Patterns and Drivers of Arsenic Bioaccumulation in Boreal Freshwater Fish of Ontario, Canada

by

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Abstract

Wild fish consumption can be an important pathway for metal exposure to subsistence and recreational fishers. Elevated levels of arsenic (As) have been reported by monitoring programs and previous research in several fish species in the province of Ontario, Canada. This is of particular concern for First Nation communities in remote northern areas that rely on locally sourced freshwater fish for subsistence. However, provincial monitoring for As in fish is less extensive than for other contaminants (e.g. mercury) and less is known about how As behaves in aquatic systems under various conditions. The goal of this thesis was to improve understanding of patterns in As accumulation across freshwater systems. More specifically, I investigated the spatial variability of total As in fish muscle and its ecological, physical and chemical drivers in lakes and rivers across Ontario. To do this, I amalgamated As data from previous research and a long-term contaminant monitoring program, resulting in a dataset of total arsenic concentrations ([As]) in 3200 fish across 30 species and 152 waterbodies sampled between 2008 and 2018. Additional datasets of water chemistry parameters (e.g., pH, DOC), landscape variables (e.g., geology, watershed area), and stable carbon and nitrogen isotopes (measures of fish trophic ecology) were also amassed from governmental and open-source databases to examine the influence of these variables on As bioaccumulation in fish.

Results show that [As] were generally low across most fish species and most waterbodies sampled. However, fish from large northern rivers draining into the ocean had up to 23-fold higher concentrations of As compared to fish from landlocked sites. In general, [As] increased slightly with fish size, although relationships varied among fish species and sites. Evidence of biomagnification of As across fish species was also observed in several lake sites. Furthermore, principal component scores, representing landscape and water chemistry variables, were related to [As] in fish, but the relationships varied among species. These results will help improve the efficacy of fish contaminant monitoring in freshwater systems by identifying physical and ecological variables related to higher concentrations of As in fish while also emphasizing the value of repurposing existing datasets and utilizing open data sources.

Keywords

Arsenic, bioaccumulation, biomagnification, stable isotopes, fish, freshwater

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List of Acronyms

$\delta^{13}C$	Stable carbon isotope ratio
$\delta^{15}N$	Stable nitrogen isotope ratio
AICc	Akaike's Information Criteria for small sample sizes
ANCOVA	Analysis of covariance
As	Arsenic
[As]	Total arsenic concentration
BsM	Broad-scale Monitoring program
Ca	Calcium
CTI	Compound topographic index
DA:LA	Drainage area to lake area ratio
DEM	Digital elevation model
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
ICP-MS	Inductively coupled plasma mass spectrometry
INLA	Integrated nested Laplace approximation
K	Potassium
LMEM	Linear mixed effects model
MDL	Method detection limit
MECP	Ontario Ministry of the Environment, Conservation and Parks
Mg	Magnesium
MNRF	Ontario Ministry of Natural Resources and Forestry
Na	Sodium
NH_3/NH_4^+	Ammonia/ammonium
NO_3/NO_4	Nitrites/nitrates
PC	Principal component
PCA	Principal component analysis
QAQC	Quality assurance/quality control
SO_4	Sulfate
SIMM	Stable isotope mixing model
TKN	Total Kjeldahl nitrogen
TP	Total phosphorus

1. Introduction

Consuming wild-caught fish is a healthy source of protein and omega fatty acids. However, it also provides a potential pathway of contaminant exposure to recreational and subsistence fishers. This concern includes arsenic (As), a metalloid known to have adverse effects to human health (Kapaj et al. 2006). While most As in fish is in organic forms (e.g., arsenobetaine) usually considered less harmful (ATSDR 2007), toxic inorganic species (i.e., As(III) and As(V)) can be found at varying concentrations (Tanamal et al. 2021). Arsenic in fish is typically monitored as total As, and a certain amount (i.e., 15-20%) is then assumed to be in the inorganic forms during risk assessments.

Although it is generally more abundant in marine organisms, total As has been detected at varying concentrations in freshwater fish, sometimes at levels that pose a risk to consumers. As a result, the Ontario Ministry of the Environment, Conservation and Parks (MECP) has issued consumption guidelines, suggesting consumers limit to varying degree the amount of fish they eat, based on these observed concentrations (MECP 2017). Furthermore, As has been reported at elevated concentrations that exceed thresholds for safe consumption in muscle tissue of several fish species in northern Ontario by Lescord et al. (2020) and other regions across Ontario through long-term contaminant monitoring by MECP. However, more work is needed to understand patterns in As accumulation across fish species and waterbodies.

Arsenic enters freshwater systems through natural weathering and leaching processes and as a result of mining and other anthropogenic sources (Smedley and Kinniburgh 2002). However, factors affecting subsequent bioaccumulation of As into aquatic biota are not fully understood, particularly under natural conditions. Total As concentrations ([As]) tend to be significantly higher in zooplankton and invertebrates than fish (Kuroiwa et al. 1994; Revenga et al. 2012) and the mechanisms of trophic transfer of As among fish species are not fully understood. In lab-based experiments, dietary exposure has been a significant pathway for accumulation of As in some freshwater fish (Erickson et al. 2011), suggesting foraging habits (e.g. benthic versus pelagic feeders) and trophic ecology influence As bioaccumulation. In lakes from the southeastern United States, Burger et al. (2002) found higher [As] in higher trophic level fish. In contrast, others have found biodilution of As with increasing trophic level for fish species in a Patagonia lake (Revenga et al. 2012). Furthermore, Lescord et al. (2020) found positive and negative effects of fish characteristics (trophic position, diet, age, round weight) on [As] in fish from the far north in Ontario, but these results varied across species and sampling locations. Such inconsistencies in the literature suggest the role of fish characteristics on [As] may vary with region and food web composition, indicating the importance of examining links between these biological and ecological factors and fish metal concentrations from waterbodies in Ontario.

Recent research has also suggested that site-related effects influence As accumulation in several fish species from northern watersheds (Lescord et al. 2020). These site characteristics could include differences in water chemistry (e.g., pH, aqueous [As]) and landscape features (e.g., watershed area, geology). Concentration of waterborne As is also an important factor in determining As bioaccumulation in aquatic biota, however it is not always an effective predictor of fish tissue concentrations in natural settings (Robinson et al. 1995). Arsenic in water and sediments undergoes numerous transformations which may indirectly affect bioaccumulative potential (Kumari et al. 2017). Water chemistry parameters that are known to influence As transformations (e.g., pH, salinity) have received little attention in terms of how they affect bioaccumulation in fish. Anthropogenic sources of As can also be of concern, with mining of nonferrous metals as a main contributor to As-contamination in Canada (Wang and Mulligan 2006). Contemporary technologies limit As pollution by the mining and metals industry, however legacy impacts still affect freshwater systems in Ontario (Zheng et al. 2003).

Understanding the influence of trophic ecology, water chemistry, and landscape factors on [As] in fish is important in risk assessments for both consumer and ecosystem health. To develop this understanding, I amassed a dataset of [As] in muscle tissue samples from the MECP's contaminant monitoring program and other related research projects from across the province of Ontario, Canada. The resulting dataset represented 30 fish species, across 152 waterbodies spanning an area of approximately 1,000,000 km^2 with three distinct ecozones and varying degrees of anthropogenic influences. My study objectives were to: 1) assess spatial patterns of [As] in freshwater fish across this diverse region; 2) examine links between fish weight, diet and trophic position and fish [As]; 3) investigate the influence of water chemistry and landscape-level variables on As bioaccumulation in Ontario fish.

2. Methods

2.1. Data sources and fish collection

Fish collected and analyzed for [As] in muscle tissue were provided by three sources: MECP (n = 2666), Lescord et al. (2020; n = 438), and the Université de Montréal (n = 96).

Fish total [As] data provided by MECP were collected between 2008 and 2018 through the Ontario Ministry of Natural Resources and Forestry's (MNRF) Broad-scale Monitoring program (BsM) and represents lacustrine and riverine fish communities sampled across the province of Ontario, Canada. Fish collected by BsM were subsequently analyzed for [As] by MECP as part of its long-term contaminant monitoring program which provides consumption guidelines for fisheries across Ontario (i.e., the Guide to Eating Ontario Fish; MECP 2017).

Data provided by Lescord et al. and the Université de Montréal (collectively herein referred to as the Lescord dataset, n = 534) were collected as part of a separate research project based in the far north of Ontario (approximately north of 51° N) between 2012 and 2016. The majority of these sampling sites are located in the Attawapiskat River drainage basin including sites along the Attawapiskat River and surrounding lakes. The mouths of several additional coastal rivers, which drain into Hudson or James Bay, were also sampled.

All fish included in the dataset were collected during the open-water season at a time when lake sites had become stratified (i.e., approximately May to September). Fish collected through the BsM program were captured using North American (NA1) and Ontario small mesh (ON2) gillnets while fish collected for the Lescord dataset were captured using a combination of the same gill nets as well as angling gear (Patterson et al. 2020). A skinless and boneless epaxial muscle tissue sample was removed and frozen in Whirl-paks until laboratory analysis. Whenever possible, individuals across a broad size range, within a given species, were sampled in this way for each waterbody.

2.2. Study area and fish species

Sampling sites within the overall combined dataset (total n = 201 sites with [As] data from at least one fish species) span a vast spatial range across the province, representing large climatic, geologic and biological gradients. The majority of sampling sites are located within the Ontario Shield ecozone, which is part of the Canadian Boreal Shield ecozone overlying Precambrian bedrock (Crins et al. 2009). Bedrock in this ecozone is primarily composed of granites and gneisses with exposed bedrock accounting for a significant amount of area. Where soils exist, they are often relatively thin and acidic, with organic soils found in wetlands (covering approximately 20% of the Ontario Shield) being low in oxygen (Urquizo et al. 2000). Additionally, many sites in the far north of Ontario are located within the Hudson Bay Lowlands Ecozone (n = 46 sites) of which wetlands and peatlands make up 90% of the land area (Martini 1989; Patterson et al. 2020). Many of the major rivers in this ecozone (e.g., the Attawapiskat River) drain into the marine environment of either Hudson or James Bay.

Land use also varies across the province, with more intense developments in southern regions and along the shores of the Great Lakes. There are currently over 40 operating metal mines in Ontario with most occurring below 50° N (Natural Resources Canada 2021). There has been little development of industry in the far north of Ontario although plans for major mining operations in a large mineral-rich region known as the "Ring of Fire" have been proposed (Ministry of Energy Northern Development and Mines 2019). While infrastructure and road networks are relatively scarce in the far north when compared to more southerly locations, there are many remote First Nation communities across this region (Ontario 2019). Members from these communities may rely on the freshwater fish as a means of subsistence, emphasizing the importance of understanding aquatic contaminant cycling and behaviour in this region (Chan et al. 2021).

My overall dataset contains contaminant information for a total of 30 species, some of which had a low number of replicates per waterbody, limiting the inclusion of many species in statistical analyses. Species of primary interest (i.e., those with the greatest replication per waterbody and covering the largest spatial range for landscape level analysis) included walleye (*Sander vitreus*), northern pike (*Esox lucius*), lake whitefish (*Coregonus clupeaformis*) and white sucker (*Catostomus commersoni*). All four species are commonly found in lakes and rivers throughout most of Ontario, including coastal rivers, where lake whitefish will occasionally enter brackish water and northern pike to some degree rely on marine-derived resources as they prey on species migrating from the sea (DeJong 2017). In lakes, walleye and northern pike are primarily piscivorous, mesothermal species which prefer near-shore environments while lake whitefish are cold water invertivores which primarily inhabit off-shore areas (Scott and Crossman 1973; Casselman and Lewis 1996). White sucker are bottom feeding detritivores which spend the majority of their adult lives in the benthic zones of lakes and rivers (Scott and Crossman 1973). Species of secondary interest (i.e., those less commonly sampled but still comprising a relatively large proportion of records) included smallmouth bass (*Micropterus dolomieu*), cisco (*Coregonus artedi*), lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*) and longnose sucker (*Catostomus Catostomus*); however, statistical analyses of these species were often limited due to small sample sizes.

2.3. Laboratory Analysis

2.3.1. Total Arsenic Analysis

Fish collected by BsM were analyzed for [As] at the MECP's Laboratory Services Branch following method BIOTA-E3461. For these analyses, total [As] was extracted from ~1 g of frozen fish using an Anton Paar Multiwave 3000 microwave digester with 16 MF-100 Teflon digestion vessels. All samples were microwaved with 5 mL deionized water, 5 mL of Optima-grade nitric acid and 1 mL Ultrex Ultrapure hydrogen peroxide for a ramp time of 25 minutes, followed by a 15 min hold time at 180°C. Digested samples were allowed to cool to room temperature, rinsed into 50 mL centrifuge tubes and brought to volume will ultrapure water, then further diluted by transferring 2 mL sample to 12-15 mL test tubes for a final acid concentration of 2% nitric acid. Fish [As] were then analyzed using a Varian 820 inductively coupled plasma mass spectrometer (ICP-MS). Method detection limit (MDL) for these analyses was 0.05 µg/g.

Prior to analysis, fish collected by Lescord et al. (2020) were freeze dried using a Labconco FreeZone 12 Bulk Tray Dryer and ground to fine power using a Retsch MM400 ball miller. Total As concentrations were analyzed at the ISO 17025 accredited Biotron trace-metal laboratory at the University of Western Ontario (n = 438) following similar methods as MECP (EPA method 3052 and 200.8). Briefly, 2 mL of trace-metal grade nitric acid were used to digest 0.1 g of freeze-dried muscle tissue in a microwave digestion system and analyzed for [As] by ICP-MS (method detection limits for total As were 0.144 µg/g dry weight, assuming 78% moisture; a detailed description of this laboratory analysis can be found in Lescord et al. 2020). An additional 96 fish from the far north of Ontario were also analyzed at the Université de Montréal in 2020 for [As] as part of ongoing research. Approximately 600 µL of pure nitric acid was added to 10 mg of freeze-dried fish muscle in a 6 mL polytetrafluoroethylene vial. Samples were extracted for three hours in an industrial pressure cooker at 121 °C at a pressure of 1.4 kg/cm^2 (20 psi). Approximately 250 µL of hydrogen peroxide (30%) and 150 µL hydrochloric acid were added after samples were cooled to room temperature and allowed to sit overnight. Samples were diluted to 15 mL to a final dilution of 4% nitric acid and 1% hydrochloric acid then analyzed for [As] using a Agilent ICP-MS Triple Quad 8900.

Because samples analyzed by Lescord et al. and Université de Montréal were reported as dry weight concentrations, these concentrations were converted to wet weight assuming 78% moisture (Lavoie et al. 2013):

$$[As]_{\text{wet wt.}} = [As]_{\text{dry wt.}} (1 - 0.78)$$

Quality assurance and control (QAQC) employed by Lescord et al., MECP and Université de Montréal include reagent blanks, spikes and standard reference materials (DORM-3 was used by Lescord et al. and MECP; additionally, DOLT-4, NIST-1946 and NIST-1947 were used by MECP; TORT-2 was used by Université de Montréal). Duplicates were also used by Lescord et al. and MECP. In-house QAQC standards were met for all MECP samples analyzed (Ministry of the Environment Labratory Services Branch 2010). All QAQC results were within accredited standards for samples analyzed by Lescord et al. (QAQC data presented in Lescord et al. (2020)) and Université de Montréal (% recovery of TORT-2 = 91±3, n = 4). Université de Montréal set of inter-laboratory checks were employed (through Proficiency Testing Canada) to verify calibration curves met acceptability criteria. Method detection limits of [As] for MECP, Lescord et al. and Université de Montréal were 0.05 µg/g, 0.03 µg/g, and 0.02 µg/g, respectively. Actual instrument readings for [As] < MDL were reported for fish analyzed by MECP (Bhavsar, personal comm, 2021). For Lescord et al., [As] < MDL were assigned a random number between zero and one half of the MDL. No observations were below the [As] MDL for fish analyzed by Université de Montréal.

