapement (adult return) data for Cultus Lake were obtained from Fisheries and Oceans Canada (DFO), who operate an enumeration fence on the migration corridor (Sweltzer Creek). These escapement records extend back more than a century and provide a critical baseline for linking reconstructed biomarker and isotope data with historical salmon population dynamics.

2.7 Statistical Analyses

All analyses were conducted in R (v4.3.2; R Core Team, 2012) using mgcv (v1.8-31; Wood, 2017) for generalized additive modelling and gratia (v0.9.0; Simpson, 2024) for diagnostics and visualization. Ages for all sediment intervals were derived from the Bayesian ^{210}Pb - ^{14}C age-depth model using posterior median estimates. Sedimentary brGDGT fractional

abundances, brGDGT-based temperature calibrations (Cal₁-Cal₁₀), and $\delta^{15}\text{N}$ values were assigned calendar ages using the Bayesian age-depth model, after which these records were aligned by year with instrumental climate records (PDO, ENSO, and MAAT), and Pacific salmon escapement data, for direct comparisons. Long-term temporal variabilities in sedimentary brGDGTs and calibration-derived temperatures were modelled using generalized additive models (GAMs) of the form $y = s(\text{age})$, where $s(\text{age})$ is a penalized thin-plate regression spline. Smoothness parameters were estimated using restricted maximum likelihood (REML), and basis dimensions were selected conservatively to avoid overfitting while resolving multi-decadal structure. GAM-smoothed predictions and 95% confidence intervals were generated on a regular age grid, for visualization and subsequent analyses.

Because the PDO operates as a phase-structured climate system, we evaluated brGDGT fractional abundances, and lake-specific temperature calibrations, across predefined PDO regime intervals representing the major twentieth-century transitions (1924-1945 warm vs. 1945-1976 cool; 1976-2006 warm vs. 2006-2022 cool). For each compound and calibration, regime contrasts were quantified as the difference between the mean values of the warm and cool phases ($\Delta = \text{Mean}_{\text{warm}} - \text{Mean}_{\text{cool}}$). Uncertainty in Δ was estimated using a moving block bootstrap, that preserves temporal autocorrelation within the time series. Bootstrap resampling was performed using blocks of two consecutive observations and repeated 5,000 times, to generate empirical distributions of Δ , from which 95% confidence intervals were derived. To facilitate comparisons among proxies of differing magnitudes and variances, standardized effect sizes were also calculated, using pooled standard deviations. Because multiple compounds and calibrations were evaluated simultaneously, p-values from regime comparisons were adjusted using the Benjamini-Hochberg false discovery rate (FDR) procedure, to control for Type I error (Fithian & Lei, 2022; Romano et al, 2008). Adjusted significance levels are reported as q-values, and contrasts were considered statistically significant, when $q < 0.05$. For sedimentary brGDGT proxy correlations with MAAT, we used 10-year resampling blocks, and 2000 bootstrap iterations, with significance assessed from 95% confidence intervals.

3.0 Results

3.1 Relationship between PDO, ENSO, and MAAT of Cultus Lake

MAAT at Cultus Lake exhibited a statistically significant relationship with large-scale Pacific climate variability, as expressed by the PDO and the ENSO indices (Figure 5.2). Across the full 130-year instrumental record, MAAT increases with increasing PDO index values, yielding a positive Pearson correlation ($r = 0.45$, $p < 0.001$; $n = 130$) (Figure 5.2A). Seasonal separation revealed pronounced variability in the strength of the PDO-Cultus Lake air temperature relationship (Figure S5.1). The strongest association occurred during the November-April period, when mean air temperature was strongly correlated with the PDO index ($r = 0.57$, $p < 0.001$, $n = 130$). Correlations weakened during the May-October interval ($r = 0.24$, $p = 0.006$, $n = 130$) and were lowest during the July-December period ($r = 0.20$, $p = 0.025$, $n = 131$), although all seasonal relationships remained statistically significant. In all cases, correlations were positive, indicating coherent directional covariability between PDO phase and air temperature across seasons.

MAAT at Cultus Lake also showed a statistically significant relationship with ENSO variability, as quantified by the Niño index, at both annual and seasonal timescales (Figure 5.2B; Figure S5.1). At the annual scale, MAAT was strongly and positively correlated with the Niño index ($r = 0.50$, $p < 0.001$, $n = 73$), indicating strong interannual covariability. Seasonal analyses mirror the PDO results, with the strongest ENSO-Cultus Lake air temperature coupling observed during the November-April period ($r = 0.5$, $p < 0.001$, $n = 74$). Weaker but still significant positive correlations were observed during the May-October interval ($r = 0.41$, $p < 0.001$, $n = 73$) and the July-December period ($r = 0.33$, $p = 0.004$, $n = 75$). Across all seasonal windows and at the annual scale, correlation slopes between MAAT and the Niño index were consistently positive, and confidence intervals indicated robust directional relationships despite differences in correlation strength. Our results demonstrated that both PDO and ENSO variability are significantly associated with MAAT at Cultus Lake, with the strongest coupling occurring during the cold season and progressively weaker associations during transitional and warm-season periods.

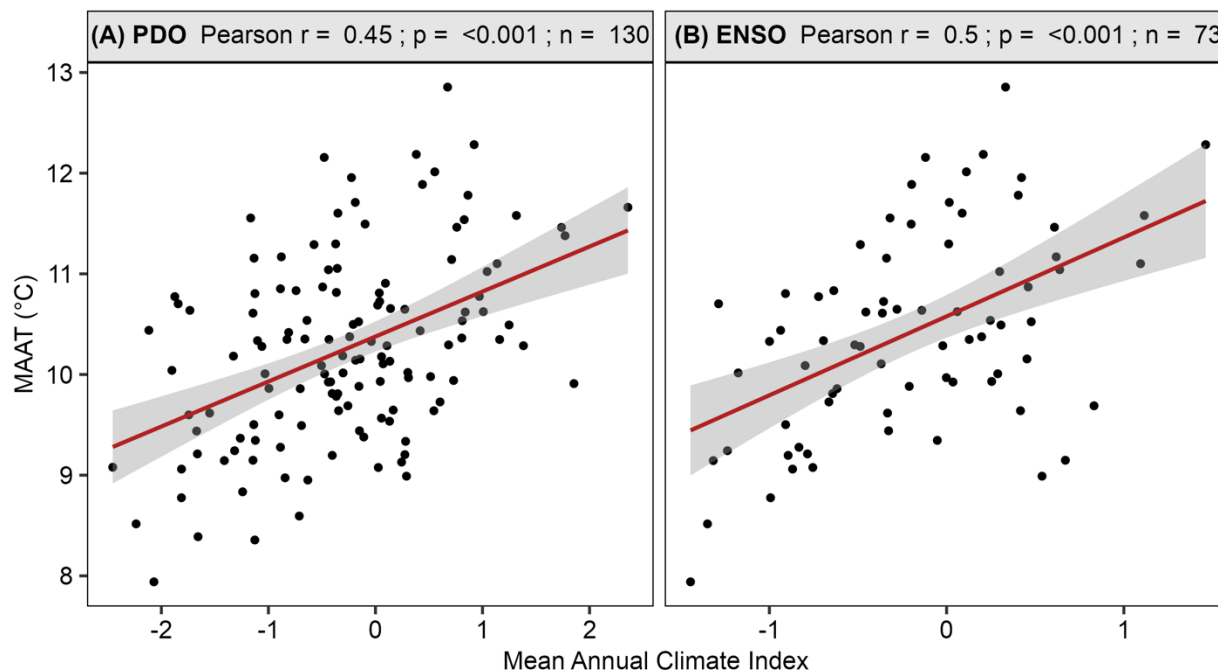


Figure 5.2 Relationship between large-scale climate indices and mean annual air temperature (MAAT, °C) at Cultus Lake. (A) MAAT plotted against the annual Pacific Decadal Oscillation (PDO) index, showing a significant positive association (Pearson's $r = 0.45$, $p < 0.001$, $n = 130$). (B) MAAT plotted against the annual El Niño-Southern Oscillation (ENSO) index, also exhibiting a significant positive relationship (Pearson's $r = 0.50$, $p < 0.001$, $n = 73$).

3.2 Age-Depth Model

A Bayesian age-depth model was developed by integrating the ^{210}Pb CRS chronology with radiocarbon ages from terrestrial macrofossils (twigs) to establish a continuous sedimentary timeline for Cultus Lake. Model diagnostics indicated an excellent Markov Chain Monte Carlo (MCMC) convergence and well-resolved posterior distributions for sediment accumulation rate, memory, and process variability (Figure S5.2, upper panel). The ^{210}Pb CRS model provided high-precision age estimates through the upper ~15 cm of the core, corresponding to approximately the past 150 - 170 years. Supported and unsupported ^{210}Pb activities declined predictably with depth, and the Bayesian model closely tracked the CRS age structure with narrow uncertainty envelopes. Below the limit of ^{210}Pb dating, calibrated ^{14}C ages from macrofossils provided chronological control for the deeper portion of the core (20 - 40 cm). Here, the model

incorporated the broader calibrated radiocarbon probability ranges while maintaining coherent and well-bounded uncertainty.

The final chronology showed a smooth, monotonic increase in age with depth, with no evidence of stratigraphic breaks, erosion, or rapid depositional events. The combined ^{210}Pb and ^{14}C Bayesian model yielded a stable and internally consistent age model for the past ~600 years. This chronology formed the essential foundation for interpreting long-term brGDGT-derived temperature variability and $\delta^{15}\text{N}$ dynamics in Cultus Lake sediment.

3.3 Dynamics of Fractional Abundance of brGDGT-a Series Versus MAAT, and PDO

Block-bootstrap correlation analyses showed compound-specific relationships between individual brGDGT *a-series* fractional abundances and MAAT in Cultus Lake sediments (Table 5.1). Among all *a-series* compounds, brGDGT-IIIa exhibited the strongest and only statistically significant negative linear relationship with MAAT ($r = -0.57$, 95% CI: -0.67 to -0.08). In contrast, brGDGT-IIIa' displayed a similarly strong positive correlation with MAAT ($r = 0.56$), but the associated confidence interval overlapped zero (95% CI: -0.07 to 0.64), indicating a non-statistically significant relationship despite its relatively high correlation coefficient.

Phase-resolved analysis revealed a compound-specific sensitivity of brGDGT fractional abundances to PDO-defined climate regimes (Figure 5.3; Table S5.4). Across the early 20th-century transition (1924-1945 warm vs 1945-1976 cool), brGDGT-IIa' exhibited a pronounced and statistically robust regime shift, with significantly lower fractional abundances during the warm phase ($\Delta = -0.049$, 95% CI: -0.061 to -0.034, $q < 0.001$). The magnitude of this contrast is substantial (standardized effect size = -3.09), indicating that a marked change in the brGDGT abundances coincided with the PDO phase change. The persistence of this signal after FDR correction confirms that it represents a reproducible regime-scale response rather than a multiple-testing artifact. Although brGDGT-Ia, IIa, IIIa, and IIIa' do not achieve FDR-adjusted significance, they exhibited coherent and internally consistent PDO phase-aligned shifts. brGDGT-IIa is elevated during the warm phase and lower during the cool phase, whereas brGDGT-Ia and IIIa' tend to decrease during the warm phase. During the later transition (1976-2006 warm vs 2006-2022 cool), fractional abundances again displayed consistent phase-dependent structure: IIa' and Ia trend lower during warm phases, while IIa trends higher.

Although these contrasts do not exceed FDR-corrected thresholds, their recurrence across both regime transitions supports a systematic PDO imprint on brGDGT composition.

Table 5.1: Block-bootstrap Pearson correlation coefficients (r) and 95% confidence intervals (CI) between down-core brGDGT a-series fractional abundances and mean annual air temperature (MAAT, °C) at Cultus Lake. Correlations were calculated using GAM-smoothed sediment records aligned to MAAT. Confidence intervals excluding zero indicate statistically significant relationships and are marked with asterisks (*).

Compound	r (MAAT)	95% CI
BrGDGT-Ia	-0.04	[-0.49, 0.41]
BrGDGT-IIa	0.16	[-0.12, 0.44]
BrGDGT-IIa'	-0.16	[-0.44, 0.19]
BrGDGT-IIIa	-0.57	[-0.67, -0.08] *
BrGDGT-IIIa'	0.56	[-0.07, 0.64]

Moving-block bootstrap, B = 2000, block length = 10.

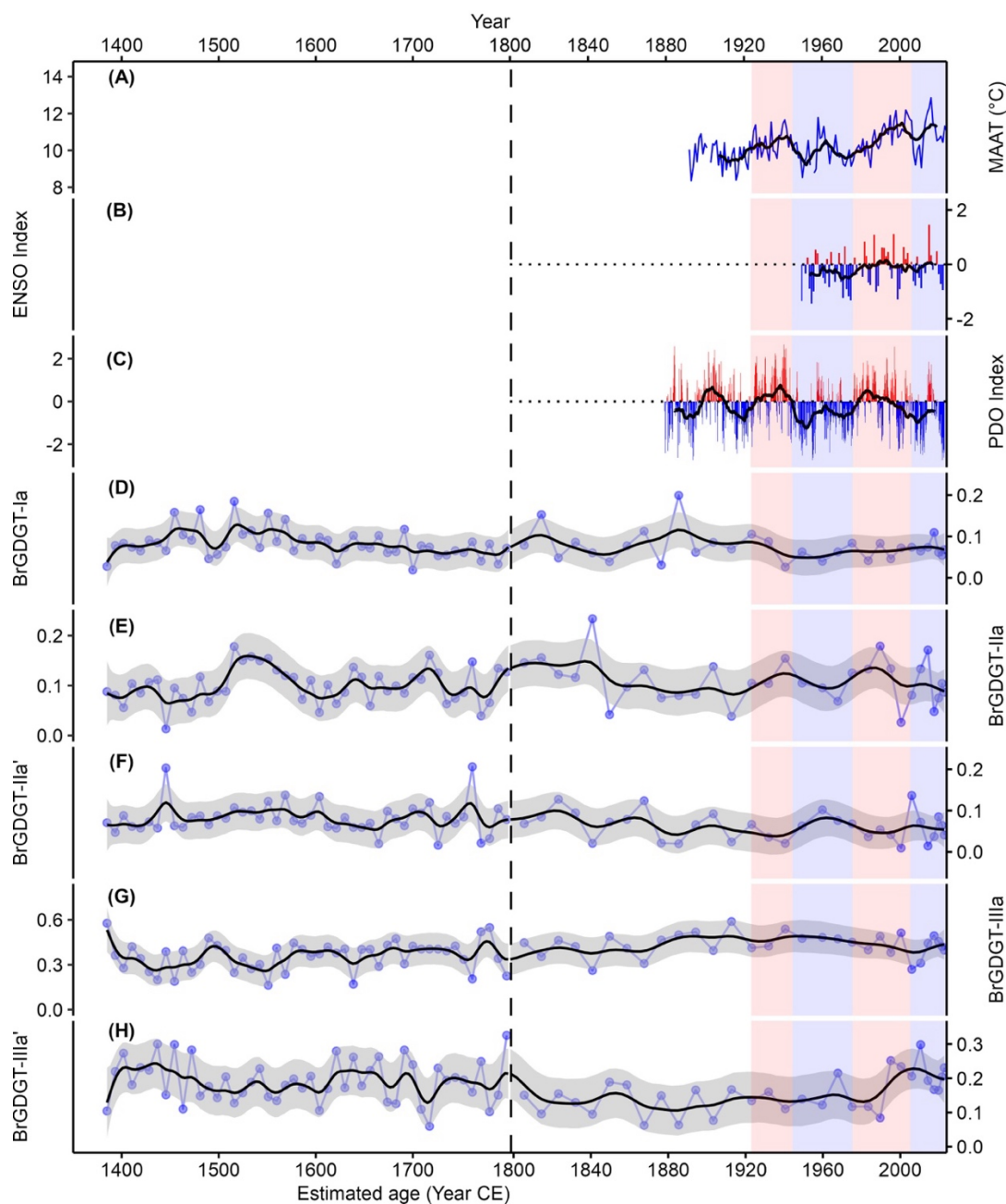


Figure 5.3 Downcore dynamics of fractional abundances for brGDGT- Ia, IIa, IIa', IIIa, and IIIa' compared with mean annual air temperature (MAAT, °C), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

3.4 Fractional Abundance of brGDGT-*b* Series, MAAT, and PDO

The brGDGT *b*-series compounds occurred at substantially lower fractional abundances than the corresponding *a*-series in Cultus Lake sediments (Figure 5.4). Despite their reduced abundance, a clear structure in sensitivity to MAAT is evident. Across the *b*-series, brGDGT-IIIb' emerged as the most MAAT-responsive compound, exhibiting a statistically significant negative association with MAAT ($r = -0.47$, 95% CI: -0.64 to -0.15, Table 5.2). The remaining *b*-series, including brGDGT-IIb' and IIIb, showed moderate positive correlations, whereas brGDGT-Ib and IIb exhibited weak negative tendencies; however, none of these reached statistical significance.

Despite their comparatively low fractional abundances, the brGDGT *b*-series compounds exhibited discrete but identifiable PDO-linked variability (Figure 5.4, Table S5.5). During the early PDO transition (1924-1945 warm vs 1945-1976 cool), brGDGT-Ib showed a clear and statistically significant phase contrast, with elevated abundances under warm conditions ($\Delta = 0.0039$; 95% CI: 0.0003 to 0.0075; $q < 0.001$). The magnitude of this shift (standardized effect size = 1.62) indicated a substantial relative change within this otherwise low-abundance pool. This response remained significant following FDR correction, identifying brGDGT-Ib as the principal regime-sensitive component of the *b*-series during the early 20th century. The remaining compounds (IIb, IIb', IIIb, and IIIb') do not exhibit statistically supported differences across this transition. Although minor directional tendencies are evident, brGDGT-IIb and IIIb were slightly elevated in warm phases, and IIIb' marginally reduced confidence intervals overlap zero, indicating that these changes fall within background variability. In contrast to the early transition, the later PDO shift (1976-2006 warm vs 2006-2022 cool) was not associated with statistically significant contrasts for any *b*-series compound. Nonetheless, a structured pattern persists: Ib, IIb', and IIIb' showed positive Δ values (higher during the warm phase and lower during the cool phase), whereas IIb trends lower during the warm phase. While these differences remained below FDR-adjusted significance thresholds, their recurrence suggests that PDO forcing modulates the *b*-series assemblage in a consistent, albeit lower-amplitude, manner.

Table 5.2: Block-bootstrap Pearson correlation coefficients (r) and 95% confidence intervals (CI) between down-core brGDGT b-series fractional abundances and mean annual air temperature (MAAT, °C) at Cultus Lake. Correlations are based on GAM-smoothed sediment records aligned to annual MAAT. Confidence intervals excluding zero indicate statistically significant relationships and are marked with asterisks (*).

Compound	r (MAAT)	95% CI
BrGDGT-Ib	-0.03	[-0.31, 0.20]
BrGDGT-IIb	-0.18	[-0.38, 0.14]
BrGDGT-IIb'	0.27	[-0.07, 0.49]
BrGDGT-IIIb	0.23	[-0.10, 0.43]
BrGDGT-IIIb'	-0.47	[-0.64, -0.15] *

Moving-block bootstrap, B = 2000, block length = 10.

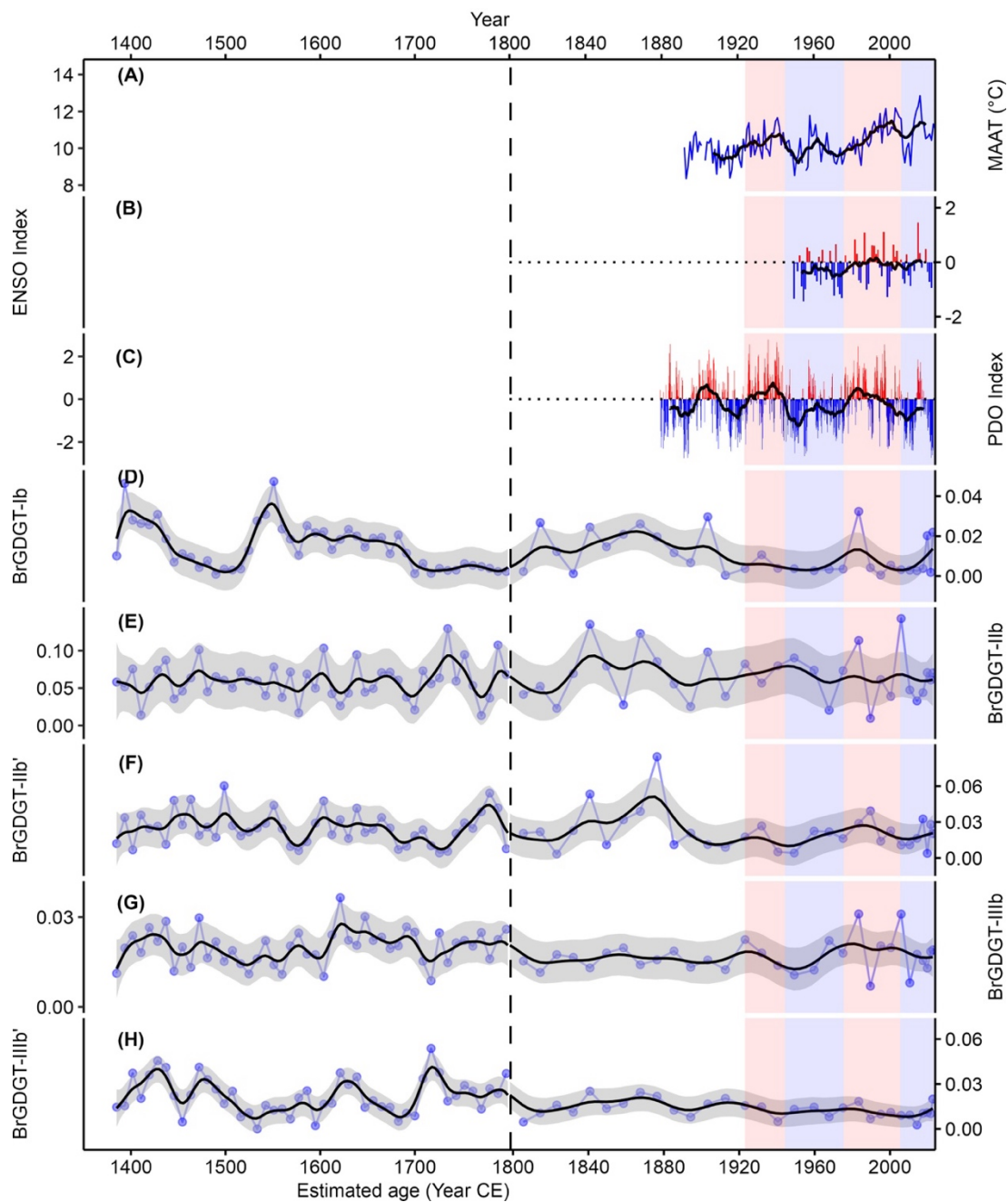


Figure 5.4 Downcore dynamics of fractional abundances for brGDGT- Ib, IIb, IIb', IIIb, and IIIb' compared with mean annual air temperature (MAAT, °C), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent raw fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

3.5 Fractional Abundance of BrGDGT-*c* Series, MAAT, and PDO

Among the evaluated *c*-series brGDGT compounds, brGDGT-IIIc' exhibited a statistically significant negative correlation with MAAT ($r = -0.12$, 95% CI: -0.47 to -0.08) (Table S5.3). BrGDGT-IIc' showed the strongest positive correlation coefficient within the *c*-series ($r = 0.49$); however, the associated confidence interval overlapped zero (95% CI: -0.14 to 0.63), precluding statistical support. Similarly, brGDGT-IIIc displayed a moderate positive association ($r = 0.27$, 95% CI: -0.08 to 0.52), and brGDGT-Ic showed a weak negative tendency ($r = -0.15$, 95% CI: -0.38 to 0.23), both with confidence intervals encompassing zero. BrGDGT-IIc exhibited no meaningful association with MAAT ($r = -0.01$; 95% CI: -0.48 to 0.61) (Table S5.3).

The *c*-series compounds occurred at the lowest fractional abundances relative to the *a*- and *b*-series; nevertheless, they exhibited clear and compound-specific restructuring across PDO-defined climate regimes (Figure 5.5; Table S5.6). During the early transition (1924-1945 warm vs 1945-1976 cool), brGDGT-IIIc' showed a pronounced and statistically significant abundance under warm conditions ($\Delta = 0.0033$, 95% CI: 0.0013 to 0.0052, $q < 0.001$), with a large, standardized effect size (2.45). BrGDGT-IIIc also increased during warm phases ($\Delta = 0.0034$; 95% CI: 0.0002 to 0.006), though this contrast does not remain significant after FDR correction. BrGDGT-IIc displayed a moderate warm-phase abundance ($\Delta = 0.0027$, 95% CI: 0.0004 to 0.0057, $q = 0.056$), approaching statistical support, whereas brGDGT-Ic showed overlapping confidence intervals, and brGDGT-IIc' exhibited no discernible regime contrast. In the later transition (1976-2006 warm vs 2006-2022 cool), the dominant response to the PDO phases shifted to BrGDGT-IIc, which exhibited a strong and FDR-supported increase during warm phases ($\Delta = 0.0035$, 95% CI: 0.0023 to 0.005; $q < 0.001$, effect size = 2.48). Other *c*-series compounds displayed comparatively small and statistically unresolved differences.

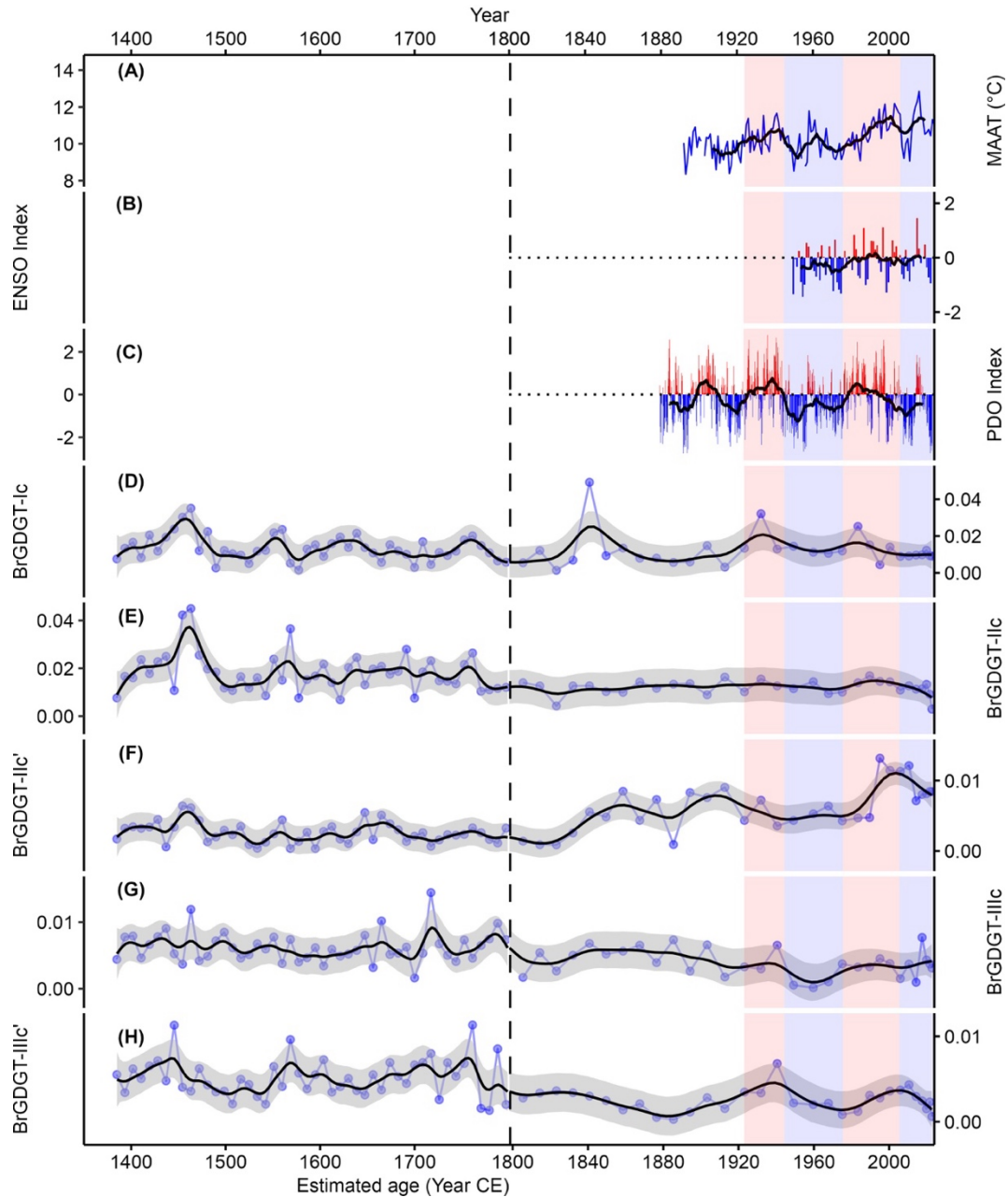


Figure 5.5 Downcore dynamics of fractional abundances for brGDGT- Ic, IIc, IIc', IIIc, and IIIc' compared with mean annual air temperature (MAAT, °C), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent raw fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

3.6 Application of Cultus Lake Specific Calibrations to Reconstruct PDO, and Integration with $\delta^{15}\text{N}$ Values

To evaluate the temporal performance of the Cultus Lake-specific temperature calibrations, the $\text{MBT}'_{5\text{ME}}$ index and ten lake-specific multivariate calibrations (Cal_1 - Cal_{10}) were applied to the dated sediment core (Dagodzo et al. in preparation). Downcore fractional abundances and $\text{MBT}'_{5\text{ME}}$ values were calculated, and reconstructed temperatures were generated for each calibration. These reconstructions were then compared to the PDO records. Calibration-derived temperature reconstructions exhibited pronounced multi-centennial variability from ~1400 CE onward (Figure 5.6B & 5.6C). Across the ensemble, GAM-smoothed trajectories revealed oscillatory structure, characterized by elevated reconstructed temperatures during portions of the 15th-16th centuries, reduced values through parts of the 17th-18th centuries, and a gradual increase into the late 19th and 20th centuries. Superimposed on this centennial variability are shorter-term fluctuations during the 20th century corresponding to PDO-defined regime intervals, which were concordant with the smoothed MAAT and ENSO patterns (Figure S5.3 & S5.4).

Cal_4 displayed persistent centennial-scale variability across the full record, including higher reconstructed temperatures during the 15th-16th centuries, lower values during the mid- to late-18th century, and sustained increases into the 20th century. Distinct separation was evident between the early 20th-century warm (1924-1945) and subsequent cool (1945-1976) phases was evident in the GAM-smoothed trajectory, supported by a statistically significant regime contrast ($\Delta = 1.05\text{ }^{\circ}\text{C}$, 95% CI: 0.17 to 1.62, $q < 0.001$) (Table S5.7).

Cal_2 similarly exhibited a coherent multi-centennial structure, with sustained variability across the pre-industrial interval and clear late-19th-20th-century amplification (Figure 6B). The GAM-smoothed curve showed consistent directional differences between PDO-defined warm and cool phases, in both 20th-century transitions (positive Δ values in each case), although these contrasts do not retain statistical significance following FDR correction. Notably, Cal_2 maintained stable PDO regime directionality across both transitions without inversion.

Cal_1 similarly showed centennial-scale variability but with an inverse regime contrast during the early 20th century ($\Delta = -2.65\text{ }^{\circ}\text{C}$, 95% CI: -4.16 to -0.91, $q < 0.001$) (Table S5.7 & Figure S5.3). The remaining calibrations (Cal_3 , Cal_5 - Cal_{10}) exhibited multi-centennial fluctuations of varying amplitude, with visible 20th-century regime shifts in the GAM-smoothed

curves; however, regime contrasts outside Cal₁ and Cal₄ are not statistically supported after FDR correction. For the later transition (1976-2006 warm vs 2006-2022 cool), GAM trajectories indicated modest phase-dependent deviations across several calibrations, but none yielded statistically significant contrasts following multiple-testing correction. The MBT'_{5ME} index exhibited centennial variability comparable in magnitude to several lake-specific calibrations but does not show statistically supported PDO regime separation in either transition (Figure S5.5).

Together, these results indicated that PDO-related regime restructuring is captured selectively within the calibration framework, with Cal₁, Cal₂ and Cal₄ demonstrating strong early 20th-century sensitivity, while most calibrations and MBT'_{5ME} exhibited more muted or statistically unresolved phase contrasts. The GAM-smoothed reconstructions further illustrated that regime-dependent variability is concentrated in the early transition and becomes less distinct in the later PDO phase shift.