A subset of fish (n = 77) was analyzed by both MECP and Lescord et al. (2020) and showed good agreement in [As] between the two analyses ($R^2 = 0.93$, $p < 0.001$; Figure SI-1). When there was overlap between samples, fish analyzed by MECP were used in the final dataset. Nine samples were also analyzed by both Lescord et al. and Université de Montréal, also showing good agreement between samples ($R^2 = 0.99$; Figure SI-2) and fish from Lescord *et al.* were chosen when there was overlap among these datasets.

2.3.2. Stable isotope analysis

All fish from the Lescord dataset and a subset from the MECP dataset (n = 705) were analyzed for stable nitrogen ($\delta^{15}N$) and carbon isotopes ratios ($\delta^{13}C$; n = 1239). The subset of fish analyzed for [As] by MECP were also analyzed for $\delta^{15}N$ and $\delta^{13}C$ as part of the MNRF Boreal Food Webs research program. Data from these two databases were matched by aligning waterbody, sampling date, fish species, sex, total length and round weight. Stable isotope analysis was completed following procedures outlined in Jardine et al. (2003) at the University of New Brunswick Stable Isotopes in Nature Laboratory. Stable nitrogen and carbon isotope values are expressed as the ratio of heavier isotopes (i.e., N^{15} and C^{13}) to their lighter, more common counterparts. Delta^{15}N values characterize relative trophic position of organisms within their food web, allowing for inferences to be made on the effect of predator-prey relationships on metal concentrations in fish (Post 2002). The effect of basal nutrient sources can be investigated using $\delta^{13}C$ values; in lakes, more depleted $\delta^{13}C$ values in fish indicates a reliance on pelagic carbon, while more enriched values suggest more consumption of benthic carbon sources (Post 2002).

2.3.3. Water chemistry analysis

Water chemistry data were provided by the MECP and MNRF and all water chemistry analyses were performed at the MECP laboratories (Dorset and Etobicoke, Ontario, Canada) following standard protocols (Ontario Ministry of the Environment, 1983). Water samples were collected from the top ~1m of water over the deepest section of each lake. Because fish and water were often sampled at different times, a cut-off of four years was used when matching fish sampling and water sampling dates in order to maximize the use of available data. In total, 77 lake sites had water chemistry meeting this four-year criterion. However, 72 of these lakes were sampled for both fish and water within two years, over which short-term periods Ontario Shield and Hudson Bay lakes are not likely to experience large changes in water chemistry (Macleod et al. 2017). Similar to fish, all water sampling for lakes occurred between the months of May and September. Water chemistry parameters used in the final dataset included: alkalinity, ammonia/ammonium (NH_3/NH_4^+), calcium (Ca), conductivity, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), magnesium (Mg), nitrate/nitrite (NO_3/NO_2), pH, potassium (K), sodium (Na), sulphate (SO_4), total Kjeldahl nitrogen (TKN), total phosphorus (TP) and true colour.

2.4. GIS methods

All watershed processing was performed using R (v. 3.6.1) and all descriptive maps (i.e., Figure 1) created using QGIS (v. 3.14.16-Pi). Watersheds were delineated only for lake sites (n =

152) because water chemistry data were not available for most river sites. Digital elevation models (DEMs) used to delineate watersheds were sourced from Ontario Integrated Hydrology data packages available through Ontario GeoHub (Ontario Ministry of Natural Resources and Forestry 2011). The ‘whitebox’ package (v 1.4.0) was used to breach depressions in DEMs using a least-cost pathway method; breached DEMs were then used to generate D8 flow pointer, slope and compound topographic index (CTI) rasters. Watersheds were then delineated using the D8 flow pointer rasters with lake polygons converted to rasters as the pour point.

Statistics for slope, elevation and CTI were calculated for each watershed using the ‘exactextractr’ package (v 0.6.1) in R, with lakes removed from slope and CTI rasters to avoid low-biasing measuring. Surface area for lakes and watersheds were calculated using package ‘sf’ (v 1.0.3). Drainage area/lake area ratio (DA:LA) was calculated by removing lakes from watershed polygons then dividing watershed surface area by lake surface area. Proportion of quaternary geological material was calculated for each watershed based on the ‘Quaternary Geology of Ontario Seamless Coverage’ data set (Ontario Geological Survey, 1997) and predominant geology was determined by identifying the quaternary material with the highest proportion within watersheds. Presence or absence of abandoned mine sites within a watershed was determined by intersecting watershed polygons with point data from the Abandoned Mines Information System which contains all known abandoned and inactive mine sites in Ontario (Ontario Ministry of Northern Development and Mines 2018). Only class “A” and “B” abandoned mine sites were used, as these sites are considered a potential concern to receiving environments. Gold mines represented 75% of abandoned sites within watersheds observed herein, though other commodities such as iron, nickel, zinc, copper and pyrite were also included, and sites were often associated with more than one type of metal mining.

2.5. Statistical analysis

All data handling, graphing, and statistical analyses were performed using R (v. 3.6.1) and alpha was set to 0.05 for all statistical tests.

2.5.1. Species differences and the effect of fish size on total [As]

To compare relationships of [As] to fish size among fish species, a series of analyses of covariance (ANCOVA) were performed using the 'car' package (v 3.0.11), with fish weight as a covariate. This was done because previous studies have found some effect of fish size on [As], which therefore could influence observed species differences (Lescord et al. 2020). To compare [As] – fish weight relationships across fish species, a subset of data was used including a total of 13 sites (11 lakes and two river sites), as these sites contained the highest number of replicates available per species in the total dataset ($n \geq 6$ per species and $n \geq 3$ species per site). This also allowed for comparing [As] across species while accounting for the variance of fish weight when relationships to fish weight were homogenous across species. A separate ANCOVA was performed for each waterbody using a type III sum of squares with sum-to-zero contrasts, with fish weight as the covariate and $\log_e$-transformed [As] as the response variable.

To investigate the effect of fish size on [As] among waterbodies, a second series of ANCOVAs were performed, using the same package and approach as described above. For these models, data were subset by species, and included waterbodies with ≥ 10 observations. This allowed for modelling of six species (with a separate model for each species), ranging from 3-13 waterbodies per species (see Table 2 for sample sizes). For both sets of ANCOVAs, homogeneity of variance of [As] across main effects were tested using Levene's Test. Variances were found to be equal across species for all within-waterbody ANCOVAs, but unequal variances were observed for walleye and white sucker in their respective within-species ANCOVA models. Assumptions of independence of the covariate and main effects were tested using a Welch's Analysis of Variance to test for differences in fish weight among species/waterbodies. This assumption was not met for fish weight across species in Clay, Ball, Bigwood, Long (Sudbury, Ontario), Panache and Goods Lakes or within any species when comparing fish weight across waterbodies. Homogeneity of regression slope models were performed with an interaction term between the covariate and main effects included to determine whether the [As]–fish weight regression slopes were homogenous across species/waterbody groups. Where regression slopes were homogenous, an ANCOVA was performed, allowing for interpretation of main effects. Model diagnostic plots were examined to determine homogeneity of variance and normality of residuals. Outliers were identified by examining scatterplots of $\log_e$ [As] vs fish weight, model residuals vs predicted values and using Cook's distance to identify influential observations. Where outliers were identified, models were rerun to see if diagnostics were improved. Results did not change significantly when outliers were removed for all models except when modelling Ball Lake, Separation Lake and Fishtrap Lake where one outlier was removed from each of these lakes and results were reported with outliers removed. When a significant relationship between main effects and [As] were observed, Tukey's Post-Hoc Tests were performed for post-hoc pairwise comparisons between fish species or waterbodies.

2.5.2. Effect of trophic ecology on total [As] in Ontario fish

To test the effect of dietary carbon sources on [As] in individual fish, stable isotope mixing models (SIMMs) within a Bayesian framework were used. Specifically, an estimate of the proportion of a fish's diet derived from pelagic-based carbon was calculated using the 'simmr' package (v 0.4.5; Parnell and Inger 2016). Estimates were made for individual fish based on their $\delta^{13}C$ and $\delta^{15}N$ in comparison to baseline signatures (Parnell et al. 2013). Delta^{13}C and $\delta^{15}N$ values (available through the Boreal Food Webs Database) from clams and snails were used as baseline indicators of pelagic and benthic signatures, respectively. In total, modeling was performed in nine lakes which had a minimum of $n \geq 5$ clams and snails and $n \geq 7$ fish per lake across all species. A clear separation between baseline (i.e., clam and snail) $\delta^{13}C$ and $\delta^{15}N$ signatures was observed for most lakes; except Crooked Lake, Fishtrap Lake and Kakakiwaganda Lake, where there was less separation between benthic and pelagic isotopic signatures of baseline organisms (Figure SI-3). Furthermore, proportions of pelagic signatures produced using SIMMs represented sufficiently broad ranges (i.e., 12 – 96% pelagic carbon) for investigating the effect of pelagic diet on fish [As] across waterbodies for several species. Because dietary estimates of pelagic carbon are proportional data, values were logit-transformed to unbound these values before linear modelling. Ordinary least-squares regressions were fit using logit-transformed percent pelagic carbon as the explanatory variable and $\log_e$ [As] in fish as the response variable. This modeling was done in two ways: 1) using data pooled within sites to create a separate model for each waterbody across all species, and 2) data were pooled within species to create a separate model for lake trout, lake whitefish, northern pike, smallmouth bass and white sucker across multiple sites.

In addition to the dietary modeling, biomagnification models across individual food webs were performed by regressing logged [As] ~ $\delta^{15}N$ values to provide an indication of the rate at which a contaminant is biomagnified or biodiluted through a food web. Biomagnification models were estimated in a total of eight lake food webs, based on the number and abundance of fish species present to try to capture multiple trophic groups. Only sites with n ≥ 4 fish species/site and n ≥ 3 individuals per species were included in this modeling exercise. Across the eight models, between 18-125 fish were included across species. However, when interpreting these models it is important to note that each food web included different fish species. Direct comparisons between sites should therefore be made with caution. River sites were excluded from this modeling exercise because nearly all were coastal rivers with marine influences, which has been shown to enrich stable isotope values in some fish species, precluding the ability to model food webs by these methods (DeJong 2017; Lescord et al. 2020).

2.5.3. Determining landscape-level predictors of fish [As]

A principal component analysis (PCA) was performed using all landscape and water chemistry variables for lakes where water chemistry data were available (n = 77) to determine variability and relatedness among variables (‘stats’ package v 4.1.0). Data used in the PCA were centered and scaled through the ‘prcomp’ function by subtracting corresponding variable means from each observation and dividing by their respective standard deviation. Four lakes were identified as being highly influential and were therefore removed from PCA and regression analysis due to their strong influence on principal component (PC) 1 (Conestogo and Silver lakes, both part of the Lake Simcoe ecoregion and have notably elevated concentrations of various ions), PC3 (Lake Abitibi as having a large watershed surface area) and PC5 (Lake Abitibi, Sturgeon Lake, both of which had large lake surface areas relative to other lakes in the dataset). Six PCs with eigenvalues > 1 were retained for use in linear modelling.

Size-standardized lake-level fish [As] were predicted using a linear mixed effects model (LMEM) within a Bayesian framework using integrated nested Laplace approximation

(INLA). A separate LMEM was fit for lake whitefish, northern pike, smallmouth bass and walleye and predictions were made for median fish weight of each respective species. Fish weight was set as a fixed effect and lake site as a random effect with random intercepts and slopes. Lake-level [As] predictions for lakes with two or more fish per waterbody were retained for use in linear modelling. Separate models were also fit for the same species using all lake and river sites to generate size-standardized predictions for all sites presented in Figure 1.

Lake-level [As] predictions for lake whitefish, northern pike and walleye were used to compare [As] in watersheds with class A or B abandoned mine sites present to those without using a Welch's two sample t-test. An unequal variances test was used due to larger sample sizes of watersheds without mine sites present (Zimmerman 2004). A similar comparison using lake-level [As] in smallmouth bass was not possible due to having too few sites with mine sites present within watersheds.

Relationships among lake-level [As] predictions and landscape variables were examined by multiple regression models, which were assessed and ranked according to the Akaike information criteria corrected for small sample sizes (AICc). Models were run within fish species for lake whitefish, northern pike, smallmouth bass and walleye. Modelling of other species (e.g., white sucker) was not possible due to limited sample sizes or because a large proportion of the data had very low [As]. Size-standardized lake-level predictions of [As] were used as the response variable, and the six PCs resulting from the before mentioned PCA used as predictors, after standardization by centering means and dividing by two standard deviations. All combinations of the six PCs were modelled, allowing each model to have up to one predictor variable per 10 observations. Models with a ΔAICc of < 2 from the top model were retained and the coefficients from all top models were full model-averaged using the 'MuMIn' package (v

1.43.15). Because a relatively large proportion of size-standardized lake-level [As] predictions were below MECP's MDL (e.g., 56% of northern pike lake-level predictions), and assuming greater uncertainty at these low concentrations, separate models were built for each species using only lake-level predictions with [As] above 0.05 µg/g.

3. Results

3.1 General description of the data – spatially and among taxa

In general, fish [As] were low in most sampling sites, with 84.2% of total observations below the lowest MECP guideline of ≤ 0.25 µg/g, which corresponds to a consumption recommendation of ≥ 32 meals/month for the general population. Proportion of observations below the lowest consumption guideline varied by species, with white sucker having the greatest proportion of [As] concentrations ≤ 0.25 µg/g (i.e., 98%) and cisco having the lowest (i.e., 60.4%). However, [As] for 6.5% of total observations were ≥ 1 µg/g (i.e., corresponding to a ≤ 4 meals/month recommendation for general population) and 2.5% were ≥ 2 µg/g (i.e., corresponding to a ≤ 2 meals/month recommendation for general population and a “do not consume” recommendation for sensitive consumers; MECP 2017).

Fish collected from northern river sites that drain into the lower Hudson or James Bay had higher [As] than lacustrine fish at similar or lower latitudes (Figure 1). This trend is most pronounced for cisco and lake whitefish, populations of which had median [As] in coastal rivers that were 23 and 13 times (respectively) higher when compared to lacustrine sites (Figure 2, Table SI-1). Northern pike and walleye follow a similar trend, though fewer river sites had elevated concentrations in populations of these predatory fish species (Figure 1). The exception to this was northern pike sampled from the mouth and upper reaches of the Albany River which had median [As] of 1.26 µg/g (n = 14) and 2.19 µg/g (n = 7), respectively.