Independent non-brGDGT proxy records exhibited sustained multi-centennial variability that parallels the structure observed in the brGDGT-based reconstructions (Figure 5.6D & 5.6E). The MacDonald & Case (2005) tree-ring index showed alternating positive and negative excursions from the 15th century onward, with moderate amplitude variability during the 15th-16th centuries, subdued values through parts of the 17th-18th centuries, and renewed variability into the 19th and 20th centuries. These oscillations corresponded temporally to comparable centennial-scale fluctuations in the brGDGT-derived temperature series, including pre-industrial variability and enhanced late 19th-20th century structure. The Mt. Logan Na⁺ record (Osterberg et al., 2014) exhibited lower-amplitude variability prior to the 18th century, followed by increased variability through the late 18th and 19th centuries and sustained elevated values into the early 20th century. This longer-term structure coincided with the gradual increase and superimposed multi-decadal oscillations observed in the brGDGT-derived temperature series. During the 20th century, phase-dependent deviations in Na⁺ from the ice record occurred contemporaneously with PDO-defined intervals and correspond to regime-scale fluctuations expressed in the brGDGT reconstructions (Figure 5.6). Overall, the independent tree-ring and ice-core Na⁺ records from Mount Logan document centennial-scale oscillatory variability with periods of significant increases and decreases (red and blue bands on Figure 5.6E) and 20th-century regime shifts that occurred synchronously with patterns observed in the brGDGT-derived temperature reconstructions.

The $\delta^{15}\text{N}$ record showed a structured multi-centennial variability from ~1400 CE onward (Figure 5.6G). Between the 15th and 18th centuries, $\delta^{15}\text{N}$ fluctuated around a stable baseline, with elevated intervals during the 15th-16th centuries and reduced values through portions of the 17th-18th centuries. These centennial-scale oscillations occurred contemporaneously with variability in the brGDGT-inferred temperature reconstruction and the tree-ring indices (Figure 5.6C-D), including depressed values during the 17th-18th centuries and higher values during earlier intervals. A sustained upward shift in $\delta^{15}\text{N}$ began in the late 19th century (~1850-1900 CE), followed by persistently elevated values through the 20th century. This long-term increase parallels the late 19th-20th century rise in brGDGT-inferred temperatures and coincides with enhanced variability in the tree-ring reconstructions. The salmon returns record (Figure 5.6F) displayed strong interannual variability, and beginning in the late 20th century (~mid-1980s), returns exhibited pronounced variability and episodic declines while $\delta^{15}\text{N}$ remained elevated. This interval marked a clear decoupling between salmon returns and the $\delta^{15}\text{N}$ -brGDGT temperature trajectory. Overall, $\delta^{15}\text{N}$ closely tracked centennial-scale variability expressed in brGDGT-derived temperatures, and tree-ring indices from ~1400 CE through the mid-19th century, with divergence emerging in the late-modern period as salmon return variability becomes increasingly independent of the long-term $\delta^{15}\text{N}$ and temperature trends.

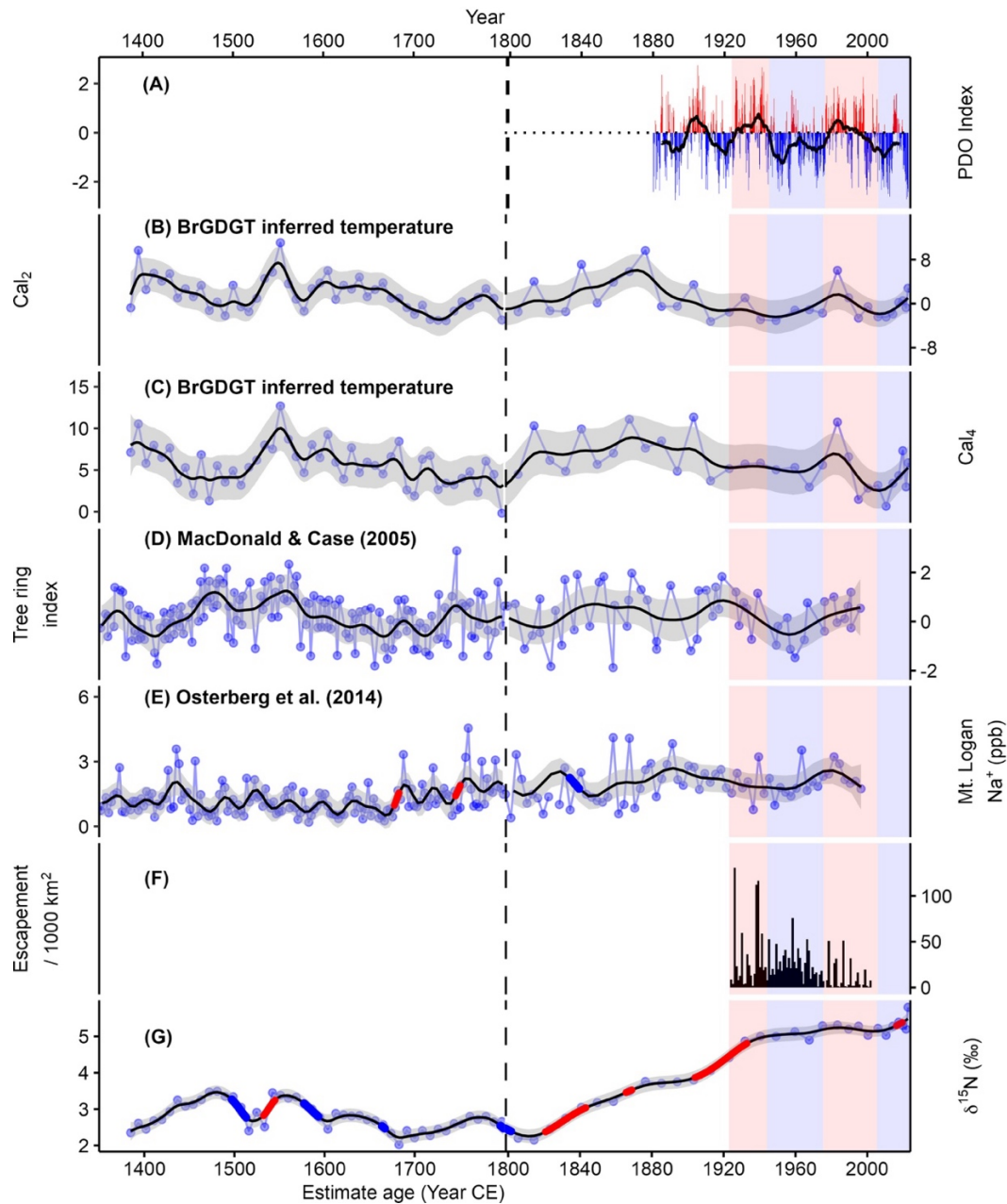


Figure 5.6 Multi-proxy comparison of (A) Pacific Decadal Oscillation (PDO) with 10-year moving average (black line). (B-C) Lake-specific brGDGT-derived temperature reconstructions (Cal₂ and Cal₄) with GAM smooths (black) and 95% confidence intervals (grey). (D) Tree-ring index (MacDonald & Case, 2005). (E) Mt. Logan Na⁺ record (Osterberg et al., 2014). (F) Salmon returns (individuals per 1000 km²). (G) Sedimentary δ¹⁵N (‰). The shaded red and blue bands denote the warm and cool PDO phases, respectively. Red and blue sections on GAMs indicate periods of significant increases and decreases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

3.7 Downcore Comparison of Lake-Specific Calibrations with Published Calibrations

We evaluated the performance of external brGDGT calibrations by applying previously published brGDGT-based MAAT and Months Above Freezing (MAF) calibrations to the Cultus Lake sediment record and quantified PDO regime contrasts using the same moving block bootstrap framework (Table S5.8; Figure S5.6, Figure S5.7). Across all published calibrations examined (Bauersachs et al., 2024; DeJong et al., 2014; Russel et al., 2018; Otiniano et al., 2023; Raberg et al., 2021), PDO-defined regime contrasts were uniformly weak and statistically unsupported following FDR correction. For the early 20th-century transition (1924-1945 warm vs 1945-1976 cool), Δ values were small to moderate in magnitude, and confidence intervals consistently overlapped zero. Effect sizes were generally small (≤ 1.03), and no comparison retained statistical support after multiple-testing correction (all $q \geq 0.113$). Directionality was not uniform: while MAAT by Bauersachs et al. 2024 showed a slight positive Δ (0.115 °C), most other MAAT and MAF calibrations yielded negative Δ values, indicating higher reconstructed temperatures during the cool PDO phase. Similarly, for the later transition (1976-2006 warm vs 2006-2022 cool), none of the published calibrations produced statistically significant contrasts. Although MAAT (Index1) by DeJong et al. 2014 exhibited a relatively large negative Δ (−5.02 °C; 95% CI excluding zero prior to FDR adjustment), this contrast did not remain significant after correction ($q = 0.113$). All remaining reconstructions displayed overlapping confidence intervals and small-to-moderate standardized effect sizes, indicating limited regime separation. GAM-smoothed trajectories of the published calibrations showed multi-centennial variability comparable in amplitude to the lake-specific reconstructions; however, 20th-century PDO phase differentiation is muted relative to that observed in selected lake-derived calibrations. In particular, consistent warm-cool phase separation evident in Cal₄ and Cal₂ is not reproduced by the external MAAT or MAF calibrations. Collectively, these results demonstrated that previously published global or regional brGDGT calibrations applied to Cultus Lake sediments do not yield statistically robust PDO regime differentiation. In contrast, PDO-sensitive regime separation is selectively expressed within the lake-specific calibration framework, indicating enhanced phase discrimination under site-specific parameterization.

3.8 Compositional Differences Among Soil, Water, and Sedimentary BrGDGT

Principal component analysis (PCA) revealed compositional differences in brGDGT distributions among sediment core, watershed soil, and water samples (Figure 5.7). The first two principal components (PC) explained 67.3% of the total compositional variance, with PC1 accounting for 55.6% and PC2 accounting for 11.7%. Most sediment core samples formed a compact cluster at negative PC1 scores, whereas most water samples were distributed at positive PC1 scores. Watershed soil samples exhibited greater dispersion across the ordination space but generally occupied a compositional region distinct from the sediment core cluster. Sample subgroups within both the soil and water datasets also showed variable dispersion. The separation along PC1 reflected differences in brGDGT composition, with positive PC1 scores associated primarily with the less methylated compounds Ia, Ib, IIa, and IIa', whereas negative PC1 scores were associated with the more highly methylated compounds IIIb, IIIb', IIIc, and IIIc'. Accordingly, sediment core samples were characterized by relatively greater contributions of the IIIb- and IIIc-series compounds, whereas most soil and water samples were more strongly associated with the Ia-, Ib-, and IIa-series compounds.

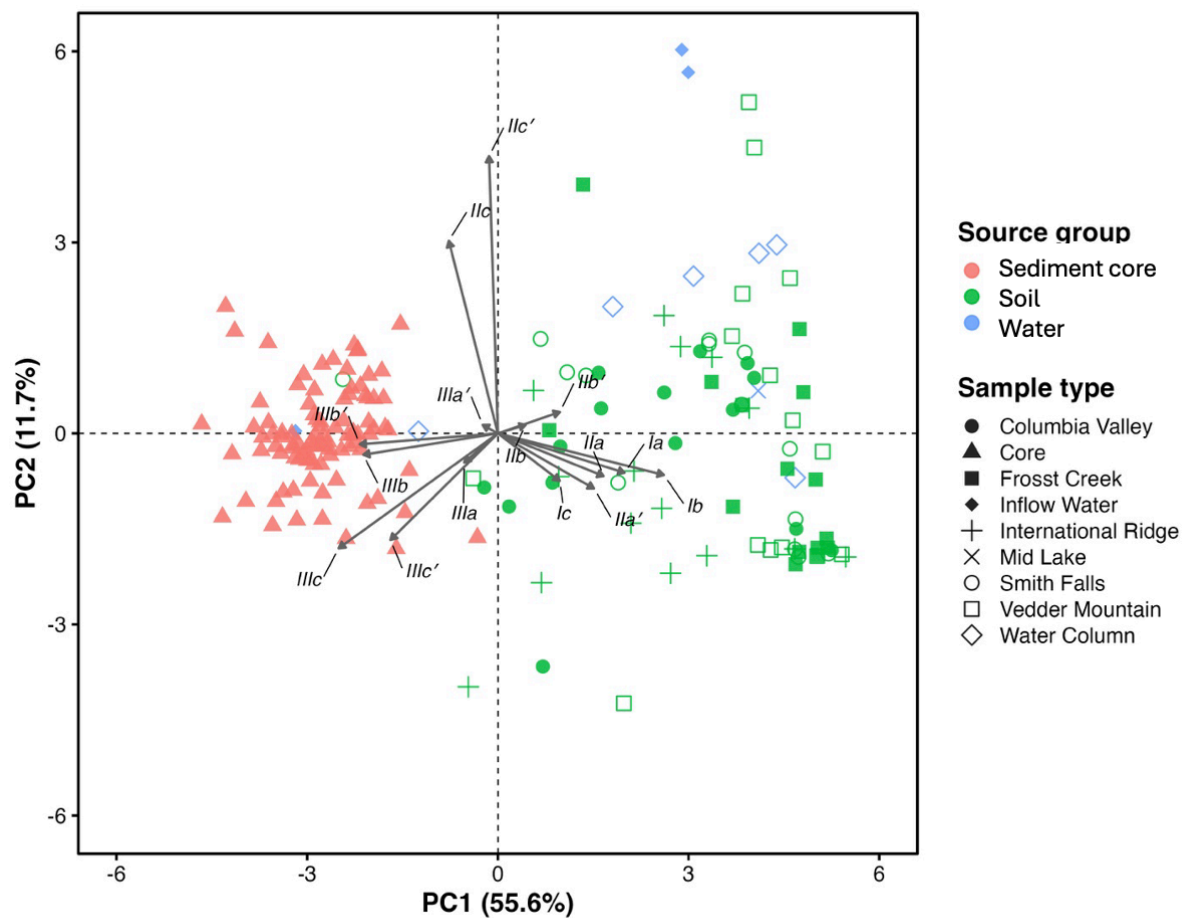


Figure 5.7 Principal component analysis (PCA) of centered log-ratio transformed fractional abundances of brGDGTs in watershed soils, water samples, and sediment core samples from Cultus Lake. PC1 and PC2 explained 55.6% and 11.7% of the total compositional variance, respectively. Arrows indicate the direction and relative contribution of individual brGDGT compounds to the ordination.

4.0 Discussion

4.1 Climatic Controls on Cultus Lake Air Temperature

MAAT at Cultus Lake exhibits a strong coupling with large-scale Pacific climate variability, particularly the PDO and ENSO, reflecting the sensitivity of lower Fraser Valley

climate to North Pacific Ocean atmosphere dynamics (Gauthier et al., 2020; Moon et al., 2015; Praskievicz et al., 2009; Selbie et al., 2022). Over the ~130-year instrumental record, MAAT is positively correlated with the PDO, such that warm PDO phases are consistently associated with elevated regional air temperatures, whereas cool phases correspond to cooler conditions. The relatively narrow confidence intervals and symmetric scatter around the fitted PDO temperature relationship indicate that this coupling has remained stable through time despite substantial interannual and decadal variability (Kiffney et al., 2002; Mantua and Hare, 2002). Independent studies documenting strong ENSO- and PDO-driven precipitation variability across the Pacific Northwest further support the presence of coherent atmospheric teleconnections that jointly influence regional hydroclimate and air temperature (Mills and Walsh, 2013; Pavia et al., 2006; Zhang et al., 2010).

The influence of the PDO on regional air temperature is strongly seasonally-modulated (Bonsal et al., 2001; Kiffney et al., 2002; Mills and Walsh, 2013). The most pronounced PDO-MAAT coupling at Cultus Lake occurs during the November-April period, corresponding to the cold season, whereas correlations weaken during the summer months. This seasonal asymmetry reflects the physical mechanisms through which the PDO modulates North Pacific atmospheric circulation, primarily via changes in the position and intensity of the Aleutian Low (Bonsal et al., 2001; Praskievicz et al., 2009; Jia and Ge, 2017). During positive PDO phases, strengthened southwesterly flows enhance the advection of maritime air masses, promoting warmer winter conditions along the Pacific coast (Bonsal et al., 2001; Mantua and Hare, 2002). In contrast, summer temperatures are more strongly governed by local radiative forcing and land-atmosphere interactions, reducing PDO influence during the warm season (Bonsal et al., 2001; Mantua and Hare, 2002). The persistence of weaker but statistically significant PDO-MAAT relationships outside the cold season indicates that PDO forcing exerts a year-round, albeit seasonally variable, influence on regional thermal conditions.