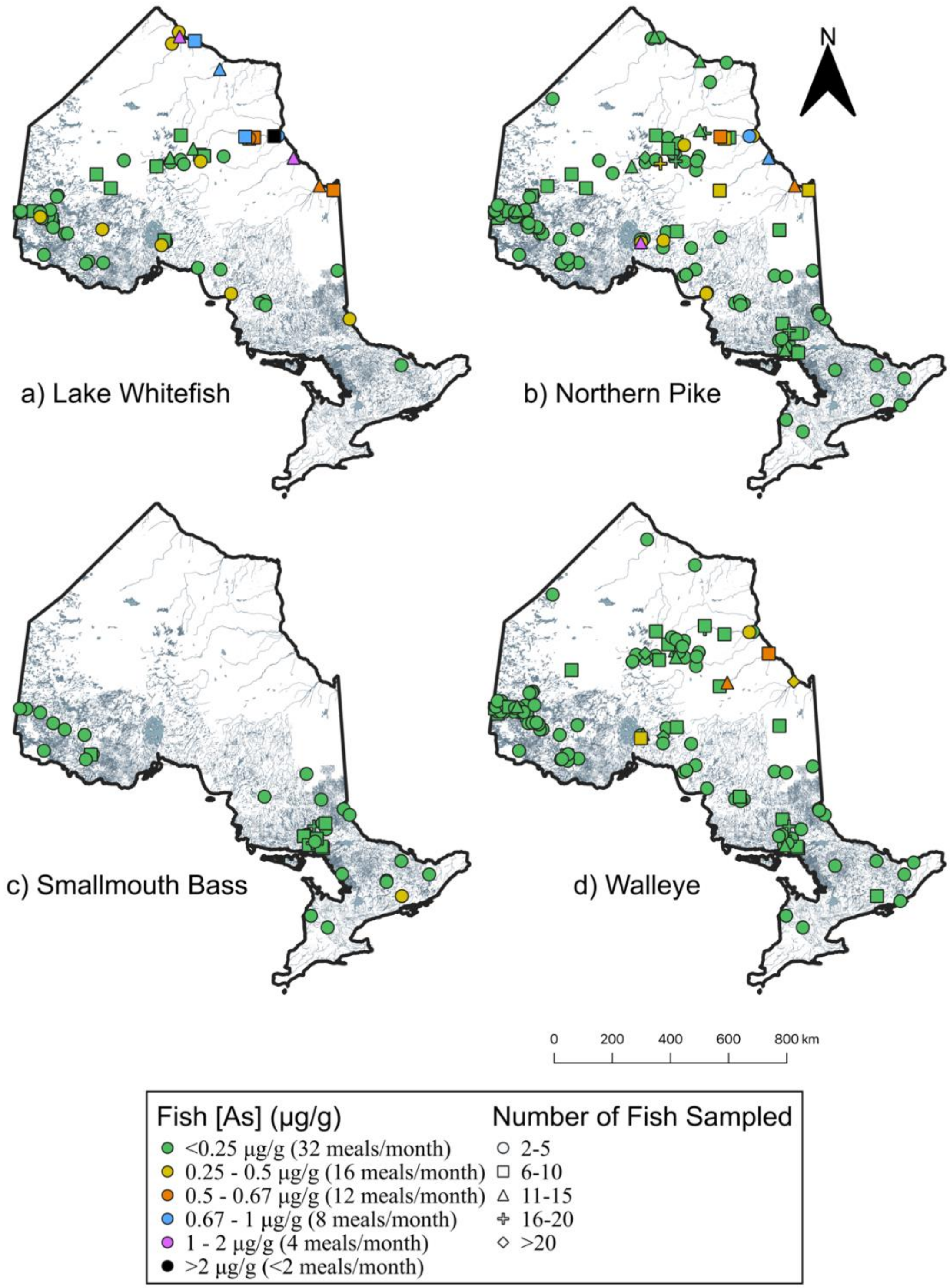

Figure 1. Maps showing total arsenic concentrations for four common fish species in Ontario. Arsenic concentrations are size-standardized predictions generated using a linear mixed effect model within a Bayesian framework using integrated nested Laplace approximation. Total arsenic concentrations were predicted at median fish weight for each respective species. Site colour indicates the [As] relative to the concentrations used for consumption guidelines by the MECP, and site shape indicates the number of fish sampled from a given site. Grey polygons in the base layer represent lakes and rivers within Ontario.

Cisco were the only species of lacustrine fish that exceeded the uppermost consumption guideline of 2 µg/g (Figure 2), which is likely due to 30 of 58 lacustrine cisco being sampled from Long Lake in Sudbury, Ontario, a site contaminated with As from historical gold mining. In addition to cisco, six northern pike were the only other lacustrine fish [As] exceeding 1 µg/g. Fish [As] from Moira Lake, another known As-contaminated site, were relatively low with smallmouth bass [As] ranging from 0.16-0.64 µg/g and all other species below 0.2 µg/g. No other sites in this dataset were contaminated with a known source of arsenic, to be able to test this potential mining landscape effect.

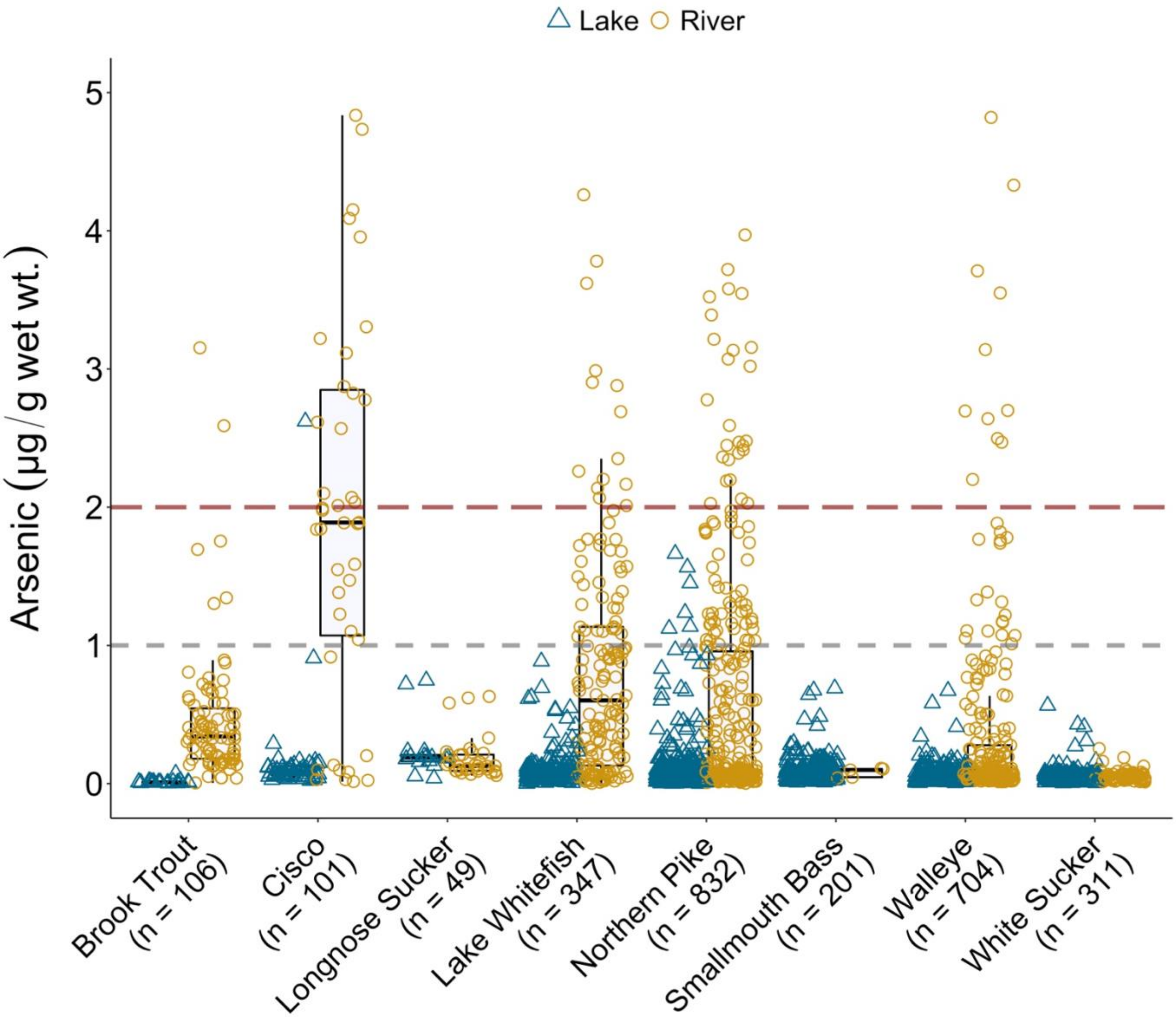

Figure 2. Muscle [As] for fish species collected from waterbodies across Ontario, Canada. Dashed lines denote meal per month recommendations provided by the Ontario MECP: the grey dashed line at 1 μg/g corresponds to ≤ 4 meals/month recommended for the general population and the red dashed line at 2 μg/g corresponds to ≤ 2 meals/month recommended for the general population and a "do not consume" recommendation for sensitive populations. Blue triangles represent fish sampled from lakes and yellow circles represent fish sampled from river sites (including several coastal river sites that drain into Hudson or James Bay). Boxes indicate the interquartile range; black lines indicate median [As]. Statistical comparisons of lake and riverine fish within species can be found in Table SI-1.

3.2. Species differences and the effect of fish size on total [As]

3.2.1. Species differences within waterbodies

A significant interaction term between fish weight and species was observed in five waterbodies, indicating that the effect of fish weight was variable among species and precluded between species comparisons in these waterbodies (Table 1). In general, several populations showed slightly positive relationships while others appeared to show no relationship between [As] and fish weight (Figure SI-4a, Figure SI-4b, Figure SI-4c). In the other eight waterbodies, where the [As]-fish weight relationships were homogenous across species within a waterbody, a significant difference in fish [As] among species was observed for all sites but Clay Lake ($F = 2.10$, $p = 0.150$) and Namewaminikan River ($F = 1.56$, $p = 0.220$). Between-species differences in [As] were somewhat inconsistent, although in several lakes where post-hoc comparisons were possible, lower-trophic-level species such as lake whitefish, longnose sucker and white sucker appeared to have higher [As] than predatory species like northern pike and walleye. Additionally, cisco appeared to have higher [As] than any other species in Long Lake, Sudbury, though they were left out of this analysis because they cover a very small range in the covariate (round weight) and had disproportionately higher sample size than other species present. When assessed alone, there appeared to be a positive relationship between $\log_e$ [As] and fish weight for cisco in Long Lake, Sudbury (slope±SE = 0.009±0.002, adjusted $R^2 = 0.30$, $p < 0.001$; data not shown).

Table 1. Results of analysis of covariance using type III sum of squares to compare relationships between arsenic muscle tissue concentrations and round weight (covariate) across fish species from 11 lakes and 2 rivers located in Ontario, Canada. Arsenic concentrations were natural log transformed to meet modelling assumptions. Relationships between [As] and round weight varied significantly among species for 5 of the 13 waterbodies presented, preventing interpretation of main effects. Tukey's HSD tests were performed for waterbodies where a significant difference between species was observed (i.e., $p < 0.05$) to compare differences among species

Waterbody	***N***	**Interaction term**			**Round weight**			**Fish Species**			**Summary of Tukey's HSD Results**
		F	*p*[a]	*d.f.*	*F*	*p*[a]	*d.f.*	*F*	*p*[a]	*d.f.*	
Attawapiskat Lake	102	1.51	0.205	4	14.14	**<0.001**	1	15.25	**<0.001**	4	LNS > LWF, NP, WALL, WS
Ball Lake	41	0.73	0.490	2	8.50	**0.006**	1	5.09	**0.027**	2	LWF > WALL
Bigwood Lake	71	6.03	**0.011**	3	—	—	—	—	—	—	—
Clay Lake	24	0.59	0.567	2	0.95	0.342	1	2.10	0.150	2	—
Fishtrap Lake	40	2.96	**0.048**	3	—	—	—	—	—	—	—
Goods Lake	41	1.85	0.158	3	6.48	**0.015**	1	8.49	**<0.001**	3	LWF > WALL, WS
Lang Lake	29	0.63	0.537	2	2.33	0.140	1	7.80	**0.002**	2	WALL > NP; WS > NP
Long Lake	67	5.21	**0.011**	2	—	—	—	—	—	—	—
Panache Lake	45	1.00	0.404	3	20.80	**<0.001**	1	8.04	**<0.001**	3	LT > NP, SMB, WALL
Separation Lake	22	11.0	**0.002**	2	—	—	—	—	—	—	—
Winisk Lake	32	1.38	0.274	3	0.39	0.535	1	9.17	**<0.001**	3	LWF > NP, WALL; WS > NP
Namewaminikan River	50	0.62	0.543	2	10.77	**0.002**	1	1.56	0.220	2	—
Blackwater River	27	6.55	**0.006**	2	—	—	—	—	—	—	—

[a] Bolded values represent significance with $p \leq 0.05$

[b] LNS = longnose sucker; LT = lake trout; LWF = lake whitefish; NP = northern pike; SMB = smallmouth bass; WALL = walleye; WS = white sucker

3.2.2. Effect of fish weight on [As] among waterbodies

When observing the effect of fish weight for individual species across several lakes, [As]-fish weight regression slopes were heterogenous among lakes for lake whitefish, northern pike, walleye and white sucker (Table 2). However, while the majority of these relationships were weak, there were more positive trends (significant and insignificant) than negative ones (Figure SI-5), suggesting some fish accumulate slightly higher [As] as they grow larger.

Lake trout and smallmouth bass were the only species where homogenous [As]-fish weight relationships were observed across waterbodies, possibly influenced by smaller sample sizes compared to other species with considerably more lakes included in the analysis (e.g., $n = 3$ lakes with ≥ 10 fish sampled for smallmouth bass, vs $n = 13$ for northern pike and $n = 12$ for walleye). A positive relationship was observed between individual lake trout [As] and fish weight (slope±SE = 0.0003±0.00008, $F = 15.70$, $p < 0.001$), but no significant relationship was observed for smallmouth bass ($F = 0.41$, $p = 0.527$; Table 2); significant differences in [As] among waterbodies were observed for both species. Lake trout [As] were highest in Panache Lake, when compared to the three other lakes considered. Smallmouth bass [As] were highest in Round Lake, which is known to be contaminated by [As] from historical mining processes.

Table 2. Results of analysis of covariance using type III sum of squares to compare relationships between arsenic muscle tissue concentrations and round weight (covariate) across lakes for several fish species. Arsenic concentrations were natural log transformed to meet modelling assumptions. Relationships between [As] and round weight varied significantly among lakes for 4 of the 6 species presented, preventing interpretation of main effects. Tukey's HSD tests were performed for waterbodies where a significant difference between lakes was observed (i.e., $p < 0.05$) to compare differences among lakes.

Fish species	***N***	**Interaction term**			**Round weight**			**Waterbody**			**Summary of Tukey's HSD Results**
		F	*p*[a]	*d.f.*	*F*	*p*[a]	*d.f.*	*F*	*p*[a]	*d.f.*	
Lake trout	54	2.13	0.109	3	15.70	**<0.001**	1	35.74	**<0.001**	3	Panache > Bigwood, Chiniguchi, Kukagami; Chiniguchi > Bigwood
Lake whitefish	64	6.21	**<0.001**	5	—	—	—	—	—	—	—
Northern pike	194	1.93	**0.034**	12	—	—	—	—	—	—	—
Smallmouth bass	56	1.85	0.170	3	0.41	0.527	1	26.42	**<0.001**	2	Round > Bigwood, Panache
Walleye	213	2.26	**0.021**	9	—	—	—	—	—	—	—
White sucker	64	6.09	**0.001**	3	—	—	—	—	—	—	—

[a] Bolded values represent significance with $p \leq 0.05$

3.3. Effect of trophic ecology on [As] in lacustrine fish

Lacustrine fish studied herein had variable diets, with pelagic carbon accounting for 12-96% of their diet across all fish species and sites. Lake whitefish and white sucker tended to have lower proportions of pelagic carbon in their diet in comparison to other species, while lake trout had consistently higher proportions. Overall, most species observed had similarly broad ranges in proportions of pelagic carbon in their diet, except lake trout which did not have any pelagic contributions below 40% (Figure 3). Ranges of pelagic signatures within lakes were notably limited in some cases, particularly Fishtrap Lake (10-20%) and Lang Lake (60-70%) which had small ranges in pelagic signatures for all fish species, despite Lang Lake having a relatively large spread of fish isotopic signatures and reasonable separation between baseline isotopic signatures (Figure SI-3).

Significant positive relationships between fish [As] and percent pelagic signature in fish diet was observed for walleye, northern pike and smallmouth bass when data were pooled across multiple lakes (Figure 3), indicating higher [As] in fish with high proportions of pelagic carbon in their diets within species. Overall, the strongest relationship was observed in walleye (R^2 = 0.55; Figure 3). Pelagic signatures in fish from Fishtrap Lake and Lang Lake represented narrow ranges in percent pelagic signatures while also having some of the lowest fish [As]; nevertheless, a significant relationship still existed for walleye and northern pike even with these lakes removed (smallmouth bass were not present in these lakes). Total As concentrations in lake trout did not appear to be related to the proportion of dietary pelagic carbon, potentially due to the more limited range in the latter variable when compared to other predatory fish (Figure 3). Furthermore, no such significant relationship was observed in lake whitefish or white sucker, both species with broader ranges in dietary percent pelagic carbon (Figure SI-6).

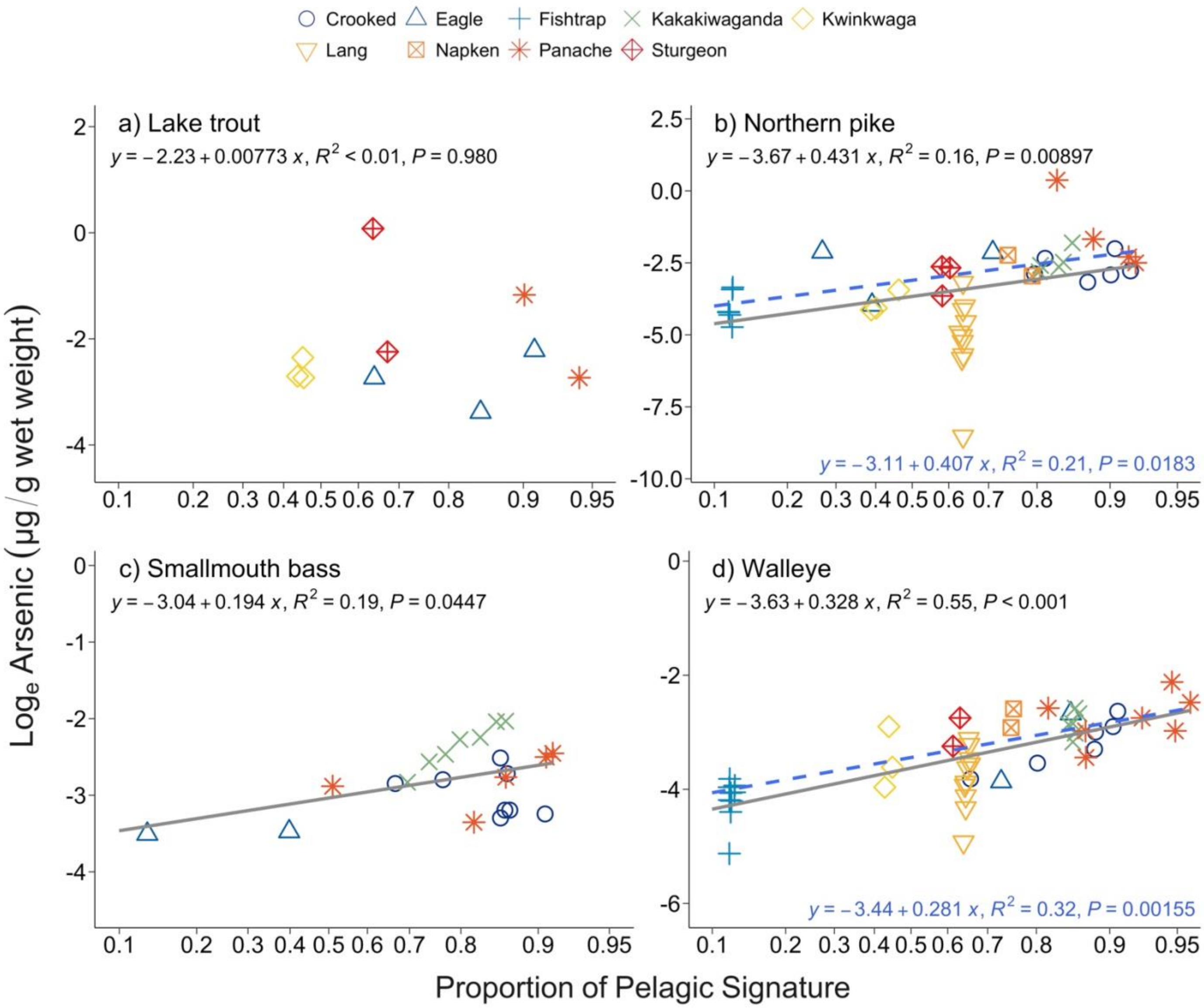

Figure 3. Relationships between $\log_e$ total arsenic in fish muscle tissue and proportion of fish diet from pelagic sources derived from SIMMs. Equations, R^2 and p-values are based on ordinary least square regression with logit transformed proportion of pelagic signature; grey lines of best fit are shown for significant regressions ($p < 0.05$) using full dataset; because fish from Fishtrap lake and Lang Lake show relatively small ranges in pelagic signatures, alternative modelling results and line of best fit excluding data from these sites are shown in blue for walleye and northern pike plots.

When examining individual food webs (i.e., within lakes, across fish species), the proportion of pelagic diet did not have any effect on fish [As] for most lakes considered (Figure SI-7). Statistically significant relationships between [As] and proportions of dietary pelagic carbon were observed for Fishtrap Lake and Kwinkwaga Lake, despite these two lakes having narrow ranges in pelagic signatures (i.e., 10-20% and 40-50% pelagic carbon, respectively). It should be noted that slope estimates for these models are very high, likely due to small ranges in pelagic signatures. Other lakes had greater ranges in pelagic signatures (e.g., Eagle Lake, 13-91% pelagic carbon, Figure SI-7) and no relationship to fish [As] was observed in these lakes, suggesting no bioaccumulation pattern within these lake food webs.

Variable patterns in As biomagnification were observed across the eight modelled food webs (Figure 4, Figure SI-8). Four of the eight sites showed no indication of biomagnification or biodilution among fish species. Three sites showed a significantly positive relationship with $\delta^{15}N$, suggesting an increase in [As] with increasing trophic level. Conversely, one site had a significantly negative relationship with $\delta^{15}N$, indicating a decrease in [As] with increasing trophic level (Figure 4, Figure SI-8). While each model contained different fish species, it is notable that a site with all predatory fish (i.e., Bigwood Lake) and one with mostly insectivorous fish (i.e., Lake Temiskaming) showed similarly positive slopes, suggesting [As] increase throughout their food webs regardless of the span in trophic levels included (Figure 4). However, it is unclear why lake trout [As] from Bigwood Lake showed an opposing negative relationship with $\delta^{15}N$ to the rest of the fish species in this lake (not statistically tested; Figure 4a).

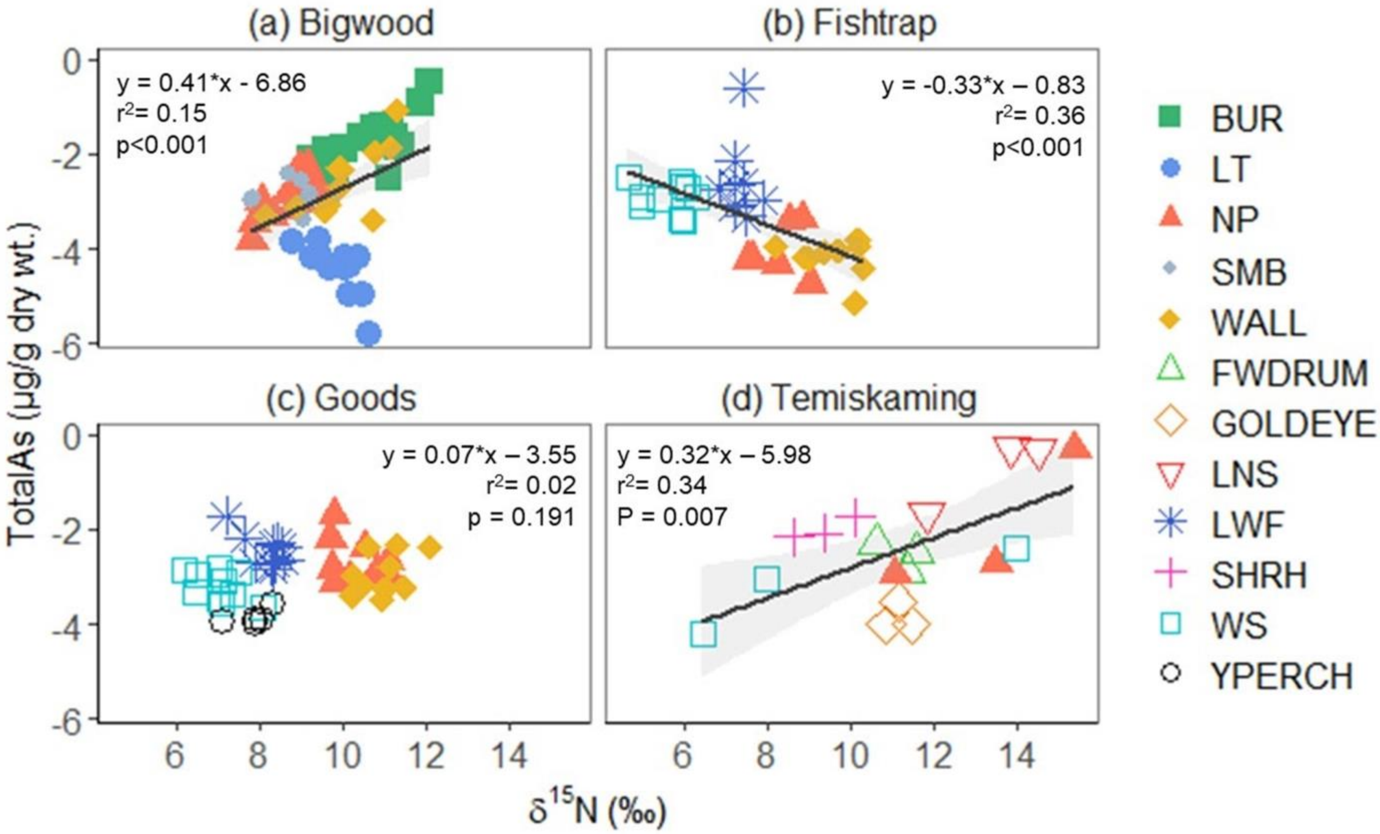

Figure 4. Arsenic biomagnification models through the food webs of four lakes. Note that different fish species are present in each of the four models. Line significance was assessed using a linear regression. Species abbreviations: BUR = burbot, LT = lake trout, NP = northern pike, SMB = smallmouth bass, WALL = walleye, FWDRUM = freshwater drum, GOLDEYE = golden eye, LNS = longnose sucker, LWF = lake whitefish, SHRH = shorthead red horse, WS = white sucker, YPERCH = yellow perch. Four additional biomagnification models are shown in Figure SI-8.

3.4. Effect of water chemistry and landscape characteristics

The PCA of landscape and water chemistry variables identified six PCs with eigenvalues ≥ 1 (Table SI-2), which were used as predictor variables in AICc-ranked model selection and averaging of fish [As]. Based on variable loadings (Table SI-2), ions (i.e., Ca, Mg, Conductivity, DIC) and pH dominated and loaded negatively on PC1; PC2 was dominated by Na, true colour, DOC and SO_4; PC3 was dominated by watershed area, DA:LA and NO_3^-/NO_2^-; PC4 was dominated by NH_3/NH_4^+, mean CTI, K and NO_3^-/NO_2^-; PC5 was dominated by lake area; PC6 was dominated by elevation, TP and NH_3/NH_4^+. In general, higher values of DOC, True colour, mean CTI, TKN, and TP were associated with higher latitudes, while higher slope, and SO_4 were associated with lower latitudes (Figure 5).

No observable differences in fish [As] were identified between predominant geology types, however limited sample sizes of different predominant geology types prevented any statistical comparisons (Figure SI-9). Significant differences in fish [As] were identified between presence/absence of class A and B abandoned mine sites for lake whitefish ($t = -2.11$, 19 d.f., $p = 0.048$), northern pike ($t = -3.19$, 45 d.f., $p = 0.003$) and walleye ($t = -3.63$, 47 d.f., $p < 0.001$; Figure SI-10), suggesting that lakes in watersheds with abandoned mine sites had higher [As]; however, these differences were small, with fish from sites with abandoned mines in their watershed having approximately 0.02-0.04 µg/g higher [As] than the same species of fish from sites without abandoned mines present.