ENSO also exerts a stronger control on Cultus Lake air temperature at interannual timescales, as reflected by higher correlations with the ENSO index. El Niño events are associated with anomalously warm conditions, whereas La Niña events correspond to cooler temperatures in southwestern British Columbia (Bonsal et al., 2001; Mills and Walsh, 2013). Similar to the PDO, ENSO temperature coupling is strongest during the cold season, underscoring the dominant role of winter atmospheric teleconnections in shaping regional

temperature variability. ENSO's influence remains significant across all seasons, suggesting that ENSO impacts are not confined to peak winter atmospheric circulation anomalies. This persistence likely reflects delayed ocean-atmosphere feedbacks and regional climate memory effects that prolong ENSO's influence beyond its core dynamical phase (Fang and Yang, 2015; Mantua and Hare, 2002).

Collectively, these results demonstrate that Cultus Lake MAAT integrates both interannual ENSO variability and decadal-scale PDO forcing, with the strongest coupling expressed during the cold season when large-scale atmospheric circulation exerts maximum control. This climatic framework is directly relevant for interpreting brGDGT-based temperature proxies in Cultus Lake sediments. Previous work on epilimnetic suspended particulate matter (SPM) from Cultus Lake shows that individual brGDGT compounds respond sensitively to short-term lake-water temperature variability across stratified to transitional seasons, reflecting rapid microbial membrane adaptation to contemporaneous thermal conditions (Dagodzo et al., in preparation). Because the Cultus Lake temperature calibration is derived from SPM collected between July and December, it characterizes brGDGT temperature relationships under stratified and overturn conditions, rather than explicitly resolving seasonal production or export dynamics. When transferred to the sedimentary archive, brGDGT distributions therefore integrate lipid production over annual- to multi-annual-timescales, reflecting the persistence and magnitude of lake thermal regimes rather than instantaneous temperature variability. Within this context, the strong instrumental coupling between Pacific climate indices and regional air temperatures provides a robust mechanistic foundation for evaluating whether sedimentary brGDGT-inferred temperatures track MAAT, ENSO, and PDO variability through time, while acknowledging that proxy sensitivity is shaped by the limnological conditions represented in the calibration dataset.

4.2 Regional Air Temperature Variability (MAAT) versus brGDGTs in Cultus Lake

Among all measured brGDGT structures in Cultus Lake sediments, brGDGT-IIIa shows the strongest inverse relationship with MAAT, exceeding that of any other compound. A weaker negative association is observed for brGDGT-IIIb', whereas the remaining brGDGTs display weak or non-significant relationships with MAAT. This pattern demonstrates that temperature

sensitivity within the sedimentary brGDGT pool is strongly compound-specific rather than uniformly expressed across structural classes (Foster et al., 2015; Naafs et al., 2017). Recognition of such compound-level variability has motivated the development of refined regional calibrations that explicitly account for environmental setting, thereby improving the accuracy and reliability of paleotemperature reconstructions derived from these bacterial lipid biomarkers (Bauersachs et al., 2024; Foster et al., 2015; Naafs et al., 2017; Otiniano et al., 2023; Zhao et al., 2021). The inverse relationship between brGDGT-IIIa abundance and MAAT indicates that cooler climatic conditions are systematically associated with elevated relative abundance of IIIa in Cultus Lake sediments, while warmer intervals correspond to reduced brGDGT-IIIa abundances. This behaviour reflects the integrated expression of temperature over the timescales relevant to sediment accumulation and archiving. This sedimentary response closely parallels observations from other lacustrine sediment studies, particularly those reported by Otiniano et al. (2023), where brGDGT-IIIa emerged as a dominant temperature-sensitive compound and formed the basis for warm-season temperature reconstructions across the AK-YK-NWT region. Although those reconstructions focused on warm-season metrics, the consistent inverse relationship between IIIa and temperature across climatically distinct lake systems supports the interpretation that sedimentary brGDGT-IIIa reliably tracks cooler thermal states. Similar behaviour is also implicit in the MAAT calibration of Bauersachs et al. (2024), which is strongly influenced by brGDGT-IIIa and Ib, two structures that capture a substantial fraction of the temperature signal in those sediments and are dominated by the brGDGT-IIIa response in Cultus Lake.

The selective emergence of brGDGT-IIIa, and IIIb', as MAAT-responsive compounds in Cultus Lake, highlights a distinction between sedimentary brGDGT behaviour and short-term or water-column signals. While multiple brGDGTs may exhibit temperature sensitivity under modern or transient conditions (Dagodzo et al. in preparation), only a restricted subset retains a robust and interpretable temperature signal once integrated into sediments over decadal to multidecadal timescales.

When the temperature transfer function of Otiniano et al. (2023), based on the fractional abundance of brGDGT-IIIa, is applied to the Cultus Lake sediment record, the resulting reconstructed temperatures show a strong positive relationship with instrumental MAAT. This apparent reversal in sign relative to the direct IIIa MAAT correlation arises from the structure of

the transfer function itself, in which IIIa is inversely related to temperature ($MAF = -13.29 \times IIIa + 11.30$). Consequently, higher IIIa abundances associated with cooler conditions are mathematically translated into lower reconstructed temperatures, yielding positive covariance between reconstructed temperatures and observed MAAT. This internal consistency confirms that the negative temperature sensitivity of sedimentary IIIa is preserved through the calibration framework. The successful application of both the Otiniano et al. (2023) and Bauersachs et al. (2024) calibrations to Cultus Lake indicates that the MAAT signal encoded by sedimentary brGDGT-IIIa is compatible with both warm-season and annual temperature reconstructions. Cultus Lake is now a warm monomictic system that does not freeze, such that the distinction between MAAT and mean temperature of months above freezing (MAF) is comparatively small relative to seasonally ice-covered or high-latitude lakes. Under these conditions, warm-season and annual temperature variability are expected to covary strongly at interannual to decadal timescales. Accordingly, sedimentary brGDGT-IIIa in Cultus Lake records integrated thermal conditions representative of the lake's overall temperature regime, rather than a narrowly defined seasonal signal.

4.3 Large-scale climate (ENSO and PDO) dynamics versus brGDGTs in Cultus Lake

The response of sedimentary brGDGTs to interannual ENSO variability and decadal PDO variability differs fundamentally from their response to mean annual air temperature (MAAT), reflecting distinct modes of climatic forcing (Mantua et al., 1997; Mantua & Hare, 2002; Trenberth et al., 2002). MAAT directly governs local atmospheric energy balance and surface-water heat exchange, thereby establishing the baseline thermal conditions under which temperature-sensitive brGDGTs are produced and ultimately archived in sediments. In contrast, ENSO influences regional climate indirectly through tropical Pacific sea surface temperature anomalies that reorganize large-scale atmospheric circulation, producing episodic regional temperature anomalies rather than sustained shifts in local thermal state (Wang, 2002; Trenberth et al., 2002). The PDO similarly operates as a basin-scale reorganization of North Pacific sea surface temperature and atmospheric pressure fields expressed in multi-decadal phase structure (Mantua et al., 1997; Mantua & Hare, 2002). Because these large-scale systems are inherently phase-based, interpretation of sedimentary brGDGT signals and especially calibration-derived

reconstructions must likewise be evaluated within a phase framework rather than solely through linear correlations with annual temperature.

Across the full record (~1400 CE onward), lake-specific calibrations resolve multi-centennial oscillations that align closely with independent terrestrial and atmospheric proxies (Biondi et al., 2001; Gedalof & Smith, 2001; Gedalof et al., 2002; MacDonald & Case, 2005; Osterberg et al., 2014). Periods of elevated reconstructed temperatures during the 15th-16th centuries, depressed intervals through parts of the 17th-18th centuries, and progressive increases into the late 19th and 20th centuries are reproduced in regional tree-ring and Mt. Logan Na⁺ records (MacDonald & Case, 2005; Osterberg et al., 2014). The shared temporal structure across lacustrine lipids, dendroclimatological indices, and atmospheric circulation proxies indicates that sedimentary brGDGTs record regionally coherent North Pacific-driven thermal variability rather than local ecological noise. This multi-proxy concordance supports interpretation of the brGDGT record as an integrative archive of basin-scale climate dynamics.

Superimposed on centennial variability, selected lake-specific calibrations resolve 20th-century PDO regime shifts, particularly during the early transition (1924-1945 warm vs. 1945-1976 cool). Statistically-supported regime differentiation in Cal₄ and consistent phase directionality in Cal₂, demonstrate that site-specific parameterization enhances sensitivity to decadal forcing (Dagodzo et al. in preparation). Notably, phase structure is more clearly expressed in selected multivariate calibrations than in most individual compounds or broader indices such as MBT'_{5ME}. This indicates that PDO sensitivity emerges through compound interactions rather than through single structures acting independently. Cal₄, which integrates brGDGT-Ib and IIIa', exhibits pronounced PDO phase separation despite IIIa' alone showing limited PDO sensitivity. Thus calibration performance depends on structural complementarity and weighting within the model. In contrast, Cal₁, combining brGDGT-Ib and IIa', the two strongest water-column temperature indicators in Cultus Lake, showed an inverse tracking of the PDO phases (i.e., high in cool phase and low in warm phase). The inclusion of IIa', which exhibits an inverse phase response relative to Ib in sediments, dilutes regime discrimination. These contrasts underscore that optimal PDO sensitivity is achieved not by maximizing short-term temperature responsiveness, but by selecting compounds that preserve coherent phase-aligned signals after sedimentary integration.

Published global and regional calibrations similarly fail to reproduce robust PDO phase structure in Cultus Lake. Although they capture broad multi-centennial variability, regime differentiation is weak or statistically unsupported when applied to this site (Bauersachs et al., 2024; DeJong et al., 2014; Otiniano et al., 2023; Raberg et al., 2021). This attenuation arises because externally derived calibrations incorporate compounds that are only weakly responsive to PDO variability in Cultus Lake sediments, thereby diluting site-specific phase information. In contrast, lake-specific calibrations that emphasize structurally selective, phase-coherent compounds preserve decadal PDO expression.

Together, these findings demonstrate that sedimentary brGDGTs encode climate variability across multiple temporal scales, but that decadal phase sensitivity depends critically on compound selection and calibration structure. In Cultus Lake, PDO signals are not uniformly expressed across the brGDGT pool; rather, they emerge through selective molecular integration within site-optimized transfer functions. This reinforces the importance of lake-specific calibration development when reconstructing phase-organized climate variability from sedimentary biomarkers.

4.4 $\delta^{15}\text{N}$ and Salmon Returns in Relation to PDO Variability in Cultus Lake

The sedimentary $\delta^{15}\text{N}$ record provides an independent ecological dimension to the climatic framework reconstructed from brGDGTs and regional proxy archives. Prior to the industrial era (~1400-1850 CE), $\delta^{15}\text{N}$ variability broadly parallels centennial-scale fluctuations observed in the brGDGT-inferred temperature record and independent regional climate proxies (MacDonald & Case, 2005; Osterberg et al., 2014). This coherence suggests that marine-derived nitrogen delivery to Cultus Lake was indirectly modulated by large-scale North Pacific climate variability (Mantua et al., 1997; Mantua & Hare, 2002). Climatically driven shifts in ocean productivity, salmon survival, and migration dynamics have been shown to influence the magnitude of marine nutrient subsidies to freshwater ecosystems, linking salmon-derived nitrogen inputs to basin-scale climatic regimes such as the PDO (Dagodzo et al. 2025; Finney et al. 2002; Mantua et al., 1997; Mantua & Hare, 2002). The agreement between $\delta^{15}\text{N}$ values, temperature reconstructions, and regional dendroclimatological and atmospheric proxies therefore implies that salmon-mediated nutrient transfer operated within the broader climate system over pre-industrial timescales.

A sustained upward shift in $\delta^{15}\text{N}$ beginning in the late nineteenth century marks a transition from this relatively stable pre-industrial regime. Euro-American settlement began in the Columbia Valley in the late 1800s (Schaepe & Ts'elxwéyeqw, 2017), and the timing of the shift in $\delta^{15}\text{N}$ coincides with the onset of widespread watershed modification and expanding human activity within the Cultus Lake catchment and the surrounding Fraser Valley (Gauthier et al. 2021; Putt et al., 2019). Paleolimnological and watershed studies from Cultus Lake indicate that nitrogen loading to the lake has increased substantially during the past century through a combination of agricultural runoff, septic leachate, atmospheric deposition, and avian input (Belzer et al. 1997; Gauthier et al. 2021; Putt et al., 2019). Elevated $\delta^{15}\text{N}$ values during this interval are therefore consistent with enhanced delivery of isotopically enriched nitrogen sources associated with anthropogenic nutrient inputs. Agricultural manure, wastewater effluent, and watershed-derived organic wastes are characteristically enriched in ^{15}N relative to natural background sources, and similar isotopic enrichment has been widely documented in lakes undergoing cultural eutrophication (Bachmann et al., 2013; Leavitt et al., 2006; Putt et al., 2019). Consequently, the long-term rise in $\delta^{15}\text{N}$ most likely reflects a superposition of anthropogenic nitrogen enrichment on the background signal associated with marine-derived nutrients from Pacific salmon.

This transition also coincides with the long-term warming trajectory inferred from the brGDGT-based temperature reconstructions. Warmer regional conditions since the late 19th century may have further amplified nutrient loading and internal cycling within the lake through enhanced watershed runoff, intensified stratification, and increased biological productivity (Putt et al., 2019). Climate warming has been shown to amplify eutrophication processes in temperate lakes by strengthening stratification and altering nutrient stoichiometry, thereby promoting greater internal nutrient recycling and shifts in planktonic community structure (De Senerpont Domis et al., 2012; Gerten & Adrian, 2002; Trolle et al., 2015). In Cultus Lake, the combined effects of warming and increasing anthropogenic nutrient inputs likely contributed to the persistent elevation in sedimentary $\delta^{15}\text{N}$ observed during the modern period.

A pronounced decoupling between Pacific salmon returns and $\delta^{15}\text{N}$ values emerges during the late twentieth century. While escapement records show strong interannual variability and substantial declines beginning in the 1970s-1980s, $\delta^{15}\text{N}$ values remain persistently elevated. This divergence indicates that the sedimentary nitrogen signal is no longer primarily controlled

by marine-derived salmon inputs. Instead, nitrogen cycling within the lake and its watershed appears increasingly dominated by anthropogenic nutrient sources and altered biogeochemical processing (Gauthier et al. 2021; Putt et al., 2019). Modern nutrient budgets for Cultus Lake indicate that watershed runoff and atmospheric deposition now constitute the largest sources of nitrogen to the system, with substantial contributions from agriculturally influenced groundwater and regional atmospheric emissions (Putt et al., 2019). The decoupling of $\delta^{15}\text{N}$ from Pacific salmon returns therefore reflects a broader reorganization of nitrogen cycling within the lake ecosystem. Under pre-industrial conditions, marine-derived nutrients transported by spawning salmon likely represented a significant driver of nitrogen delivery to the lake, linking salmon population dynamics to regional climate variability. In contrast, the modern nitrogen cycle of Cultus Lake is increasingly governed by anthropogenic nutrient inputs and watershed processes, which obscure the climatic signal previously expressed through salmon-mediated nutrient transfer.

Taken together, these results indicate that the ecological linkage between Pacific salmon dynamics, regional climate variability, and lake nutrient cycling persisted for several centuries but has been progressively disrupted during the modern era. The sedimentary $\delta^{15}\text{N}$ record therefore captures a transition from a climate-regulated nutrient regime to one increasingly dominated by anthropogenic forcing.

4.5 Transferability of the Epilimnetic Calibration to the Sediment Record

The principal component analysis demonstrated that the sedimentary brGDGT assemblages preserved in the Cultus Lake core are compositionally distinct from most modern epilimnetic and watershed samples (Figure 5.7). This finding has important implications for interpreting the downcore temperature reconstructions because the lake-specific calibrations were developed using modern epilimnetic brGDGT distributions but subsequently applied to sedimentary archives (Zhao et al., 2021). Consequently, application of these calibrations assumes that the sedimentary assemblages preserve the same temperature-sensitive compositional relationships represented in the modern calibration dataset.

The PCA indicates that this assumption may not be universally valid. Most sediment core samples formed a compact cluster separated from the majority of modern epilimnetic samples,

suggesting that the buried brGDGT assemblage differs systematically from that observed in the contemporary water column. Such compositional differences provide an explanation for why several calibration-derived temperatures underestimated the expected thermal range of Cultus Lake. When the sedimentary fractional abundance distributions differ from those represented in the calibration dataset, calibration equations may attribute compositional differences arising from non-temperature processes to changes in temperature, thereby producing biased absolute temperature estimates (Zhao et al., 2021).