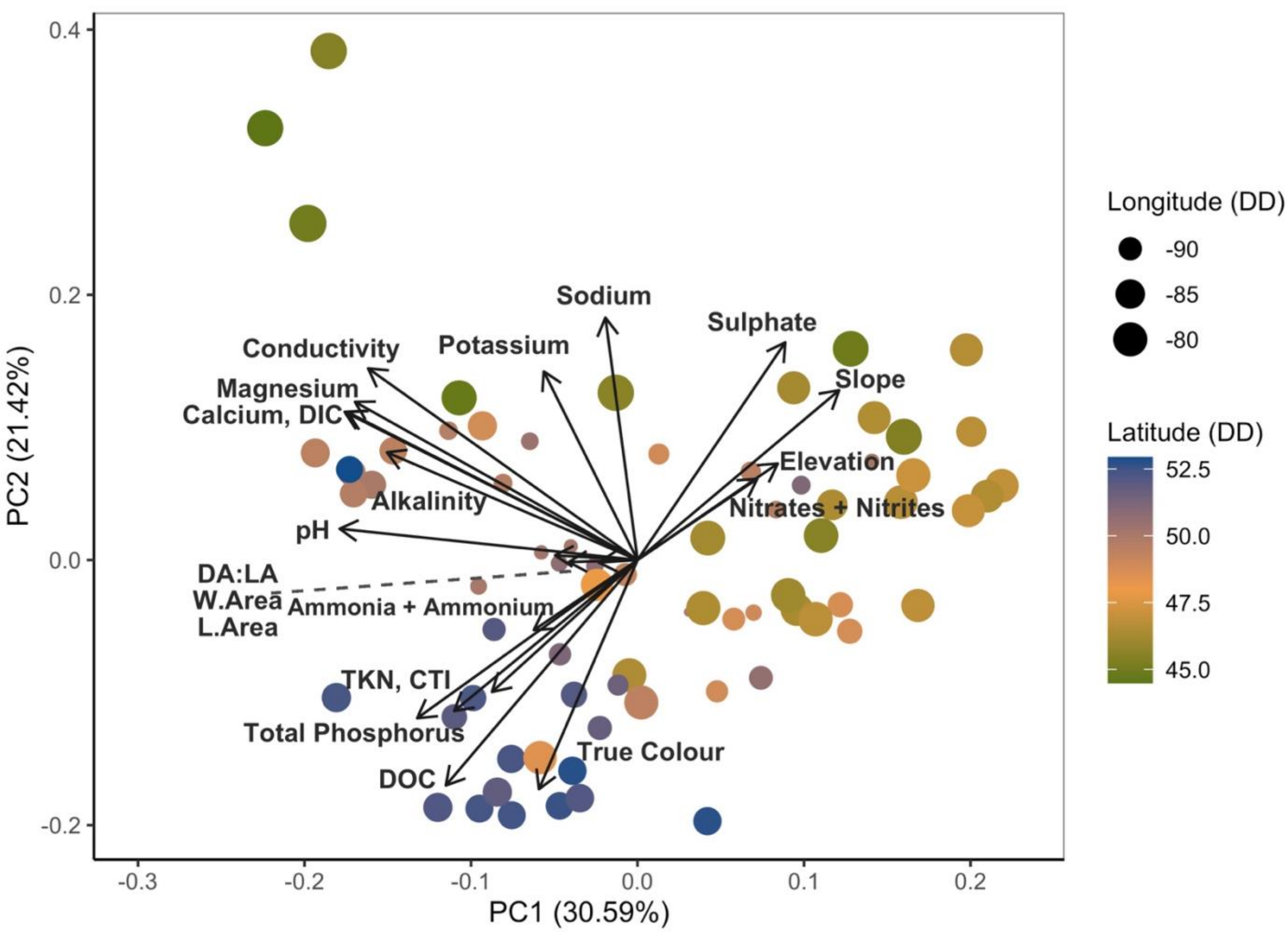

Figure 5. Bi-plot of the first two PCs resulting from a PCA including landscape and water chemistry data from 73 lakes across Ontario.

Overall, results suggest that water chemistry and landscape variables (via PCs) had differing effects on fish [As] across species (Table 3). For walleye, a strong single model (i.e., $R^2 = 0.52$, AIC weight = 1.00) was selected which showed significantly positive relationships with PC3 (representing DA:LA and NO_3^-/NO_2^-) and PC6 (elevation/TP/NH_3/NH_4^+) and significant negative relationships with PC4 (NH_3/NH_4^+/mean CTI/K/NO_3^-/NO_2^-) and PC5 (lake area; Table 3). When regressing individual PCs with $\log_e$ walleye [As], significant relationships were observed for PC4 and PC6 (Figure 6). Furthermore, NO_3^-/NO_2^- (which load positively on PC3 and negatively on PC4; Table SI-2) showed a positive relationship with walleye [As] using both the full dataset and with lake-level predictions < MDL removed (Figure 6). Additionally, a negative relationship between TP (which loads negatively on PC6) and walleye [As] was observed when modelling the full dataset, but not when observations < MDL were removed. No other significant linear relationships were observed when regressing walleye [As] with individual landscape variables represented by PCs selected in the top walleye model. PC6 was the only predictor variable selected when modelling only size-adjusted population means > MDL, but the relationship between walleye [As] and PC6 was no longer significant (0.10 ± 0.07, $p = 0.22$) after observations < MDL were removed (Figure SI-11).

A relatively strong negative relationship was observed between northern pike [As] and PC4 ($p < 0.001$; Table 3; Figure SI-12) and a weaker positive relationship with PC3 when averaging the coefficients from top-models. Similar to walleye, northern pike [As] showed a positive relationship to nitrates/nitrites ($R^2 = 0.27$, $p = <0.001$) and was the only individual predictor variable represented by PC4 that appeared to show any relationship with [As] in northern pike. Furthermore, PC4 and PC3 were not selected in any of the top models

Table 3. Results of a full model averaging exercise to determine the effect of water chemistry and landscape variables (via PCs) on [As] (log_e-transformed) in four common freshwater fish species in Ontario. Lake-level size-standardized predictions were generated from a Bayesian mixed effects model using INLA. Modelling results are shown for both the full dataset and a subset of data above 0.05 µg/g. All combinations of PCs were modelled, with a limit of predictor variables per model by allowing one predictor variable per 10 observations. Models with a ΔAICc of < 2 were selected; all models meeting this criterion were then averaged to estimate the slope of the relationship to log_e [As] in fish.

Fish species	**Data included**	**Model metrics**			[a] **Standardized coefficients (Mean ± SE)**					
		ΔAICc	Weight	R^2	PC1 (Ions)	PC2 (DOC)	PC3 (DA:LA)	PC4 (Nutrients)	PC5 (LA, mix)	PC6 (Elevation)
Walleye	All (n = 40)	0.00	1.00	0.52	—	—	[b]0.51±0.14**	[b]-0.59±0.14***	[b] -0.38±0.13**	[b] 0.56±0.13***
	> MDL (n = 14)	0.00 – 1.48	0.32 – 0.68	0.00 – 0.12	—	—	—	—	—	0.10±0.07
Northern Pike	All (n = 46)	0.00 – 0.3	0.46 – 0.54	0.33 – 0.36	—	—	0.69±0.26*	-1.18±0.26***	-0.17±0.25	—
	> MDL (n = 19)	0.00 – 1.87	0.19 – 0.49	0.22 – 0.37	-0.06±0.16	0.53±0.22*	—	—	-0.21±0.26	—
Lake Whitefish	All (n = 22)	0.00	1.00	0.51	[b]0.93±0.24***	—	—	[b]0.75±0.24**	—	—
	> MDL (n = 12)	0.00	1.00	0.38	[b]0.62±0.22*	—	—	—	—	—
Smallmouth Bass	All (n = 17)	0.00 – 1.77	0.21 – 0.51	0.00 – 0.24	—	0.32±0.37	—	-0.15±0.29	—	—

[a] Asterisks denote significance levels based on model averaged p-values (significance levels: *** p<0.001; ** p<0.01; * p<0.05, no asterisk = non-significant).
[b] Results based on single model.

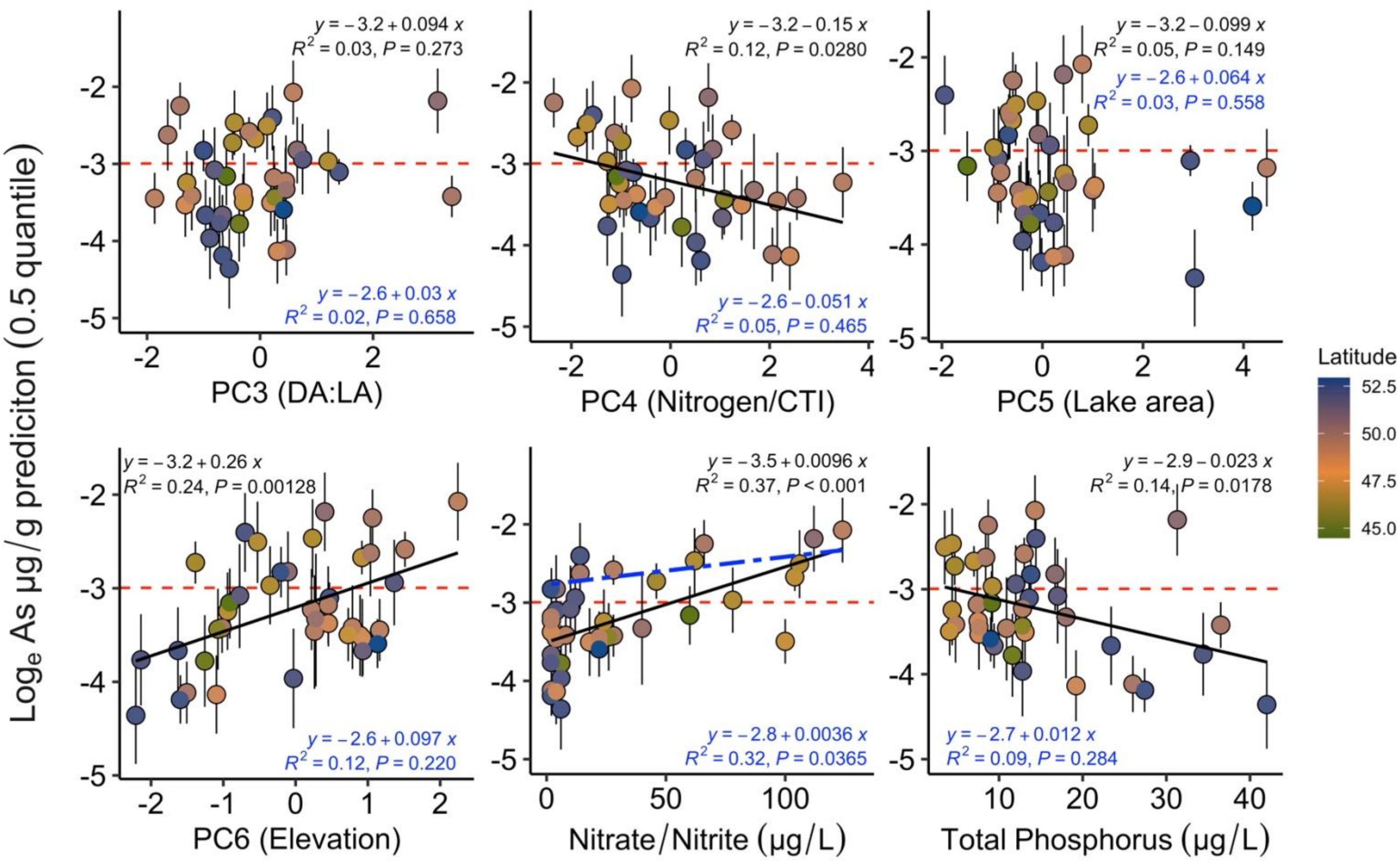

Figure 6. Relationships between loge size standardized walleye [As] predictions and PC scores. Bivariate relationships of individual predictors that dominate PC3, PC4 and PC6 are also shown. The red dashed line represents MECP MDL of 0.05 µg/g (log value = -3.0 µg/g). Fitted lines are significant ordinary least-squares regressions: black fitted lines are modelled using the full dataset and blue dashed fitted lines are modelled using only lake-level predictions > MDL. Model equations, R2 and p-values are also shown within plots and are colour coordinated with fitted lines.

when modelling only size-adjusted population means > MDL. Five lakes with the highest PC4 scores (i.e., characterized by low DOC and true colour, as well as higher sodium and sulphate concentrations) and [As] < MDL were all located within the Lake Wabigoon Ecoregion and appear to partially drive the negative relationship between northern pike [As] and PC4 (Figure SI-12). However, several lakes with the lowest PC4 scores and relatively higher [As] in northern pike were not spatially related. A positive significant relationship was also observed between northern pike [As] and PC2 only when modelling size-adjusted population means > MDL (Figure SI-12).

A positive and relatively strong (R^2 = 0.44-0.51, AIC weight = 1) relationship between lake whitefish [As] and PC1 (representing low [Ca], [Mg], Conductivity, DIC, pH) was observed when modelling both with the full dataset and the subset of data with size-adjusted population means < MDL removed (Figure 7). These results were based on a single model selected by AICc ranking (Table 3). The most dominant variables represented by PC1 (based on coefficients, Table SI-2) all had negative loadings, suggesting that fish from waterbodies with higher PC1 scores (i.e., lower pH, conductivity and ions) had slightly higher lake whitefish [As]. Figure 7 shows negative relationships of lake whitefish [As] and water chemistry variables with the highest loadings on PC1. Delaney lake had the highest lake whitefish [As] in this dataset (size-adjusted mean = 0.35 μg/g; 95% credibility interval = 0.18–0.68) and appeared to drive the relationship between [As] and ions when modelling the full dataset. With Delaney Lake removed, modelling data > MDL still showed significant or near significant negative relationships to lake whitefish [As] for alkalinity (R^2 = 0.47; p = 0.02), conductivity (R^2 = 0.40; p = 0.04), magnesium (R^2 = 0.37; p = 0.05), DIC (R^2 = 0.30; p = 0.08) and pH (R^2 = 0.33; p = 0.06). A negative significant relationship was also observed between lake whitefish [As] and

PC4, although this relationship was no longer detected when modelling only size-adjusted population means > MDL.