Several mechanisms could contribute to this compositional divergence. First, the modern calibration dataset represents only a limited seasonal sampling period (July–December) and therefore may not capture the complete annual cycle of brGDGT production. In contrast, sediments integrate material produced throughout the year, including periods during winter and spring overturn when microbial communities and environmental conditions may differ substantially from those observed during the sampling period. Consequently, the annually integrated sedimentary signal may not be fully represented by the modern epilimnetic calibration dataset.

Second, the calibration was developed primarily from epilimnetic suspended particulate material, whereas brGDGT production may also occur within the thermocline, hypolimnion, sediment-water interface, or sediments themselves. Material produced within these environments may contribute disproportionately to the buried sedimentary assemblage while remaining poorly represented in the modern calibration dataset. The compact clustering of sediment samples observed in the PCA is consistent with a relatively stable sediment-associated compositional signature, although direct production measurements or compound-specific flux data would be required to determine the relative importance of benthic production.

The PCA loading structure further supports compositional differences between modern and sedimentary assemblages. Positive PC1 scores were associated primarily with Ia, Ib, IIa, and IIa', whereas negative PC1 scores were associated with IIIb, IIIb', IIIc, and IIIc'. Because the sediment core samples were concentrated predominantly at negative PC1 scores, the buried assemblage was characterized by relatively greater contributions of IIIb- and IIIc-series compounds than most modern soil and epilimnetic samples. These differences likely reflect changes in microbial community composition, production environment, or post-depositional integration prior to burial.

Despite these compositional differences, the downcore reconstructions exhibited coherent relationships with instrumental temperature, PDO, ENSO, $\delta^{15}\text{N}$, and independent regional paleoclimate records. These agreements indicate that the sedimentary brGDGT record retains a meaningful climate-related signal. However, the PCA demonstrates that the sedimentary assemblage is not compositionally identical to the modern epilimnetic calibration dataset. Consequently, the reconstructed values should be interpreted primarily as epilimnetic calibration-derived temperature estimates that robustly capture relative temporal variability and climatic trends, whereas their absolute magnitudes, particularly values falling outside the expected thermal range of Cultus Lake should be interpreted with appropriate caution.

5.0 Conclusion

This study demonstrates that sedimentary brGDGTs from Cultus Lake preserve a coherent record of regional climate variability driven by large-scale Pacific climate systems. Instrumental observations confirm that mean annual air temperature (MAAT) at Cultus Lake is strongly influenced by both the Pacific Decadal Oscillation (PDO) and the El Niño-Southern Oscillation (ENSO), with the strongest coupling occurring during the cold season when atmospheric teleconnections exert the greatest control on regional climate. This climatic framework provides a mechanistic basis for interpreting the sedimentary brGDGT record and evaluating its sensitivity to both interannual and decadal-scale Pacific variability. Individual brGDGT compounds exhibit strongly compound-specific responses to temperature and PDO phases. Among these, brGDGT-IIIa emerges as the most robust MAAT-sensitive structure in Cultus Lake sediments, highlighting that temperature information within the brGDGT pool is selectively preserved rather than uniformly distributed across molecular classes. This compound-level variability underpins the development of lake-specific temperature calibrations that capture site-specific environmental controls more effectively than global transfer functions. Application of ten lake-specific calibrations to Cultus Lake sediments reveals pronounced multi-centennial temperature variability since ~1400 CE, characterized by relatively warm conditions during portions of the fifteenth and sixteenth centuries, cooler intervals during the seventeenth and eighteenth centuries, and a gradual warming trend beginning in the late nineteenth century. These reconstructed trends closely align with independent regional proxies, including tree-ring chronologies and the soluble sodium (Na^+) from the Mount Logan ice core record, indicating that

the brGDGT-derived temperature signal reflects regionally coherent North Pacific climate dynamics rather than localized limnological variability. Superimposed on this centennial variability, selected lake-specific calibrations resolve decadal PDO regime structure, particularly during the early twentieth-century transition (1924-1945 warm vs. 1945-1976 cool). The strongest regime differentiation occurs in calibrations that integrate complementary brGDGT structures, demonstrating that PDO sensitivity emerges through compound interactions within calibration frameworks rather than through individual molecules alone. In contrast, previously published global and regional brGDGT calibrations applied to the Cultus Lake record do not reproduce robust PDO phase structure from the Cultus Lake sediment record, underscoring the importance of site-specific calibration development for reconstructing decadal climate variability in lacustrine systems.

The sedimentary $\delta^{15}\text{N}$ record provides an ecological perspective that complements the climatic reconstructions. Prior to the late nineteenth century, $\delta^{15}\text{N}$ variability broadly parallels reconstructed temperature and regional proxy records, suggesting that marine-derived nitrogen inputs associated with Pacific salmon were indirectly influenced by basin-scale climate variability. Beginning in the late nineteenth century, however, $\delta^{15}\text{N}$ values exhibit a sustained upward shift coincident with increased watershed disturbance and anthropogenic nutrient loading in the Cultus Lake catchment. During the late twentieth century, declining salmon escapement became decoupled from $\delta^{15}\text{N}$ values, indicating that nitrogen cycling within the lake is increasingly dominated by anthropogenic inputs rather than marine-derived nutrient subsidies. Together, these findings illustrate that sedimentary brGDGTs from Cultus Lake record climate variability across multiple temporal scales, from centennial fluctuations associated with North Pacific climate regimes to decadal PDO phase transitions. At the same time, the $\delta^{15}\text{N}$ record reveals a fundamental ecological transition in which anthropogenic nutrient enrichment has progressively obscured the natural linkage between climate variability, salmon-derived nutrients, and lake biogeochemistry. The integration of lipid biomarkers, isotopic records, and independent regional climate proxies therefore provides a powerful framework for reconstructing both climatic and ecological change in Pacific Northwest Lake systems over the past six centuries.

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Conflicts of Interest

The authors declare no conflict of interest.

Open Research/Data Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Information

North Pacific Climate Variability Inferred from a 600-Year Sedimentary Lipid Biomarker Record and Ecosystem Responses in Cultus Lake, British Columbia, Canada

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Table S5.1 Weather stations' data showing distance from Cultus Lake, temporal coverage after applying a minimum completeness criterion (≥ 9 months per year), and inclusion in inter-station correlation analysis. Stations with insufficient coverage or variance were excluded from correlation statistics but retained for descriptive comparison and gap-filling in the compiled temperature dataset.

Station	Distance to Cultus Lake (km)	Years with valid annual mean	First valid year	Last valid year	Used in correlation
Cultus A	0.0	43	1946	1994	Yes
Cultus B	0.0	6	2019	2024	No
Cultus L	3.0	6	1982	1988	No
Chilliwack	3.1	2	2020	2024	No
Sumas Canal	10.3	42	1959	2022	Yes
Mission WA	22.8	61	1963	2023	Yes
Agassiz CDA	24.2	125	1892	2017	Yes

Table S5.2 Inter-station correlations of mean annual air temperature among valid weather stations near Cultus Lake.

Station 1	Station 2	Pearson r	p-value	Overlapping years (n)
Agassiz CDA	Mission WA	0.885	2.79×10^{-19}	55
Mission WA	Sumas Canal	0.882	2.52×10^{-13}	38
Agassiz CDA	Cultus A	0.868	4.67×10^{-14}	43
Cultus A	Mission WA	0.866	6.14×10^{-10}	30
Agassiz CDA	Sumas Canal	0.841	6.94×10^{-11}	37
Cultus A	Sumas Canal	0.731	2.96×10^{-3}	14

Table S5.3 Block-bootstrap Pearson correlation coefficients (r) and 95% confidence intervals (CI) between down-core brGDGT c-series fractional abundances and mean annual air temperature (MAAT, °C) at Cultus Lake. Correlations are based on GAM-smoothed sediment records aligned to annual MAAT. Confidence intervals excluding zero indicate statistically significant relationships and are marked with asterisks (*).

Compound	r (MAAT)	95% CI
BrGDGT-Ic	-0.15	[-0.38, 0.23]

Compound	r (MAAT)	95% CI
BrGDGT-IIc	-0.01	[-0.48, 0.61]
BrGDGT-IIc'	0.49	[-0.14, 0.63]
BrGDGT-IIIc	0.27	[-0.08, 0.52]
BrGDGT-IIIc'	-0.12	[-0.08, -0.47]

Moving-block bootstrap, B = 2000, block length = 10.

Table S5.4 Phase-resolved fractional abundances of brGDGT-Ia, IIa, IIa', IIIa, and IIIa' across Pacific Decadal Oscillation (PDO) regimes (1924-1945 warm; 1945-1976 cool; 1976-2006 warm; 2006-2022 cool). Values represent regime-averaged fractional abundances for each compound, together with the change (Δ ; Warm - Cool) relative to the preceding PDO phase. Uncertainty was estimated using moving block bootstrap resampling (95% CI). Standardized

effect sizes are expressed as Δ divided by pooled SD. q-values reflect Benjamini-Hochberg FDR correction across all comparisons; * indicates FDR-significant contrasts (CI excludes zero).

Compound	PDO Contrast	Δ (Warm – Cool)	95% CI	n Warm	n Cool	Std. Effect Size	q-value	Phase Direction
BrGDGT-Ia	1924–1945 vs 1945–1976	-0.0051	[-0.0448, 0.0368]	2	4	-0.19	0.931	Higher in Cool
	1976–2006 vs 2006–2022	-0.0121	[-0.0338, 0.0058]	4	6	-0.62	0.732	Higher in Cool
BrGDGT-IIa	1924–1945 vs 1945–1976	0.0307	[-0.0045, 0.0620]	2	4	1.13	0.524	Higher in Warm
	1976–2006 vs 2006–2022	0.0160	[-0.0730, 0.0720]	4	6	0.30	0.931	Higher in Warm
BrGDGT-IIa'	1924–1945 vs 1945–1976	-0.0491	[-0.0609, -0.0344] *	2	4	-3.09	<0.001	Higher in Cool
	1976–2006 vs 2006–2022	-0.0329	[-0.0950, 0.0076]	4	6	-0.93	0.524	Higher in Cool
BrGDGT-IIIa	1924–1945 vs 1945–1976	0.0112	[-0.0536, 0.0740]	2	4	0.27	0.926	Higher in Warm
	1976–2006 vs 2006–2022	0.0468	[-0.0405, 0.1794]	4	6	0.58	0.897	Higher in Warm
BrGDGT-IIIa'	1924–1945 vs 1945–1976	-0.0129	[-0.0481, 0.0341]	2	4	-0.30	0.926	Higher in Cool
	1976–2006 vs 2006–2022	-0.0337	[-0.1210, 0.0519]	4	6	-0.53	0.897	Higher in Cool

Moving-block bootstrap, B = 5000, block length = 2.

Table S5.5 Phase-resolved fractional abundances of brGDGT-Ib, IIb, IIb', IIIb, and IIIb' across Pacific Decadal Oscillation (PDO) regimes (1924-1945 warm; 1945-1976 cool; 1976-2006 warm; 2006-2022 cool). Values represent regime-averaged fractional abundances for each compound, together with the change (Δ ; Warm - Cool) relative to the preceding PDO phase. Uncertainty was estimated using moving block bootstrap resampling (95% CI). Standardized

effect sizes are expressed as Δ divided by pooled SD. q-values reflect Benjamini-Hochberg FDR correction across all comparisons; * indicates FDR-significant contrasts (CI excludes zero).

Compound	PDO Contrast	Δ (Warm – Cool)	95% CI	n Warm	n Cool	Std. Effect Size	q-value	Phase Direction
BrGDGT-Ib	1924–1945 vs 1945–1976	0.0039	[0.0003, 0.0075] *	2	4	1.62	<0.001	Higher in Warm
	1976–2006 vs 2006–2022	0.0049	[-0.0061, 0.0265]	4	6	0.46	0.897	Higher in Warm
BrGDGT-IIb	1924–1945 vs 1945–1976	0.0038	[-0.0237, 0.0214]	2	4	0.14	0.959	Higher in Warm
	1976–2006 vs 2006–2022	-0.0106	[-0.0665, 0.0548]	4	6	-0.26	0.931	Higher in Cool
BrGDGT-IIb'	1924–1945 vs 1945–1976	-0.0003	[-0.0119, 0.0130]	2	4	-0.03	0.997	Higher in Cool
	1976–2006 vs 2006–2022	0.0091	[-0.0016, 0.0190]	4	6	0.84	0.524	Higher in Warm
BrGDGT-IIIb	1924–1945 vs 1945–1976	0.0002	[-0.0040, 0.0057]	2	4	0.04	0.984	Higher in Warm
	1976–2006 vs 2006–2022	0.0023	[-0.0067, 0.0150]	4	6	0.27	0.926	Higher in Warm
BrGDGT-IIIb'	1924–1945 vs 1945–1976	-0.0027	[-0.0088, 0.0036]	2	4	-0.61	0.897	Higher in Cool
	1976–2006 vs 2006–2022	0.0028	[-0.0010, 0.0096]	4	6	0.72	0.718	Higher in Warm

Moving-block bootstrap, B = 5000, block length = 2

Table S5.6 Phase-resolved fractional abundances of brGDGT-Ic, IIc, IIc', IIIc, and IIIc' across Pacific Decadal Oscillation (PDO) regimes (1924-1945 warm; 1945-1976 cool; 1976-2006 warm; 2006-2022 cool). Values represent regime-averaged fractional abundances for each compound, together with the change (Δ ; Warm - Cool) relative to the preceding PDO phase. Uncertainty was estimated using moving block bootstrap resampling (95% CI). Standardized

effect sizes are expressed as Δ divided by pooled SD. q-values reflect Benjamini-Hochberg FDR correction across all comparisons; * indicates FDR-significant contrasts (CI excludes zero).

Compound	PDO Contrast	Δ (Warm – Cool)	95% CI	n Warm	n Cool	Std. Effect Size	q-value	Phase Direction
BrGDGT-Ic	1924–1945 vs 1945–1976	0.0105	[-0.0005, 0.0210]	2	4	1.51	0.524	Higher in Warm
	1976–2006 vs 2006–2022	0.0049	[-0.0010, 0.0153]	4	6	0.92	0.759	Higher in Warm
BrGDGT-IIc	1924–1945 vs 1945–1976	0.0027	[0.0004, 0.0057]	2	4	1.26	0.056	Higher in Warm
	1976–2006 vs 2006–2022	0.0035	[0.0023, 0.0050] *	4	6	2.48	<0.001	Higher in Warm
BrGDGT-IIc'	1924–1945 vs 1945–1976	0.0003	[-0.0018, 0.0026]	2	4	0.20	0.931	Higher in Warm
	1976–2006 vs 2006–2022	-0.0007	[-0.0052, 0.0037]	4	6	-0.22	0.931	Higher in Cool
BrGDGT-IIIc	1924–1945 vs 1945–1976	0.0034	[0.0002, 0.0060]	2	4	1.81	0.279	Higher in Warm
	1976–2006 vs 2006–2022	0.0001	[-0.0019, 0.0018]	4	6	0.03	0.959	Higher in Warm
BrGDGT-IIIc'	1924–1945 vs 1945–1976	0.0033	[0.0013, 0.0052] *	2	4	2.45	<0.001	Higher in Warm
	1976–2006 vs 2006–2022	-0.0002	[-0.0018, 0.0010]	4	6	-0.21	0.931	Higher in Cool

Moving-block bootstrap, B = 5000, block length = 2.

Table S5.7 PDO regime differences in calibration-derived temperature reconstructions from Cultus Lake sediments. Values represent mean differences (Δ = Warm - Cool) between sequential PDO phases (1924-1945 vs 1945-1976; 1976-2006 vs 2006-2022). Uncertainty was estimated using moving block bootstrap resampling (95% CI). Standardized effect sizes are Δ divided by pooled SD. q-values reflect Benjamini-Hochberg FDR correction across all tests; * indicates FDR-significant contrasts (CI excludes zero).

Compound	PDO Contrast	Δ (Warm – Cool)	95% CI	n Warm	n Cool	Std. Effect Size	q-value	Phase Direction
Cal ₁	1924–1945 vs 1945–1976	-2.654	[-4.164, -0.911] *	2	4	-1.95	<0.001	Higher in Cool
	1976–2006 vs 2006–2022	-1.273	[-6.076, 4.404]	4	6	-0.35	0.926	Higher in Cool
Cal ₂	1924–1945 vs 1945–1976	0.829	[-1.224, 3.032]	2	4	0.51	0.897	Higher in Warm
	1976–2006 vs 2006–2022	2.110	[-0.983, 7.056]	4	6	0.85	0.806	Higher in Warm
Cal ₃	1924–1945 vs 1945–1976	0.427	[-1.804, 2.717]	2	4	0.29	0.931	Higher in Warm
	1976–2006 vs 2006–2022	-0.306	[-3.904, 5.215]	4	6	-0.11	0.959	Higher in Cool
Cal ₄	1924–1945 vs 1945–1976	1.047	[0.165, 1.618] *	2	4	1.00	<0.001	Higher in Warm
	1976–2006 vs 2006–2022	1.768	[-2.126, 7.265]	4	6	0.57	0.897	Higher in Warm
Cal ₅	1924–1945 vs 1945–1976	-2.270	[-5.165, 0.668]	2	4	-1.10	0.897	Higher in Cool
	1976–2006 vs 2006–2022	-3.298	[-11.445, 1.708]	4	6	-0.72	0.826	Higher in Cool
Cal ₆	1924–1945 vs 1945–1976	0.213	[-2.890, 2.925]	2	4	0.07	0.997	Higher in Warm
	1976–2006 vs 2006–2022	-0.405	[-9.543, 6.783]	4	6	-0.09	0.971	Higher in Cool
Cal ₇	1924–1945 vs 1945–1976	-0.526	[-3.997, 3.054]	2	4	-0.22	0.931	Higher in Cool
	1976–2006 vs 2006–2022	-1.518	[-7.198, 2.453]	4	6	-0.45	0.897	Higher in Cool
Cal ₈	1924–1945 vs 1945–1976	-0.760	[-3.048, 1.044]	2	4	-0.33	0.897	Higher in Cool
	1976–2006 vs 2006–2022	0.822	[-4.389, 5.856]	4	6	0.23	0.931	Higher in Warm
Cal ₉	1924–1945 vs 1945–1976	-0.061	[-1.205, 1.244]	2	4	-0.06	0.997	Higher in Cool
	1976–2006 vs 2006–2022	0.871	[-0.182, 1.834]	4	6	0.82	0.524	Higher in Warm
Cal ₁₀	1924–1945 vs 1945–1976	0.670	[-1.305, 2.099]	2	4	0.32	0.897	Higher in Warm
	1976–2006 vs 2006–2022	2.155	[-2.718, 7.116]	4	6	0.59	0.897	Higher in Warm
MBT _{SME} Cal	1924–1945 vs 1945–1976	0.582	[-4.821, 6.212]	2	4	0.16	0.959	Higher in Warm
	1976–2006 vs 2006–2022	-1.052	[-2.683, 0.987]	4	6	-0.57	0.897	Higher in Cool

Moving-block bootstrap, B = 5000, block length = 2.

Table S5.8 PDO regime differences in published Mean Annual Air Temperature (MAAT) and Month Above Freezing (MAF) brGDGT-based temperature reconstructions applied to Cultus Lake sediments. Values represent mean differences (Δ = Warm - Cool) between sequential PDO phases (1924 -1945 vs 1945 -1976; 1976 - 2006 vs 2006 - 2022). Uncertainty was estimated using moving block bootstrap resampling (95% CI). Standardized effect sizes are Δ divided by pooled SD. q-values reflect Benjamini-Hochberg false discovery rate (FDR) correction across all comparisons.

Variable	PDO Contrast	Δ (Warm – Cool)	95% CI	n Warm	n Cool	Std. Effect Size	q-value	Direction
MAAT Bauersachs et al. (2024)	1924–1945 vs 1945–1976	0.115	[-0.976, 1.236]	2	4	0.16	0.959	Higher in Warm
	1976–2006 vs 2006–2022	-0.305	[-2.124, 2.028]	4	6	-0.23	0.931	Higher in Cool
MAF Bauersachs et al. (2024)	1924–1945 vs 1945–1976	-0.082	[-0.727, 0.597]	2	4	-0.19	0.931	Higher in Cool
	1976–2006 vs 2006–2022	-0.197	[-0.548, 0.094]	4	6	-0.62	0.732	Higher in Cool
MAAT (Index1) DeJong et al. (2014)	1924–1945 vs 1945–1976	-5.384	[-12.803, 2.583]	2	4	-1.03	0.897	Higher in Cool
	1976–2006 vs 2006–2022	-5.017	[-11.190, -0.934]	4	6	-1.19	0.113	Higher in Cool
MAAT Russel et al. (2018)	1924–1945 vs 1945–1976	-0.978	[-3.877, 2.309]	2	4	-0.44	0.897	Higher in Cool
	1976–2006 vs 2006–2022	-1.538	[-3.562, 0.229]	4	6	-0.88	0.524	Higher in Cool
MAF Otiniano et al. (2023)	1924–1945 vs 1945–1976	-0.149	[-0.983, 0.713]	2	4	-0.27	0.926	Higher in Cool
	1976–2006 vs 2006–2022	-0.622	[-2.384, 0.538]	4	6	-0.58	0.897	Higher in Cool
MAF (Fullset) Raberg et al. (2021)	1924–1945 vs 1945–1976	-0.342	[-1.909, 1.403]	2	4	-0.28	0.931	Higher in Cool
	1976–2006 vs 2006–2022	-0.278	[-1.449, 1.653]	4	6	-0.24	0.931	Higher in Cool
MAF (Methset) Raberg et al. (2021)	1924–1945 vs 1945–1976	-5.919	[-16.945, 7.181]	2	4	-0.60	0.897	Higher in Cool
	1976–2006 vs 2006–2022	4.614	[-8.698, 13.013]	4	6	0.43	0.897	Higher in Warm

Moving-block bootstrap, B = 5000, block length = 2.

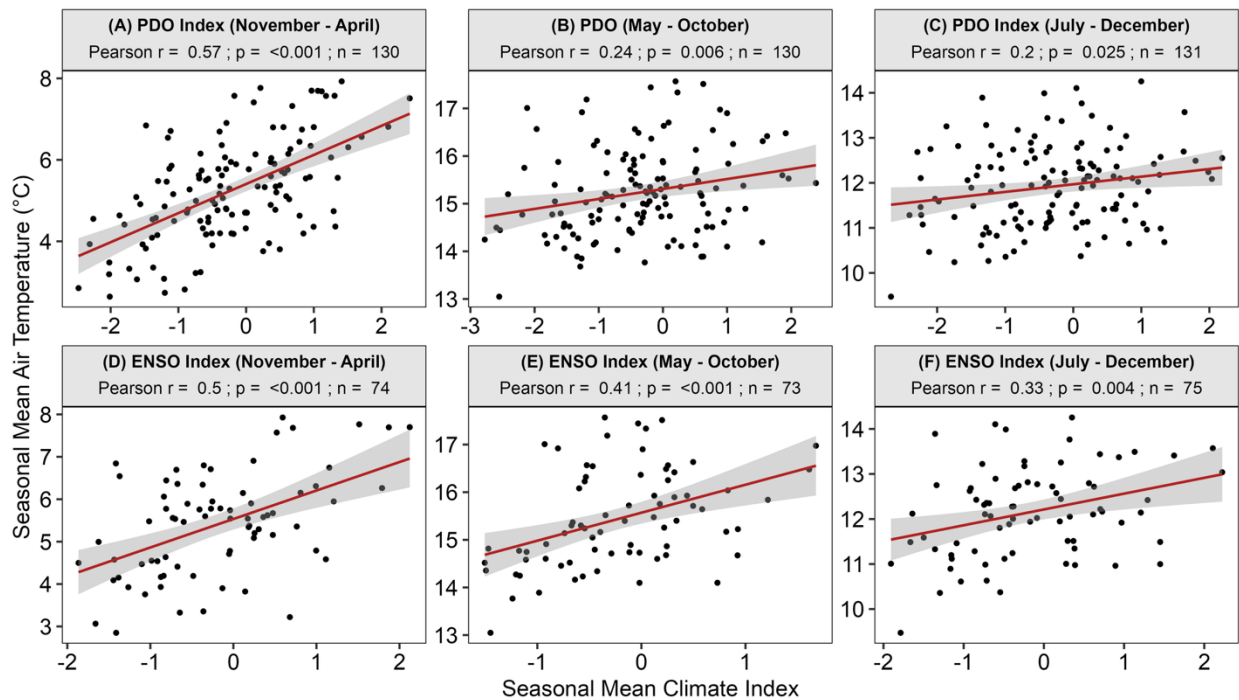


Figure S5.1 Relationships between seasonal mean climate indices and seasonal mean air temperature at Cultus Lake. Panels A-C show the relationship between the seasonal Pacific Decadal Oscillation (PDO) index and seasonal mean air temperature, whereas Panels D-E show the relationship between the seasonal El Niño-Southern Oscillation (ENSO) index and seasonal mean air temperature.

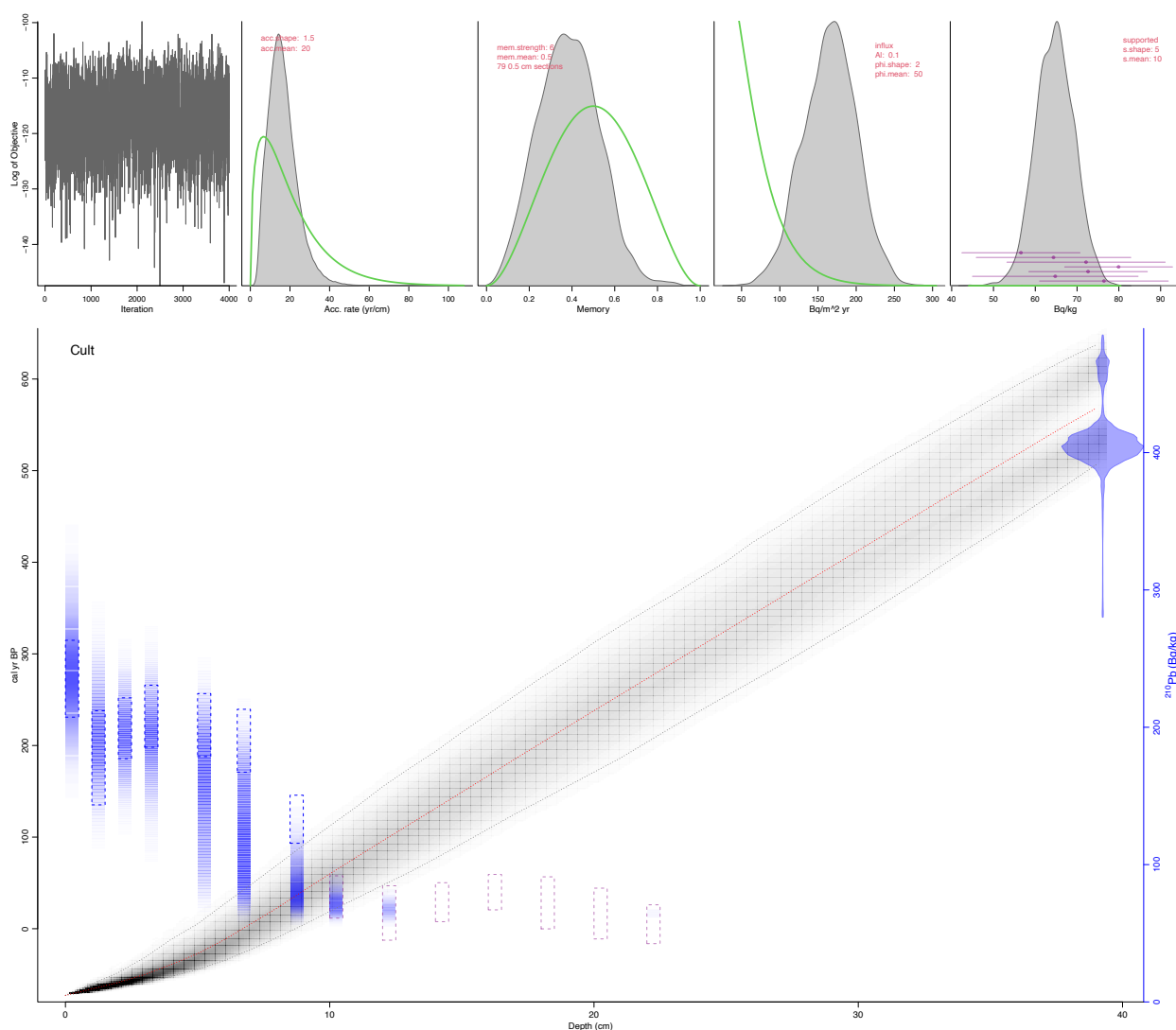


Figure S5.2 Bayesian age-depth model for the Cultus Lake sediment core constructed using the rPlum/Bacon framework. Upper panels displayed Markov Chain Monte Carlo (MCMC) diagnostics and posterior distributions for model parameters, including (from left to right) iteration stability, sediment accumulation rate (yr cm^{-1}), memory (temporal autocorrelation in accumulation rates), and accumulation variability. Green curves denote prior distributions, while grey histograms represent posterior distributions following model updating. The lower panel

showed the final age-depth relationship (black line) with 95% confidence intervals (grey shading). The red dashed line indicates the weighted mean model estimate. The resulting chronology provided a continuous, age framework spanning approximately the last 800-900 years, integrating high-resolution ^{210}Pb constraints in the upper sediments with radiocarbon control at depth.

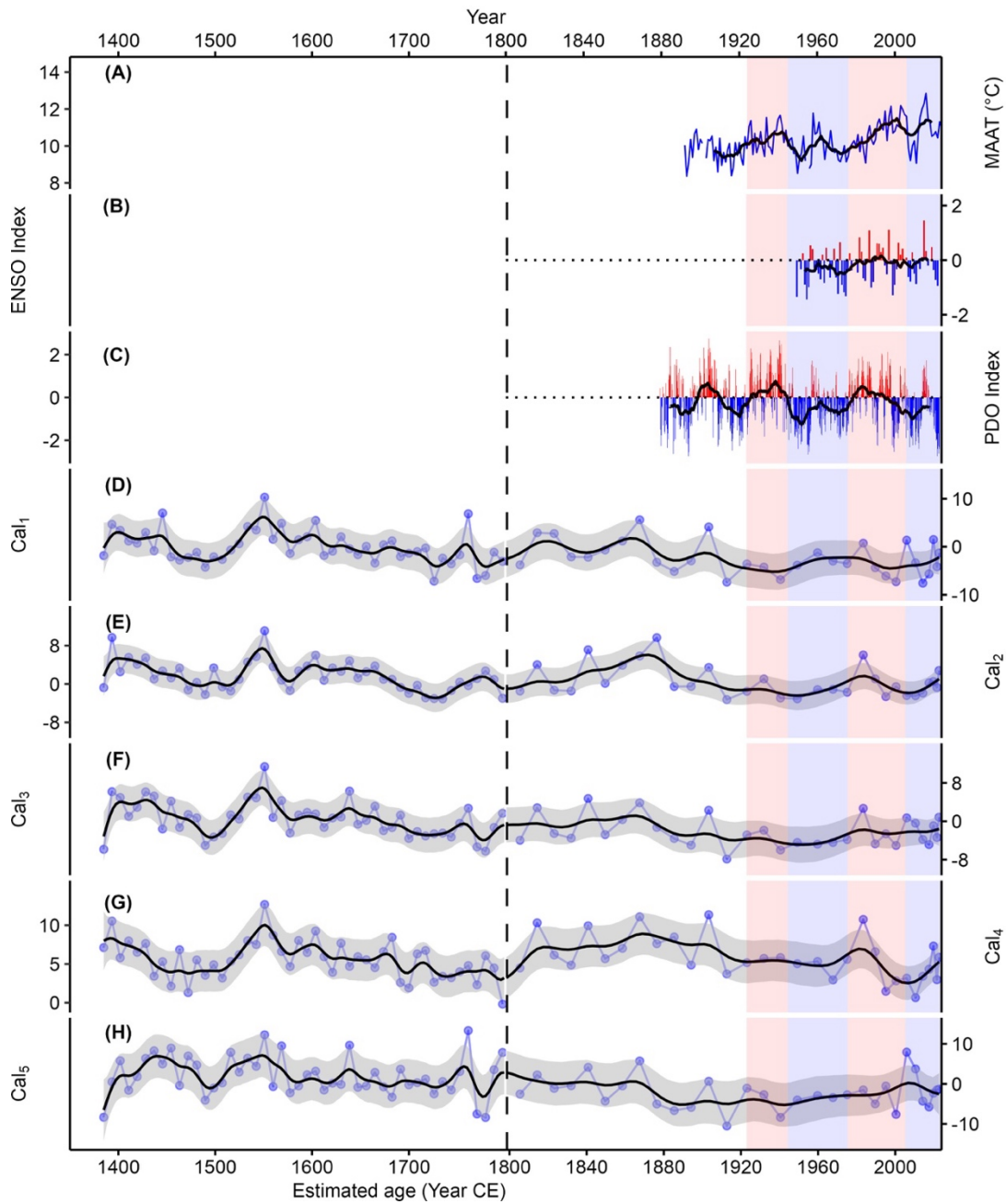


Figure S5.3 Downcore dynamics of Cultus Lake calibration-derived temperatures ($Cal_1 - Cal_5$) compared with mean air annual temperature (MAAT), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent raw fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