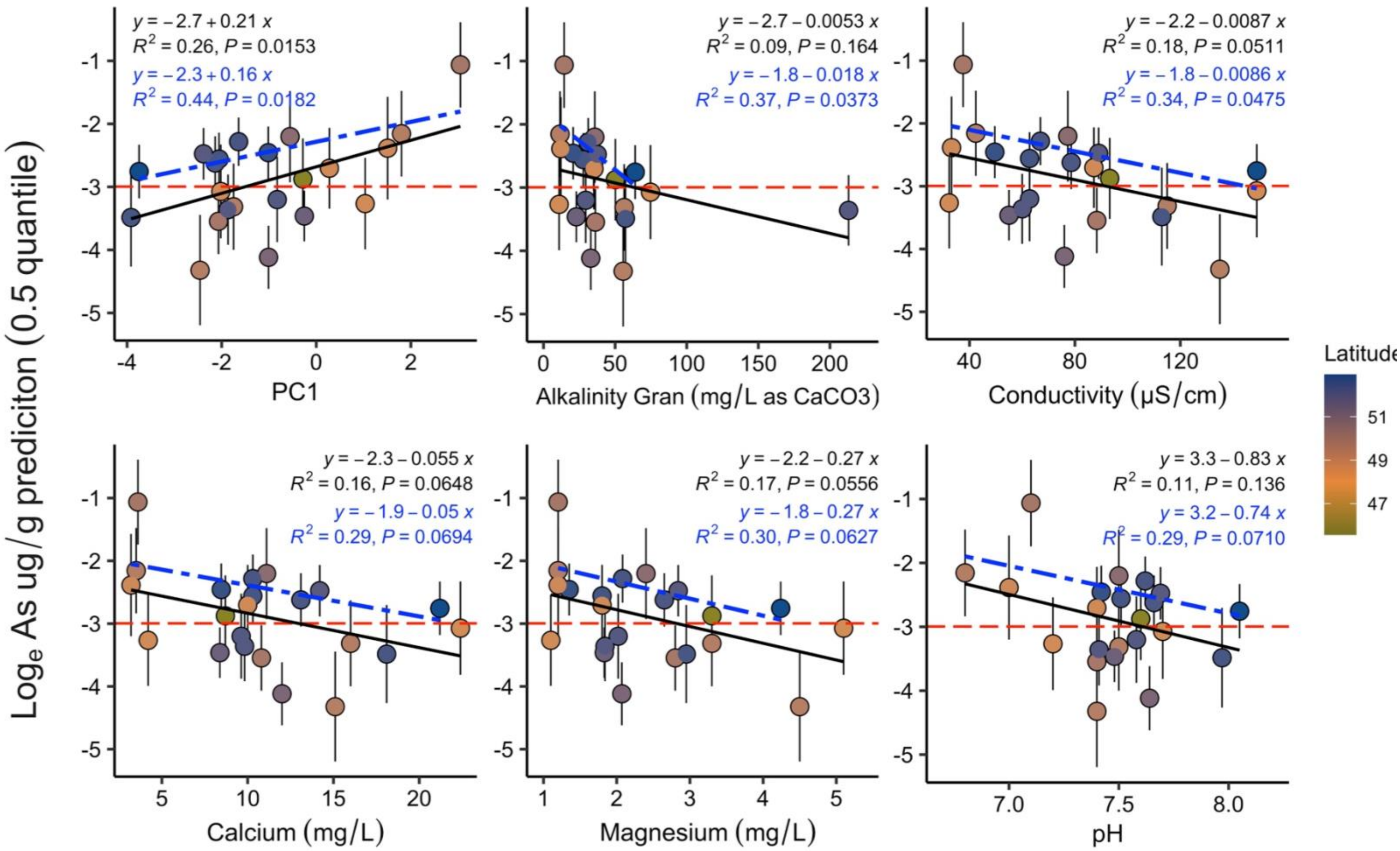

Figure 7. Relationships between $\log_e$ size standardized lake whitefish [As] predictions and PC1 scores (top-left panel). Bivariate relationships of individual predictors that dominate PC1 are also shown. The red dashed line represents MECP MDL of 0.05 µg/g (log value = -3.0 µg/g). Fitted lines are ordinary least-squares regressions: black fitted lines are modelled using the full dataset and blue dashed fitted lines are modelled using only lake-level predictions > MDL. Model equations, R^2 and p-values are also shown within plots and are colour coordinated with fitted lines.

No significant relationships were observed between smallmouth bass [As] and any PCs, despite the moderately strong model metrics (i.e., AIC weight = 0.21-0.51; Table 3 and Figure SI-13). The top model selected included only a single predictor variable (PC2, representing DOC and true colour), but this relationship along with any relationship between PC4 appears to be highly influenced by Moira Lake (Figure SI-13), a lake in eastern Ontario with a history of mining and smelting-related As-contamination (Azcue and Dixon 1994). Modelling smallmouth bass [As] using only size-adjusted population means > MDL was not possible due to limited sample size (n = 7).

4. Discussion

4.1 Total [As] in Ontario fish and implications for consumers

Total [As] in popular subsistence and gamefish species (e.g., walleye, northern pike) were generally low in most inland waterbodies, likely posing little risk to health of fish consumers. While individual fish occasionally had elevated concentrations, the vast majority of fish within this dataset had [As] that were well below all benchmarks used to determine consumption recommendations by MECP. Total arsenic concentrations were comparable to those found in freshwater systems in the northern United States by Eisler (1988), who reported total [As] ranging from <0.05-0.28 µg/g, 0.03-0.13 µg/g, <0.05-0.09 µg/g, and 0.06-0.68 µg/g in in smallmouth bass, white sucker, northern pike, and lake trout, respectively.

Fish from inland waterbodies with known As-contamination issues (i.e., Long and Moira lakes) had some of the highest [As]. In fact, 25% of lacustrine fish with [As] > 1 µg/g were cisco sampled from Long Lake, Sudbury, including the highest [As] observed from any lake site (i.e., 6.52 µg/g wet wt.). Jankong et al. (2007) similarly reported approximately 10-fold higher [As] in freshwater fish from contaminated ponds in Thailand, when compared to a reference site. There is also some evidence that planktivorous fish species, such as cisco, accumulate As at a higher rate than omnivorous species (USEPA 1999; Chen and Folt 2000); this may help further explain these elevated [As], as cisco comprise a relatively small proportion of the dataset herein (i.e., 3.2% of total observations). Blackwater River was the only river site with no marine influence in this dataset with fish that had [As] > 1 µg/g, potentially because of abandoned gold mines in the area. However, while fish from sites with abandoned mines in their watersheds had significantly higher [As] to those without, these differences were small and do not constitute a major difference in consumption risk. Nevertheless, monitoring sites such as Long Lake with known point sources of As is still important for evaluating risk of contaminant exposure. Other lakes with relatively elevated [As] in fish were Black Trout Lake and Catfish Lake, which are within approximately 1 km of each other, though I am unaware of any anthropogenic sources of As for this area.

Fish from coastal rivers draining to Hudson or James Bay had consistently higher [As] when compared to those with no potential for marine influence. This trend was most pronounced for cisco and lake whitefish, both species that are known to utilize marine environments from northern Ontario coastal rivers (DeJong 2017). Northern pike, which followed a similar trend, although to a lesser extent, have also been shown to have relatively high marine-derived resources in their diet in these northern rivers (DeJong 2017). Arsenic is often a greater concern in marine fish (Kumari et al. 2017), possibly due to higher background concentrations in sea water and higher accumulation rates by lower trophic organisms, such as marine algae (Sanders and Windom 1980). It is also possible that As biotransformation pathways differ in marine and freshwater ecosystems (Caumette et al. 2012). While these pathways are not well understood, such differences could alter As speciation profiles in organisms, which could in-turn affect As cycling and retention in fish (Erickson et al. 2019).

No assessment of As speciation was conducted on fish studied herein. Arsenic species in fish muscle are variable, however arsenobetaine (a non-toxic As species; Tao and Bolger 2014) commonly comprises > 90% of As in marine fish (Luvonga et al. 2020) and is often the most common As compound in freshwater fish. However, some studies suggest that As speciation in freshwater biota may be more variable than in marine biota (Caumette et al. 2012). A recent study by Tanamal et al. (2021) found fish from several lakes in Northwest Territories, Canada had variable arsenobetaine proportions (mean±sd = 58.6±34.5%). More studies on As speciation in boreal systems are needed, particularly for Ontario fish and those with dietary contributions from marine environments.

4.2. Influence of fish size on [As]

Increasing [As] with higher fish weight suggests the potential for As to bioaccumulate as fish grow, while a negative or non-existent relationship to fish size suggests As is not accumulating at a faster rate than it can be expelled or diluted with growth. Past studies have reported mixed effects of fish size on [As] in freshwater fish, with some studies providing evidence for both positive and negative relationships, while others found no relationship (Burger et al. 2002; Chételat et al. 2019; Lescord et al. 2020). In the present study, [As] often had a positive but weak relationship or no relationship with fish weight, although there was much variation both among species and waterbodies. It should be noted that consumption recommendations in the provincial monitoring programs are based on fish size; it is unclear what the implications of these varying relationships are for this method. As other studies have suggested, these inconsistencies may be related to differences in exposure related to background As concentrations and speciation, food web composition, and other biotic and abiotic conditions affecting As bioavailability (Azizur Rahman et al. 2012). Seasonality may further complicate this, as a study of anadromous arctic charr collected during summer months showed no significant relation to fish body size, while a relatively strong relationship was observed from fish collected post-winter (Martyniuk et al. 2020).

4.3. Influence of dietary sources and trophic ecology on fish [As]

The proportion of pelagic carbon represented in fish diet appeared to have some relation to [As] for some species when pooling data within species and comparing across multiple lakes. Several studies have examined relationships between [As] and dietary carbon, which is typically inferred from relative $\delta^{13}C$ signatures; some have reported higher [As] in fish with more depleted $\delta^{13}C$ within sites, suggestive of more pelagic-based feeding (Chen et al. 2008; Chételat et al. 2019; Donadt et al. 2021), while others reported no relationship to dietary carbon (Martyniuk et al. 2020). In my study, significant positive regressions were observed for several species (i.e., walleye, northern pike and smallmouth bass) across sites, indicating fish that occupy offshore zones in their respective lakes tend to accumulate slightly more As. This may be due to differences in As cycling in lower order organisms associated with benthic and pelagic zones (e.g., phytoplankton *vs* algae; Caumette et al. 2014), although more research is needed to understand As cycling in freshwater systems. Chételat et al. (2019) suggested the negative correlation they observed between fish [As] and $\delta^{13}C$ was possibly a result of differences among fish species (i.e., higher [As] in pelagic species such as cisco driving the relationship); the opposite may also be true, in that species differences may also make detecting a relationship between $\delta^{13}C$ and [As] more challenging. Furthermore, lakes used in this analysis cover a wide range of latitudes with variable terrestrial carbons inputs which can affect $\delta^{13}C$ in invertebrates and fish (Pace et al. 2004) and be difficult to distinguish from aquatic carbon sources (France 1997; Post 2002). Utilization of terrestrial carbon sources has been shown to vary due to factors such as lake size, (Wilkinson et al. 2013), food web composition and is assimilated by both benthic and pelagic invertebrates (Solomon et al. 2011; Scharnweber et al. 2014), however, in what way this may affect interpretation of pelagic and benthic endpoints is uncertain.

Arsenic has been shown to biodiminish from lower trophic organisms like phytoplankton, zooplankton and invertebrates to higher trophic organisms like fish (Chen and Folt 2000). Most previous studies have found similar patterns of biodilution (Rogowski et al. 2009; Donadt et al. 2021), or no relationship to trophic position among freshwater fish species (Liu et al. 2018; Chételat et al. 2019; Yang et al. 2020). My findings are consistent with the literature in four of eight lakes observed, which showed no evidence of As biomagnification among fish species and [As] were negatively correlated with $\delta^{15}N$ one lake (i.e., Fishtrap Lake). However, three lakes showed evidence of biomagnification among fish. Slope estimates from significant biomagnification models (range = 0.16-0.41) were comparable to those for mercury (global trophic magnification slope average = 0.2; Lavoie et al. 2013), suggesting relatively strong biomagnification in these particular lakes. It is noteworthy the biomagnification models herein were performed across fish species only; it is possible that the inclusion of invertebrates would nullify the trends, due to their previously mentioned elevated concentrations when compared to fish. Furthermore, because different fish species were included in each of the eight biomagnification models, direct comparisons between sites should be made with caution. Limited evidence of As biomagnification has been shown to occur in some marine food webs

(Barwick and Maher 2003; Du et al. 2021) and anadromous fish species (Martyniuk et al. 2020), although we are unaware of other studies showing evidence of As biomagnification in a freshwater system.

4.4. Influence of water chemistry and landscape variables on fish [As]

While several previous studies have identified watershed scale variables related to mercury concentrations in fish (Eagles-Smith et al. 2016; Sumner et al. 2019), none, to my knowledge, have reported significant relationships between landscape-level variables and fish [As]. Lescord et al. (2020) investigated the role of geological features on fish [As] in the far north of Ontario and suggested that the geographic scale of their dataset was too broad to detect meaningful relationships, especially within river systems where fish are mobile. I also did not observe any notable differences in [As] influenced by geology, however the ability to test these relationships statistically was limited by small sample sizes from some geology types.

Legacy mining impacts continue to present risks associated with As-contamination in surface waters, sediments and aquatic organisms in Canada (Azcue and Dixon 1994; Webster et al. 2015; Cott et al. 2016) and globally (Smedley and Kinniburgh 2002). Mining is heavily regulated in Ontario through mandatory closure plans for developers which include requirements for site rehabilitation after operations have ceased. However, all known closure dates for abandoned mine sites used in my dataset were prior to 1989. Our results suggest that higher [As] may be associated with fish from lakes with abandoned mine sites present in their watershed, emphasizing the importance of effective mine closure planning to reduce the risk of contaminants, such as As.

In this study, relationships between fish [As] and water chemistry/landscape variables (i.e., through PCs) were inconsistent among fish species. Despite these variations, I found evidence of multiple relationships with water chemistry variables and fish [As]. My results suggest that walleye and northern pike from sites rich in NO_3^-/ NO_2^- and walleye from sites low in TP had slightly higher [As]. Some evidence has shown that As mobility (which is closely related to Fe cycling) may be affected by NO_3^-in water (Smith et al. 2017; Gao et al. 2021) and higher occurrences of arsenate relative to arsenite have been observed as a result of increased NO_3^- (Senn and Hemond 2002), however understanding of how these processes may directly affect As bioaccumulation under natural conditions is currently unclear. Furthermore, arsenate uptake by basal organisms (e.g., phytoplankton) is thought to occur inadvertently through phosphate uptake mechanisms (Hellweger and Lall 2004) and may compete for binding sites in P-rich environments in some species of microalgae (Wang et al. 2017) and aquatic plants (Mkandawire et al. 2004). However, competition between As and phosphate is not ubiquitous among all aquatic species (Neff 1997; Wang et al. 2019), and little is known about how it may affect As bioaccumulation in fish. It is noteworthy that walleye and northern pike were the most abundant fish in our dataset and thus additional lakes, potentially with more variable water chemistry, were included in the modeling exercise for these species.

Relationships were also observed between lake whitefish [As] and various measures of ionic strength (i.e., PC1, pH, conductivity, ion concentrations, etc.), all of which suggested that sites with lower aqueous ionic strength had higher piscine [As]. Because variables dominating PC1 are all highly correlated, speculation of causal links between any individual variable represented in PC1 and lake whitefish [As] is difficult without similar studies for comparison. A possible explanation is that elevated concentrations of calcium carbonate (often used to remediate As-contaminated water) may reduce mobility of dissolved As through precipitation of inorganic As species (Bothe and Brown 1999). It is unclear why the negative relationship between fish [As] and ionic strength was observed in lake whitefish, but not in other fish species. In general, lake whitefish are considered an offshore invertivorous species, which is in contrast to the other predatory fish (i.e., smallmouth bass, walleye, and northern pike) examined in this modeling exercise. It is therefore possible that the effects of ionic strength on fish As accumulation are somehow related to off-shore or profundal processes, more so than near-shore.

Conclusion

Contaminant monitoring by MECP, MNRF and others has been extensive and of great benefit to fish consumers. Mercury and persistent organic pollutants have been the primary focus of monitoring programs in Ontario fish, while contaminants such as As have received comparatively less attention, largely due to As generally posing limited health risks in comparison to these other contaminants of concern and because of costs and complexity associated with analyses. My results further findings by Lescord et al. (2020) that As is a contaminant of concern in some fish from rivers in the far north of Ontario, based on [As] relative to benchmark levels. River systems in the far north of Ontario are of great importance to First Nation communities and are often used as a means of transportation and a source of subsistence fish. In addition to As, other contaminants such as mercury and chromium have also been shown to be of concern in fish from these northern regions emphasizing the need for continued monitoring, especially with proposed plans for mining development in the near future. Total arsenic concentrations in most other sampled waterbodies across the province appear to pose little risk to fish consumers with some exceptions that are typically associated with point sources of As-contamination such as mine tailings.

Arsenic may accumulate to higher concentrations in larger fish in some Ontario waterbodies, however there is so much variation in these relationships that fish size does not appear to be a reliable predictor of fish [As] in general at this time. However, employing broad-scale datasets in combination with Bayesian LMEMs may be an effective method of predicting [As] among size classes of fish that can utilize limited sample sizes and obtain measures of uncertainty for parameter estimates.

In addition to generating the fish [As] relationships that I was able to, my study demonstrates the value of using previously collected data from a combination of diverse programs and open data sources to test hypotheses while eliminating the requirement for field collections.

Through this study I provide evidence of As bioaccumulation and biomagnification across fish species in several waterbodies observed. For several species, fish with higher proportion of their diet based on pelagic carbon appeared to have higher [As] than those with more benthic-based diets. Further investigation using fish [As] and stable isotope ratios for additional waterbodies is necessary for determining the extent of As bioaccumulation and biomagnification in fish from freshwater systems in Ontario.

A mix of water chemistry and landscape variables appeared to play some role in fish [As]. Because many of the water chemistry parameters used in this study are those that are commonly analyzed for, and the landscape features I used can be readily generated using open data sources, my findings can be used to select monitoring sites for more detailed studies into the factors affecting [As] in fish. For example, the Abandoned Mines Information System in combination with watershed mapping may provide useful tools in selecting sites at greater risk of metal contamination due to legacy mining impacts. Our findings may also help with site selection for monitoring As in both disturbed and undisturbed waterbodies with high NO_3^-/NO_2^-, low TP and low concentrations of major ions such as calcium.

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Supplemental Information

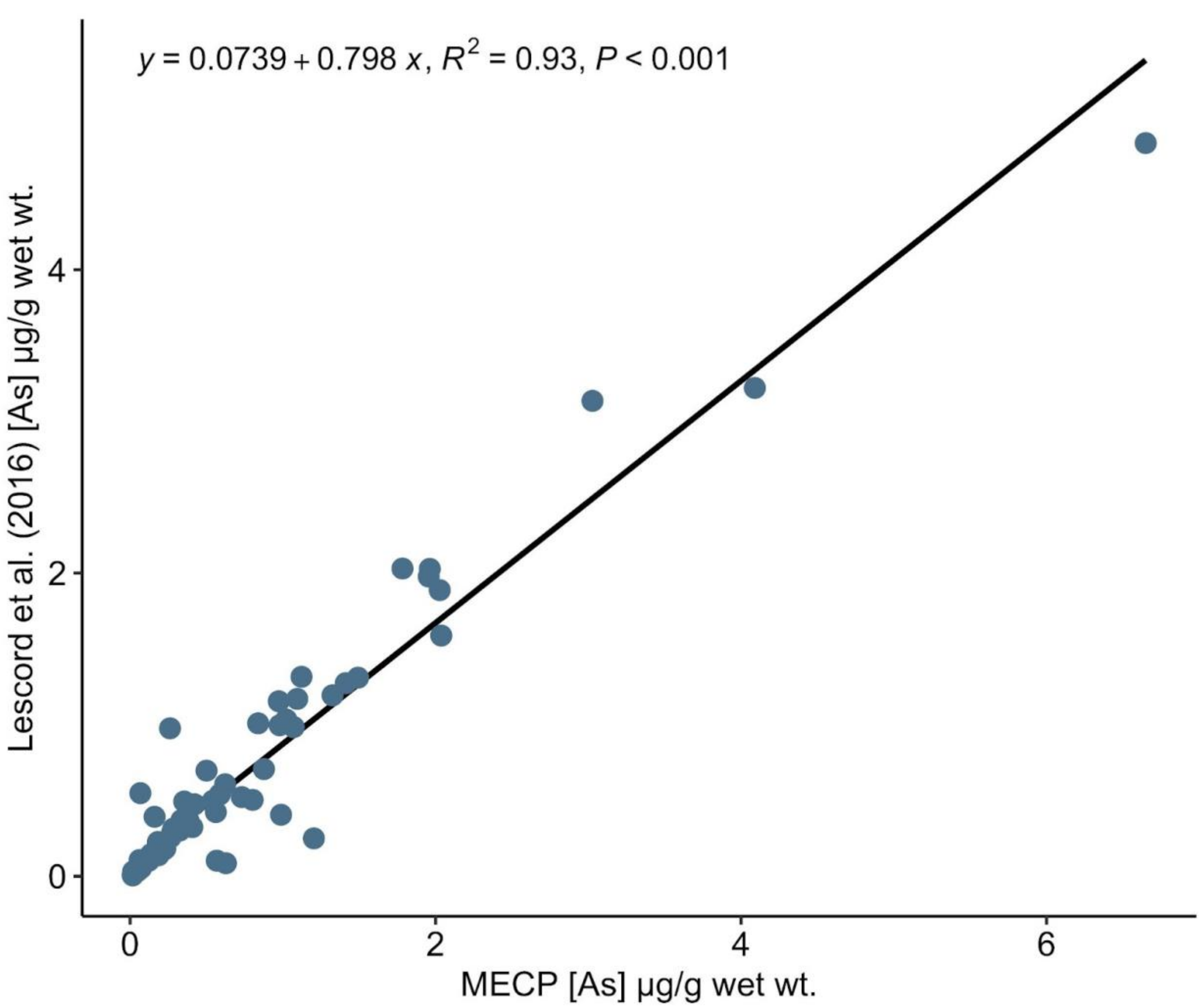

Figure SI-1. Comparison of fish (n = 77) analysed by both MECP and Lescord et al (2020). Black line represents least-squares regression fit. Model equations, R^2 and p-values are also shown within the plot.

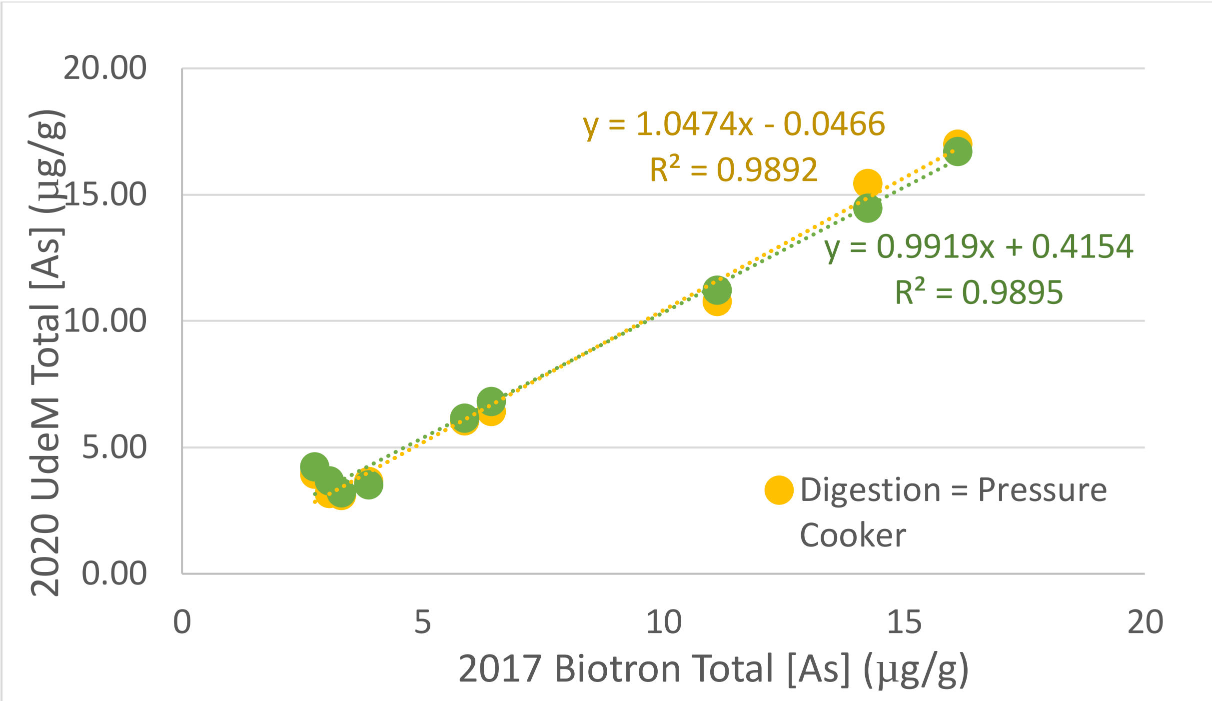

Figure SI-2. A comparison of total arsenic concentrations in 9 of the samples from this study across labs and digestion methodologies. All concentrations are on a dry weight basis. UdeM = the University of Montreal. Biotron = the Biotron Trace Metal Laboratory at Western University.

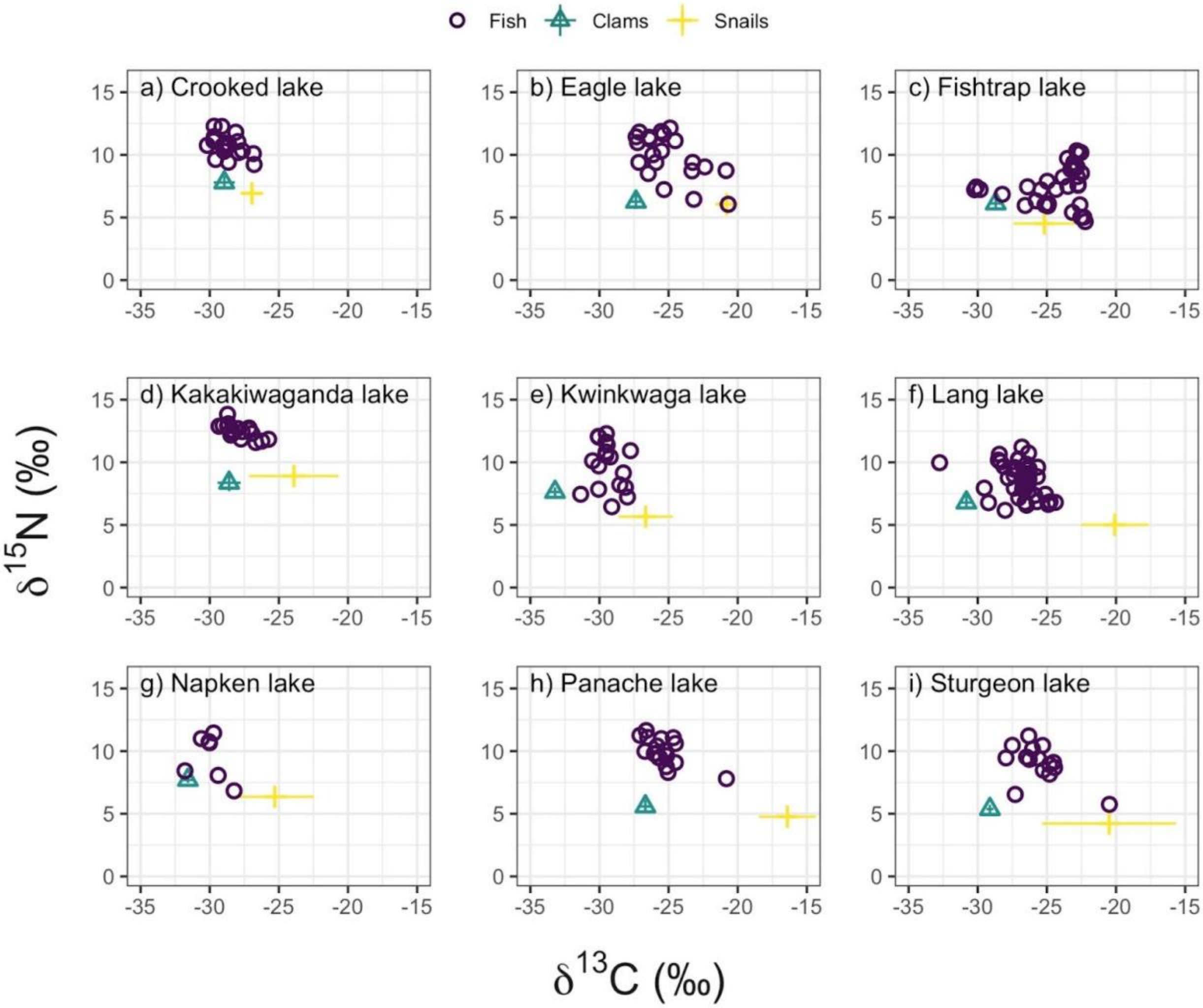

Figure SI-3. Biplots showing stable carbon and nitrogen isotope ratios for fish and baseline indicator species (i.e., clams and snails) for nine lakes in Ontario, Canada.

Table SI-1. Results of Welch's t-tests comparing total muscle arsenic concentrations between lacustrine and riverine fish. Negative t-statistics indicate higher group mean for riverine fish. Note: Size was not accounted for in this model.

Species	N lacustrine fish	N riverine fish	t	p[a]	d.f.
Brook trout	**23**	**83**	**-20.34**	**<0.001**	**51.07**
Cisco	**58**	**43**	**-7.75**	**<0.001**	**74.72**
Longnose suckers	16	33	1.06	0.301	25.65
Lake whitefish	**194**	**153**	**-12.94**	**<0.001**	**242.37**
Northern pike	**529**	**303**	**-13.34**	**<0.001**	**463.52**
Walleye	**447**	**257**	**-10.39**	**<0.001**	**326.62**
White sucker	**226**	**85**	**-2.84**	**0.005**	**205.32**
Yellow perch	**75**	**21**	**3.66**	**0.001**	**32.06**

[a] Bolded values represent significance with $p \leq 0.05$.