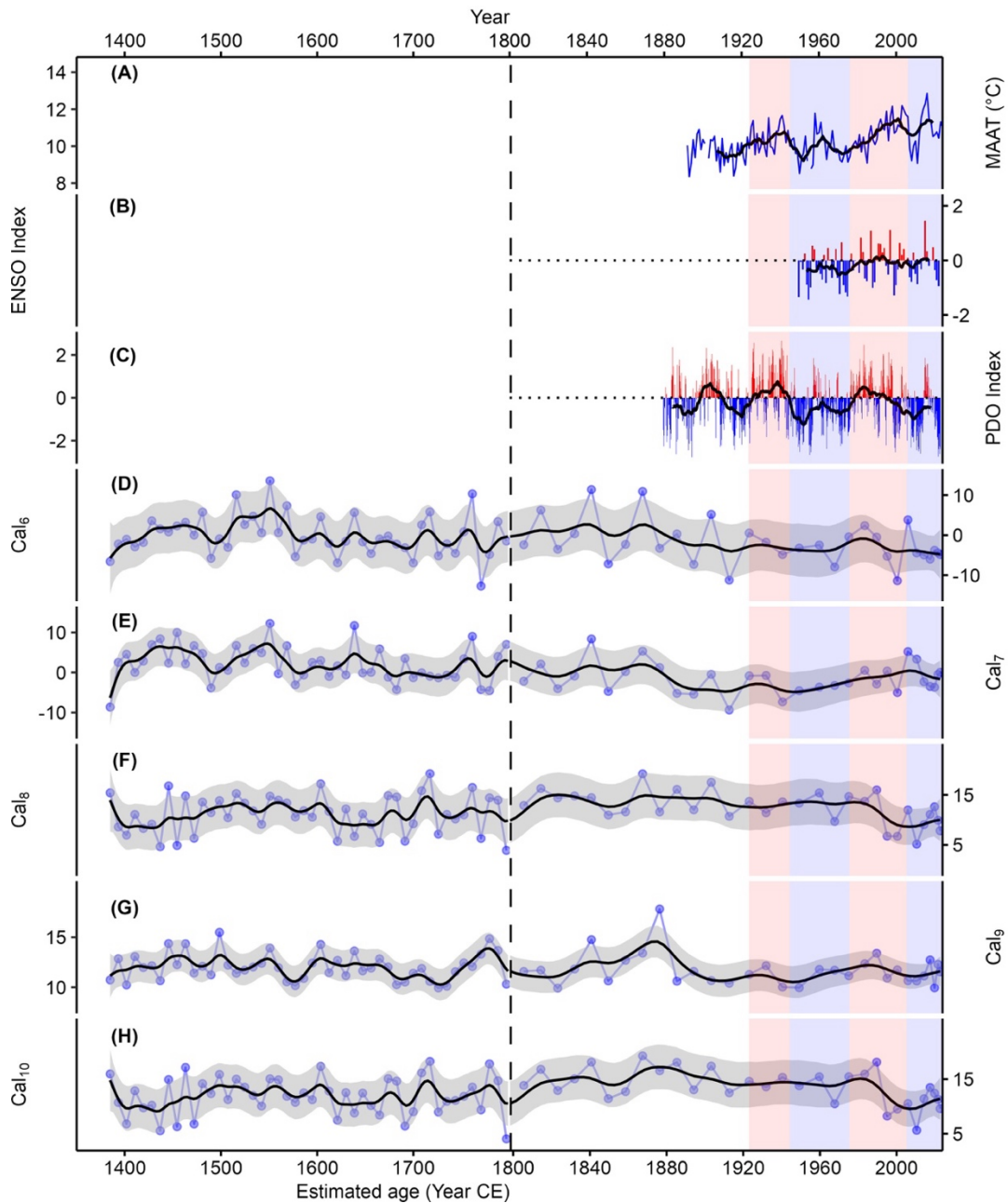


Figure S5.4 Downcore dynamics of Cultus Lake calibration-derived temperatures ($Cal_6 - Cal_{10}$) compared with mean air annual temperature (MAAT), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent raw fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

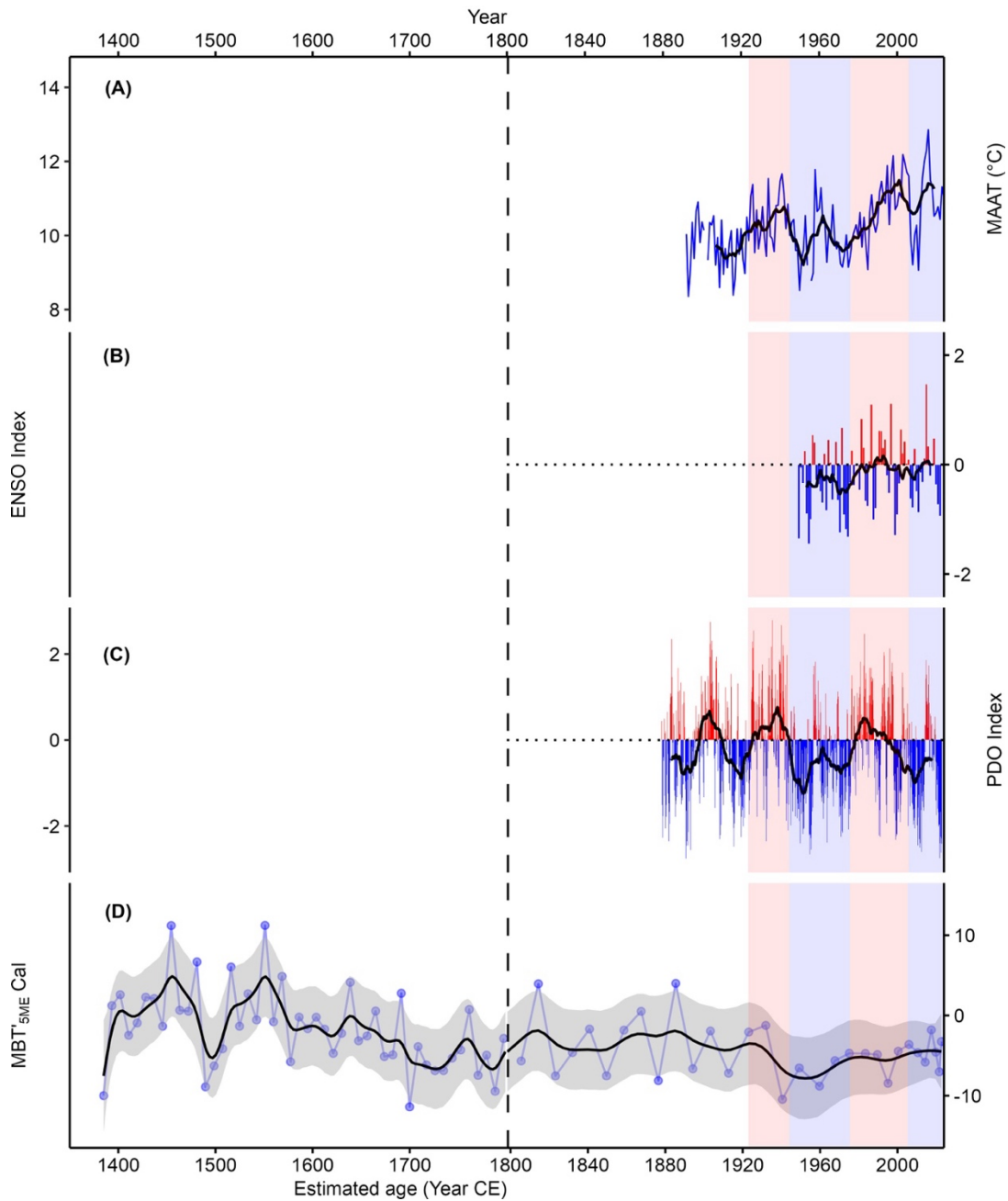


Figure S5.5 Downcore dynamics of Cultus Lake MBT'_{5ME} calibration-derived temperatures compared with mean air annual temperature (MAAT), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent raw fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

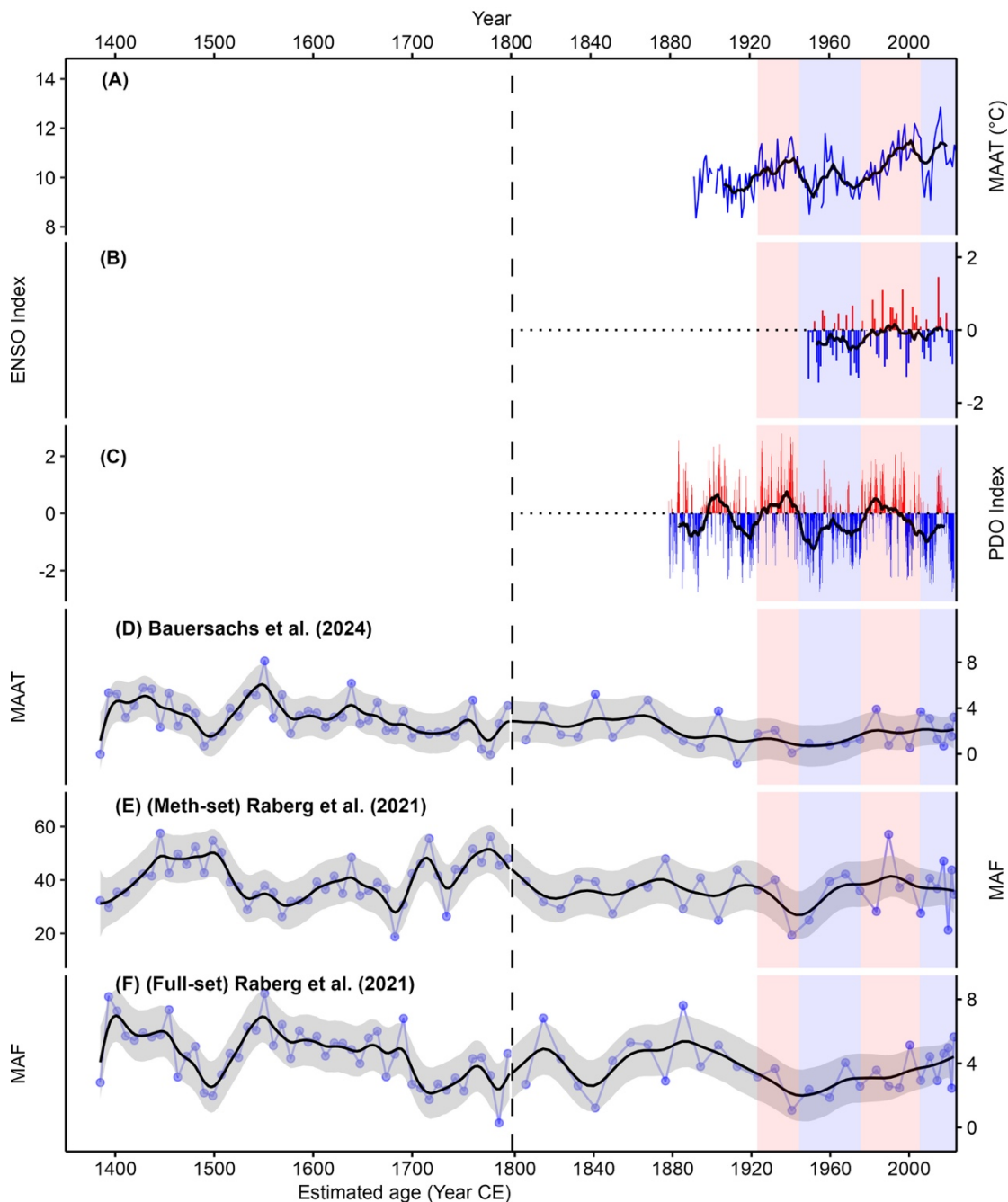


Figure S5.6 Downcore dynamics of temperature estimates from published MAAT and MAF calibration compared with mean air annual temperature (MAAT), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent raw fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on expanded scale to highlight recent variability.

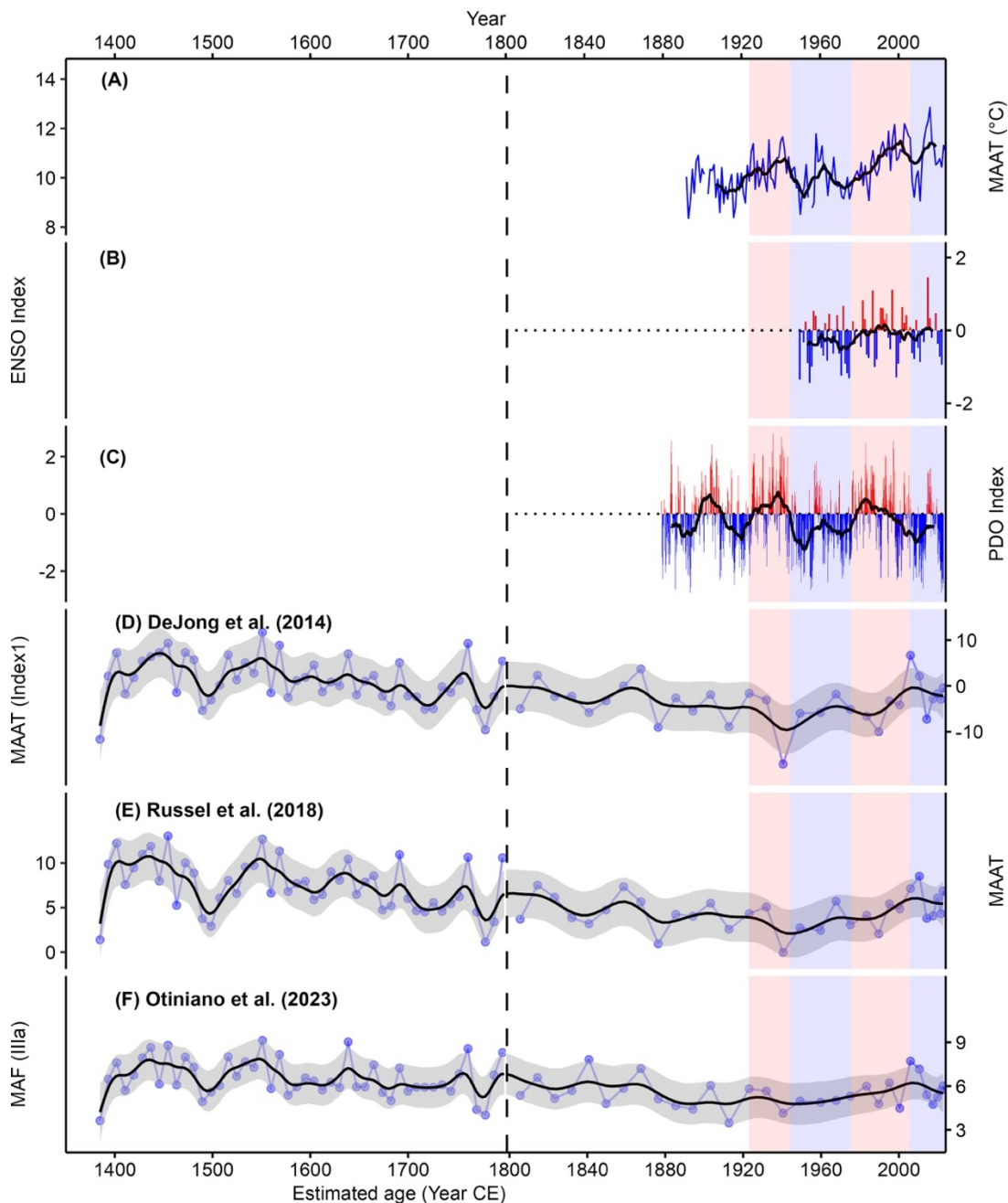


Figure S5.7 Downcore dynamics of temperature estimates from published MAAT and MAF calibration compared with mean air annual temperature (MAAT), El Niño Southern Oscillation index (ENSO), and Pacific Decadal Oscillation (PDO). Blue points represent raw fractional abundances, black lines show the generalized additive model (GAM) smooth, and gray shaded bands denote the 95% confidence interval. Red and blue vertical bars mark warm and cool PDO phases, respectively. The post 1800 CE interval is displayed on an expanded scale to highlight recent variability.

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6 CHAPTER 6: General conclusions and implications

STUDY OBJECTIVES AND IMPLICATIONS

Chapter 2

This chapter reconstructed the long-term population dynamics of double-crested cormorants and evaluated their ecosystem-scale impacts on island pond environments in eastern Lake Ontario using sedimentary geochemical and molecular biomarkers. By integrating sterol and stanol proxies with stable nitrogen isotopes in dated sediment cores from impacted (EBI) and reference ponds (CI), the study assessed the timing of colony establishment, quantified guano-driven nutrient enrichment, and examined associated shifts in sediment biogeochemistry and ecosystem productivity within a broader twentieth-century environmental context. The results of this chapter demonstrate that sedimentary biomarker approaches provide a robust and mechanistically interpretable archive of colonial waterbird population dynamics and their ecosystem consequences. The concordant increases in bird-derived sterols, stanols, and $\delta^{15}\text{N}$ values establish a geochemical signature of cormorant colonization and sustained guano input, enabling long-term reconstruction of population trajectories. Importantly, this study shows that cormorants function as powerful biovectors capable of fundamentally restructuring nutrient cycling and ecosystem functioning in small-island pond systems. The observed transition from nutrient enrichment and enhanced productivity to ecological degradation highlights the nonlinear and potentially threshold-driven nature of cormorant impacts, where prolonged nutrient loading leads to vegetation loss, altered organic matter inputs, and habitat deterioration. By placing these changes within a historical framework, the findings further suggest that recent cormorant population expansion is closely linked to broader anthropogenic drivers, including contaminant recovery and food-web restructuring in the Great Lakes. This provides critical context for ongoing management debates, indicating that current colony sizes likely represent a return toward pre-disturbance conditions rather than purely novel expansion. Overall, this chapter

underscores the value of paleolimnological approaches for establishing ecological baselines, disentangling natural variability from anthropogenic change, and informing evidence-based management of colonial waterbird populations and their ecosystem impacts.

Chapter 3

Chapter 3 extended the biomarker-based paleolimnological framework established in Chapter 2 to reconstruct sockeye salmon population dynamics and associated marine-derived nutrient inputs to freshwater ecosystems. Using sedimentary sterols, stanols, and stable nitrogen isotopes ($\delta^{15}\text{N}$) from lakes with well-documented escapement histories, this study evaluated the capacity of lipid biomarkers to serve as an independent quantitative proxy of salmon abundance. By integrating biomarker measurements with historical fisheries datasets, this chapter (i) quantified the strength and consistency of relationships between sedimentary sterols and salmon escapement, (ii) developed and validated a novel multivariate salmon sterol index (SSI), (iii) reconstructed temporal variability in salmon populations over the period of available monitoring records, and (iv) assessed the extent to which biomarker signals capture the influence of large-scale climate variability, including the Pacific Decadal Oscillation, on salmon production. The results demonstrate that sedimentary sterols and stanols derived from sockeye salmon nursery lake sediments provide a robust and quantitatively validated archive of sockeye salmon population dynamics. The strong positive relationship between cholesterol (i.e. the dominant sterol in salmon tissue) concentrations in surface sediments and salmon escapement, together with the high predictive performance of SSI_a , establishes lipid biomarkers as reliable proxies of marine-derived nutrient delivery to freshwater ecosystems. Critically, this study shows that biomarker signals resolve both direct and indirect ecological pathways of salmon influence. Cholesterol and its degradation products record direct inputs from salmon biomass, whereas the positive relationships observed for algal (fucosterol) and plant-derived sterols (sitosterol) reflect secondary ecosystem responses driven by nutrient enrichment. This dual signal highlights the role of salmon as biovectors that not only transport marine nutrients but also stimulate in-lake primary production through bottom-up ecological processes. Although this study is grounded within the temporal extent of available monitoring records, the strong agreement between biomarker proxies and observed escapement demonstrates their reliability and establishes a validated framework that can be applied to extend reconstructions into periods lacking

observational data. Furthermore, the development and application of the SSIA underscores the strength of multivariate biomarker approaches in reducing uncertainty associated with single-proxy reconstructions. By integrating multiple sterols linked to salmon-derived inputs, this index enhances both the robustness and transferability of paleolimnological reconstructions across lake systems. Overall, this chapter advances paleolimnological methodology by establishing a mechanistically grounded and quantitatively validated framework for reconstructing anadromous fish populations from lake sediments. These findings provide a critical bridge between ecological theory, biogeochemical processes, and applied fisheries management and demonstrate the power of molecular biomarkers to resolve interactions among salmon populations, climate variability, and freshwater ecosystem dynamics.

Chapter 4

Chapter 4 developed and evaluated lake-specific temperature calibrations based on branched glycerol dialkyl glycerol tetraethers (brGDGTs) for Cultus Lake, British Columbia. While brGDGTs are widely used paleotemperature proxies, the performance of global and regional calibrations often varies among lakes due to differences in microbial production, water column structure, and sedimentary processes. This chapter, therefore, aimed to develop process-informed, lake-specific calibrations that more accurately reflect in situ environmental controls. Specifically, this study (i) characterized brGDGT distributions in suspended particulate matter, watershed inputs, and surface sediments to constrain production sources, (ii) quantified relationships between individual brGDGT fractional abundances and lake-water temperature, (iii) developed multiple linear regression calibration models based on temperature-sensitive compounds, and (iv) evaluated their performance relative to established global and regional indices, including MBT'_{5ME}-based models. The results demonstrate that brGDGT-based temperature reconstructions are strongly governed by site-specific production dynamics and compound-level responses, rather than universally transferable relationships. In Cultus Lake, temperature sensitivity was highly compound-specific, with select fractional abundances exhibiting strong linear and, in some cases, nonlinear relationships with water temperature, supporting a shift from bulk indices toward compound-resolved approaches. Locally derived calibration models substantially outperformed global and regional models, yielding higher explained variance and lower prediction error. This improvement reflects the importance of

accounting for site-specific controls, including seasonal production, microbial ecology, and water column stratification. The calibration framework developed here provides a robust foundation for subsequent downcore temperature reconstructions and enables direct comparison with independent climate drivers, including large-scale variability such as the Pacific Decadal Oscillation, as well as integrated assessments of climate-ecosystem linkages, including salmon production. Overall, this chapter demonstrates that process-based, lake-specific calibrations are essential for improving the accuracy, interpretability, and ecological relevance of brGDGT-derived temperature reconstructions, reinforcing the broader thesis principle that molecular proxies must be calibrated within their environmental and biological context to reliably reconstruct past climate and ecosystem change.

Chapter 5

Chapter 5 examines how large-scale North Pacific climate variability influences regional temperature and ecosystem processes in Cultus Lake by integrating biomarker-based paleotemperature reconstructions with ecological proxies. Building on the lake-specific brGDGT calibrations developed in Chapter 4, this chapter provides a multi-proxy assessment of climate-ecosystem linkages over the past ~600 years. Specifically, this study (i) reconstructed long-term temperature variability using downcore brGDGT-based calibrations, (ii) evaluated relationships between reconstructed temperature and major Pacific climate modes, including the Pacific Decadal Oscillation (PDO) and El Niño Southern Oscillation (ENSO), (iii) assessed how climate variability influences salmon-derived nutrient inputs using sedimentary $\delta^{15}\text{N}$ records, and (iv) examined the temporal coupling among climate variability, salmon dynamics, and anthropogenic nutrient inputs to distinguish natural from human-driven ecosystem change. The results demonstrate that sedimentary biomarker and isotopic records from Cultus Lake capture climate variability across multiple temporal scales, including centennial trends and decadal regime shifts associated with North Pacific climate dynamics. The strong correspondence between reconstructed temperatures and independent climate indices indicates that the sedimentary archive reliably records basin-scale climatic forcing. Critically, the integration of climatic and ecological proxies reveals a fundamental shift in ecosystem regulation through time. Prior to the late nineteenth century, sedimentary $\delta^{15}\text{N}$ closely tracked climate-driven variability in salmon-derived nutrient inputs, indicating strong coupling between regional climate, ocean productivity,

and freshwater nutrient dynamics of the Cultus Lake. In contrast, this relationship weakened during the twentieth century as anthropogenic nutrient inputs increasingly dominated nitrogen cycling within the lake. This decoupling reflects the growing influence of human activities, including agricultural runoff, watershed modification, and atmospheric deposition, which have altered nutrient regimes and obscured natural climate-salmon linkages. As a result, modern ecosystem dynamics cannot be interpreted solely within the context of natural climate variability. Taken together, these findings highlight the interacting controls of climate variability, biovector-driven nutrient transfer, and anthropogenic forcing on lake ecosystem dynamics. By integrating temperature reconstructions, biomarker proxies, and isotopic records, this chapter provides a mechanistic framework for understanding how freshwater ecosystems respond to both natural climate variability and human disturbance. Overall, this work demonstrates that long-term sedimentary records are essential for distinguishing natural variability from anthropogenic change and for contextualizing modern ecological conditions. These insights are critical for informing conservation and management strategies aimed at sustaining Pacific salmon populations and the freshwater ecosystems they support.

Overall conclusions and implications

This thesis demonstrates that lake ecosystems are structured by the combined influences of large-scale migratory animal-driven nutrient inputs and climate variability, both of which can be quantitatively reconstructed using molecular and isotopic proxies preserved in lake sediments. By integrating sterol and stanol biomarkers, brGDGT-based temperature reconstructions, and stable nitrogen isotopes, this work provides a mechanistic and multi-proxy framework for understanding long-term ecosystem change in freshwater systems.

Chapters 2 and 3 establish that sedimentary lipid biomarkers provide robust archives of animal-mediated nutrient transfer across ecosystem boundaries. In Chapter 2, sterol and stanol, C:N ratios, and $\delta^{15}\text{N}$ records revealed that double-crested cormorants act as biovectors, driving intense nutrient enrichment and ecosystem transformation in island pond environments. Also, Chapter 3 demonstrated that sockeye salmon function as biovectors linking marine and freshwater systems, with sedimentary biomarkers capturing both direct inputs from salmon biomass and indirect stimulation of in-lake primary production. Together, these chapters show that migratory animal-driven nutrient subsidies fundamentally regulate lake biogeochemistry and

productivity. Chapters 4 and 5 extend this framework by incorporating climate forcing as a key control on lake ecosystem dynamics. Chapter 4 demonstrated that brGDGT-based temperature reconstructions are strongly governed by site-specific production processes, and that accurate paleotemperature reconstructions require process-informed, lake-specific calibrations. In Cultus Lake, brGDGTs primarily record epilimnetic water temperature during the stratified season. Building on this calibration, Chapter 5 reconstructed long-term temperature variability and demonstrated strong coupling between regional climate dynamics and lake ecosystem processes. A central finding of this thesis is the temporal shift in ecosystem regulation from climate-driven to anthropogenically influenced conditions. Prior to the late nineteenth century, sedimentary $\delta^{15}\text{N}$ records indicate that salmon-derived nutrient inputs were closely linked to climate variability, reflecting tight coupling between ocean conditions, salmon productivity, and freshwater nutrient dynamics. However, during the twentieth century, this relationship weakened as anthropogenic nutrient inputs increasingly dominated nitrogen cycling within the lake. This transition highlights a fundamental shift from natural climate-ecosystem coupling to human-modified system dynamics.

Taken together, these findings illustrate that freshwater ecosystems are governed by interacting controls operating across multiple scales: (i) climate variability, which regulates temperature and large-scale ecosystem processes, (ii) large-scale migratory animal-mediated nutrient transfer, which links ecosystems and drives productivity, and (iii) anthropogenic disturbance, which can override natural system dynamics. By resolving these controls within a unified sedimentary archive, this thesis demonstrates the power of paleolimnological approaches to disentangle complex ecological drivers through time. Beyond advancing fundamental understanding, this work has important implications for environmental management and conservation. The ability to reconstruct long-term baselines provides critical context for evaluating contemporary ecological change, particularly for systems experiencing rapid climate warming and increasing human pressure. For Pacific salmon, these results emphasize that population dynamics and ecosystem impacts cannot be understood without considering both climate forcing and anthropogenic influences on freshwater habitats. More broadly, this thesis highlights that molecular proxies must be interpreted within their environmental and ecological context to yield reliable reconstructions. Process-based calibration, multi-proxy integration, and alignment between proxy signals and environmental drivers are essential for improving the

accuracy and interpretability of paleoclimate and paleoecological records. In conclusion, this work provides a comprehensive, process-informed framework for reconstructing coupled climate-ecosystem dynamics in lake systems. By integrating molecular, isotopic, and ecological indicators, it advances the capacity of paleolimnology to resolve how natural variability and human activities interact to shape freshwater ecosystems over centennial timescales. This integrative approach is critical for informing future research, guiding ecosystem management, and predicting how lake systems will respond to ongoing environmental change.

Future Directions

This thesis establishes a process-based, multi-proxy framework for reconstructing coupled climate-ecosystem dynamics in lake systems; however, several avenues remain for advancing both methodological development and ecological interpretation. A key priority for future research is the broader application and validation of biomarker-based reconstructions across diverse lake systems. The sterol and stanol proxies developed for tracking large -scale migratory animal-driven nutrient inputs, as well as the lake-specific brGDGT calibrations, should be tested in lakes with varying morphometry, trophic status, and climatic regimes to assess their transferability and identify common versus site-specific controls. Expanding these approaches across regional gradients will help refine their general applicability and improve their use in comparative studies.

Further work is also needed to better constrain the ecological and microbial processes underlying proxy production and preservation. For brGDGTs, this includes resolving the relative contributions of water column versus sedimentary production, seasonal variability in production, and the influence of microbial community composition on compound distributions. Similarly, for sterol and stanol biomarkers, improved understanding of degradation pathways, transport mechanisms, and sedimentary incorporation will enhance the interpretation of biomarker signals as quantitative ecological proxies.

Integration of additional proxy systems represents another important direction. Combining molecular biomarkers with other paleolimnological indicators, such as diatom assemblages, pigment records, or ancient DNA (eDNA), could provide complementary insights into ecosystem structure and function. Such multi-proxy approaches would improve the ability to

disentangle overlapping environmental drivers and strengthen interpretations of past ecological change.

Future research should also focus on extending biomarker-based reconstructions into older sediment records where monitoring data are unavailable. While this thesis demonstrates strong validation of proxies within the instrumental period, applying these calibrated relationships to longer sediment sequences from salmon nursery lakes would enable reconstruction of pre-industrial baselines and natural variability over millennial timescales.

Finally, integrating paleolimnological reconstructions with predictive ecological and climate models represents a critical next step. Linking long-term proxy records with modelling frameworks could improve projections of how lake ecosystems will respond to ongoing climate warming and increasing anthropogenic pressures. This is particularly important for salmon-bearing systems, where interactions among climate variability, nutrient dynamics, and habitat conditions directly influence population sustainability.

Overall, advancing these research directions will enhance the precision, transferability, and ecological relevance of biomarker-based reconstructions, and further strengthen the role of paleolimnology in understanding and managing freshwater ecosystems under changing environmental conditions.

Closing Statement

This thesis aimed to (1) reconstruct long-term population dynamics and ecosystem change driven by nutrient inputs from large-scale migratory animals using sedimentary sterol and stanol biomarkers; (2) develop and validate lipid biomarker proxies for tracking sockeye salmon population dynamics and associated marine-derived nutrient delivery; and (3) establish a lake-specific brGDGT calibrations to reconstruct temperature variability and evaluate climate-ecosystem interactions in Cultus Lake. I demonstrated that sedimentary sterol and stanol biomarkers provide robust records of nutrient transfer mediated by large-scale migratory animals, capturing both localized impacts of colonial waterbirds and broader cross-ecosystem nutrient subsidies from sockeye salmon (Chapters 2 and 3). I further showed that lipid biomarker approaches can be quantitatively validated against observational datasets, providing reliable proxies of ecological processes such as nutrient enrichment and primary productivity. In Chapter

4, I established that brGDGT-based temperature reconstructions are strongly governed by site-specific production dynamics and that lake-specific calibrations substantially improve predictive accuracy relative to global models. Building on this framework, Chapter 5 demonstrated that sedimentary records from Cultus Lake capture the coupled influence of climate variability and ecosystem processes, while also revealing a transition from climate-driven to anthropogenically influenced nutrient dynamics in the modern era. Overall, this thesis demonstrates that integrating lipid biomarkers, isotopic records, and climate proxies provides a powerful framework for reconstructing long-term climate-ecosystem interactions. By resolving the combined influences of climate variability, nutrient inputs from migratory animals, and human disturbance, this work advances paleolimnological approaches and provides critical insights for understanding and managing freshwater ecosystems under changing environmental conditions.