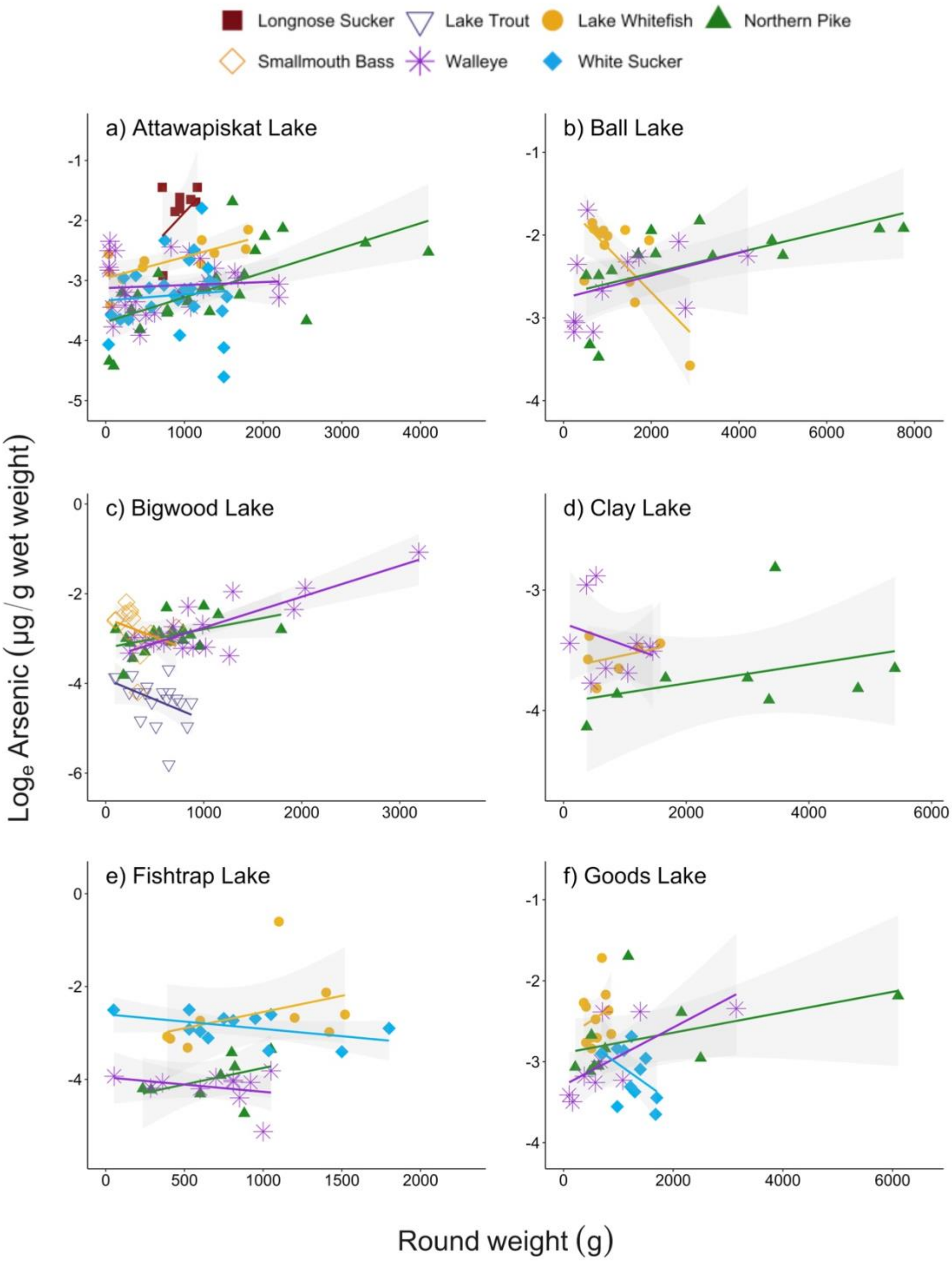

Figure SI-4a. Scatterplots showing relationship between total arsenic muscle concentrations among fish species for 6 lakes in Ontario, Canada. Lines represent ordinary least squares regression fits, with grey shading representing 95% confidence intervals.

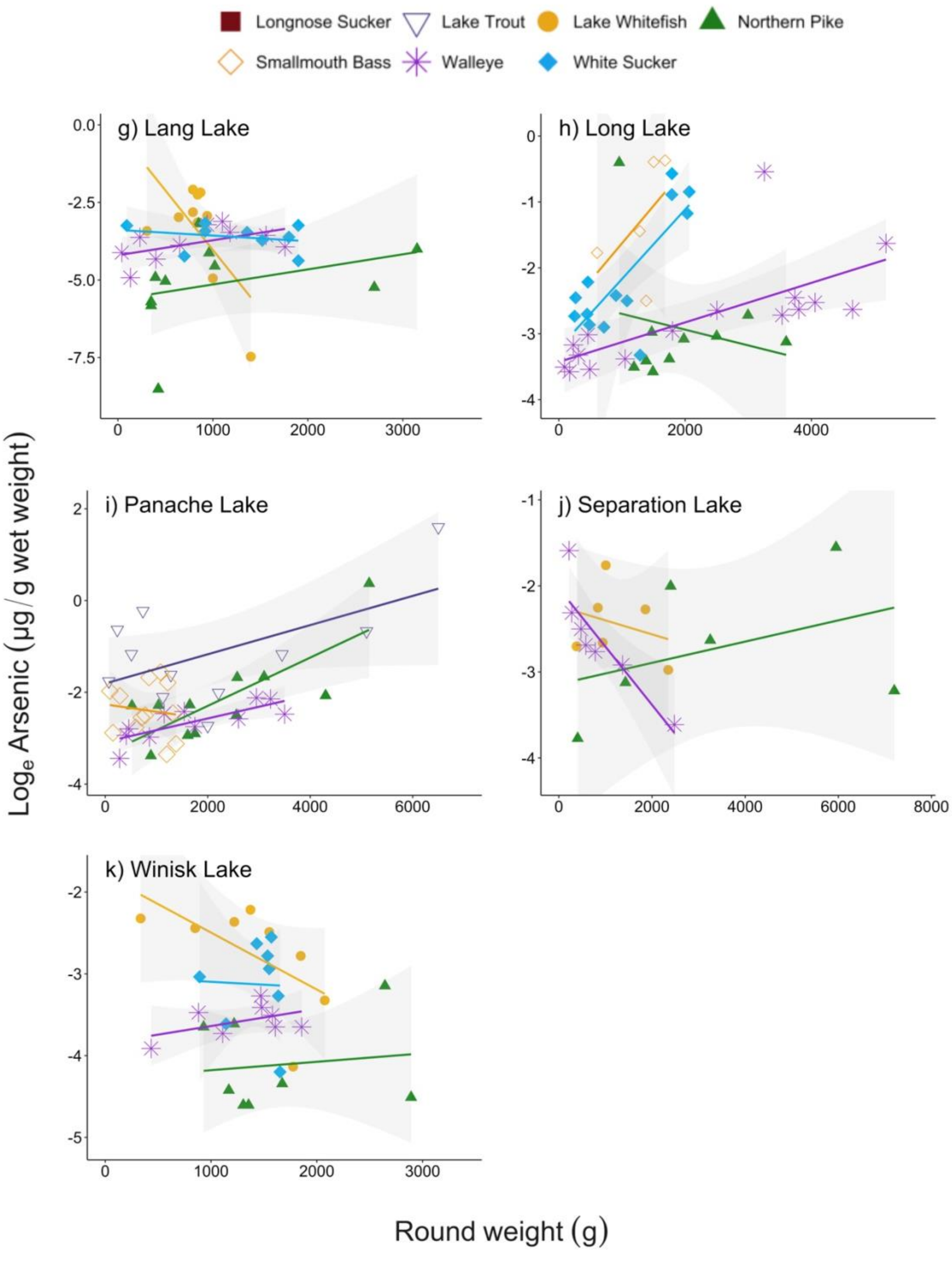

Figure SI-4b. Scatterplots showing relationship between total arsenic muscle concentrations among fish species for 5 lakes in Ontario, Canada. Lines represent ordinary least squares regression fits, with grey shading representing 95% confidence intervals.

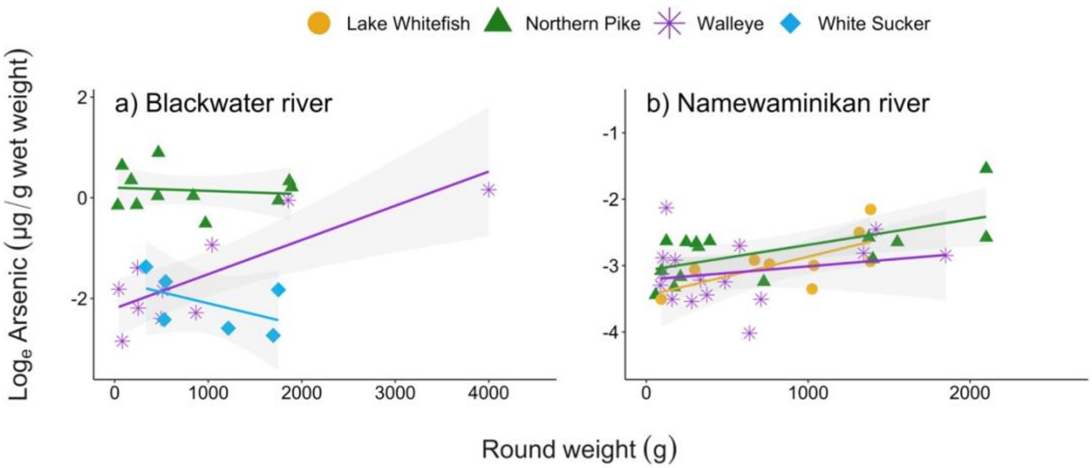

Figure SI-4c. Scatterplots showing relationship between total arsenic muscle concentrations among fish species for 2 rivers in Ontario, Canada. Lines represent ordinary least squares regression fits, with grey shading representing 95% confidence intervals.

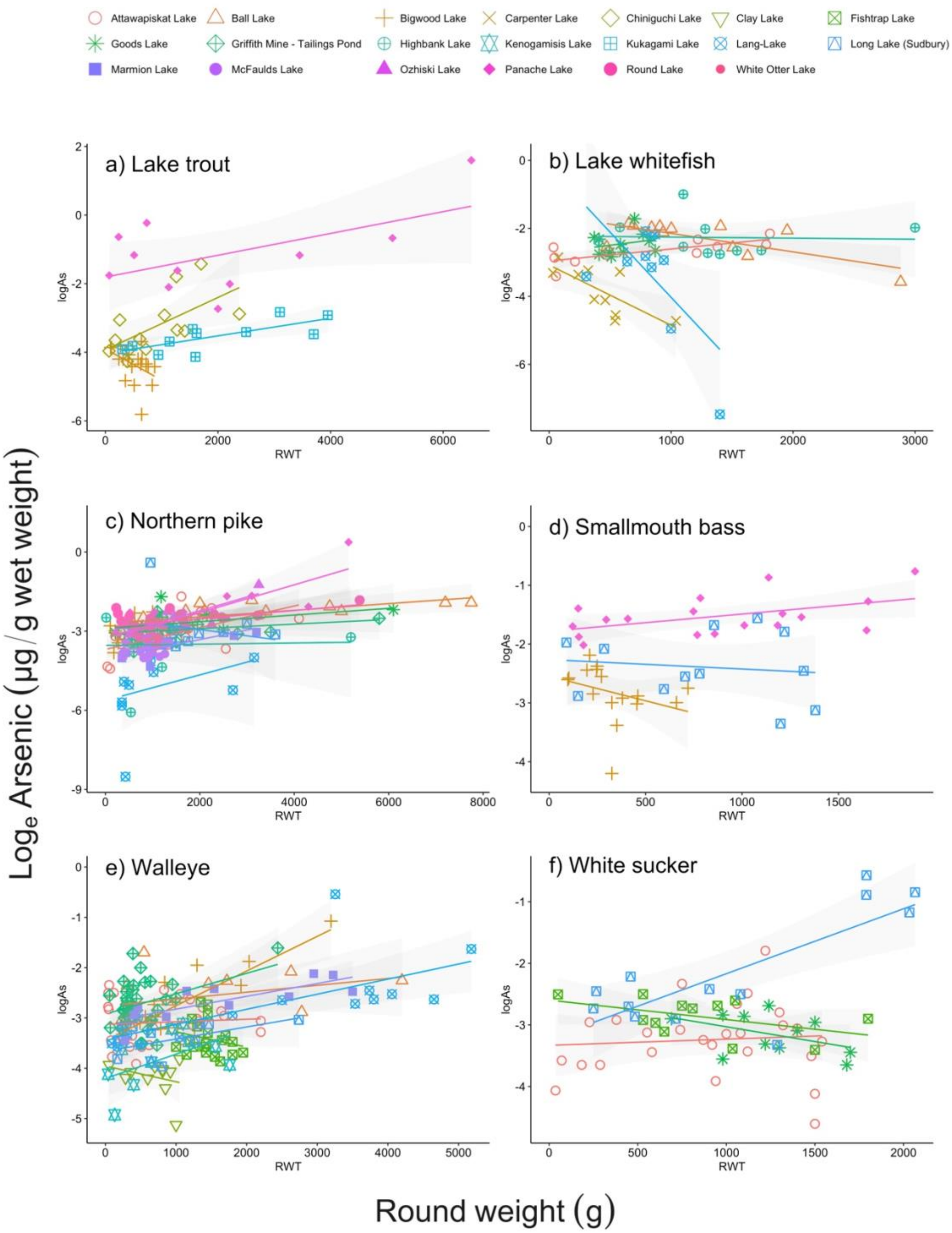

Figure SI-5. Scatterplots showing relationship between total arsenic muscle concentrations among lakes for six common fish species in Ontario, Canada. Lines represent ordinary least squares regression fits, with grey shading representing 95% confidence intervals.

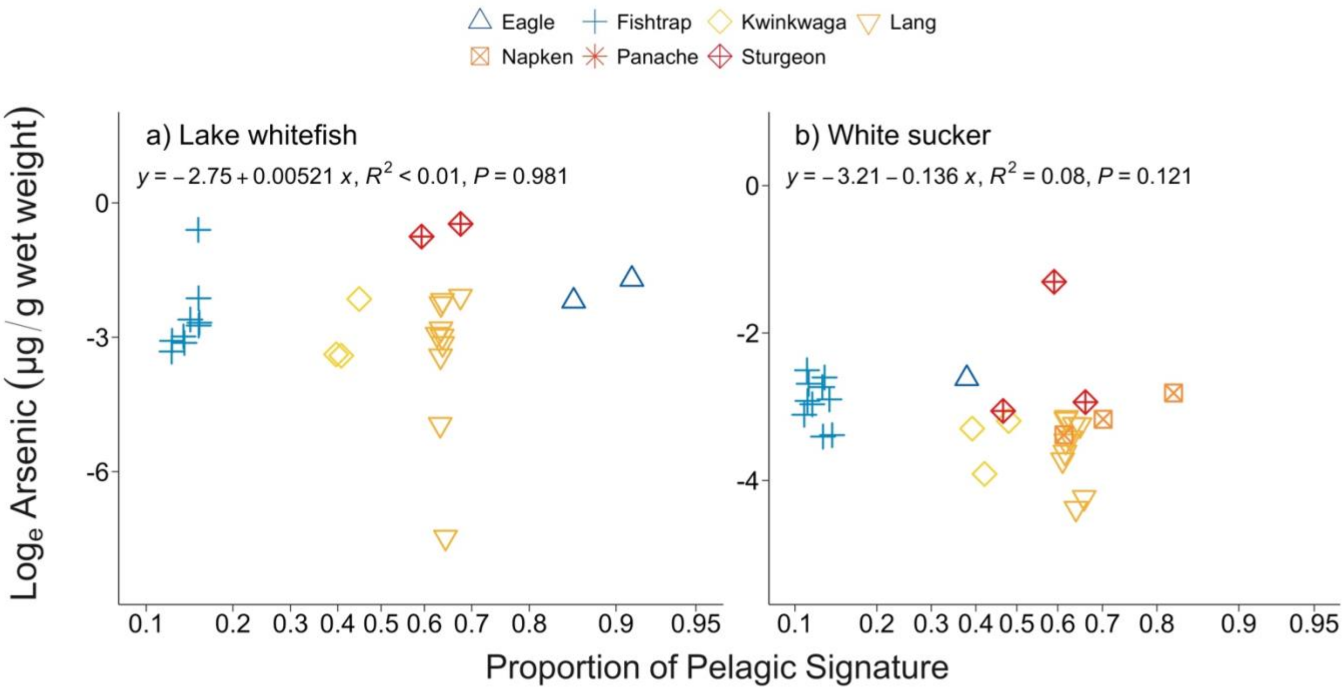

Figure SI-6. Relationships between $\log_e$ total arsenic in fish muscle tissue and proportion of fish diet from pelagic sources derived from SIMMs. Equations, R^2 and p-values are based on ordinary least square regression with logit transformed proportion of pelagic signature

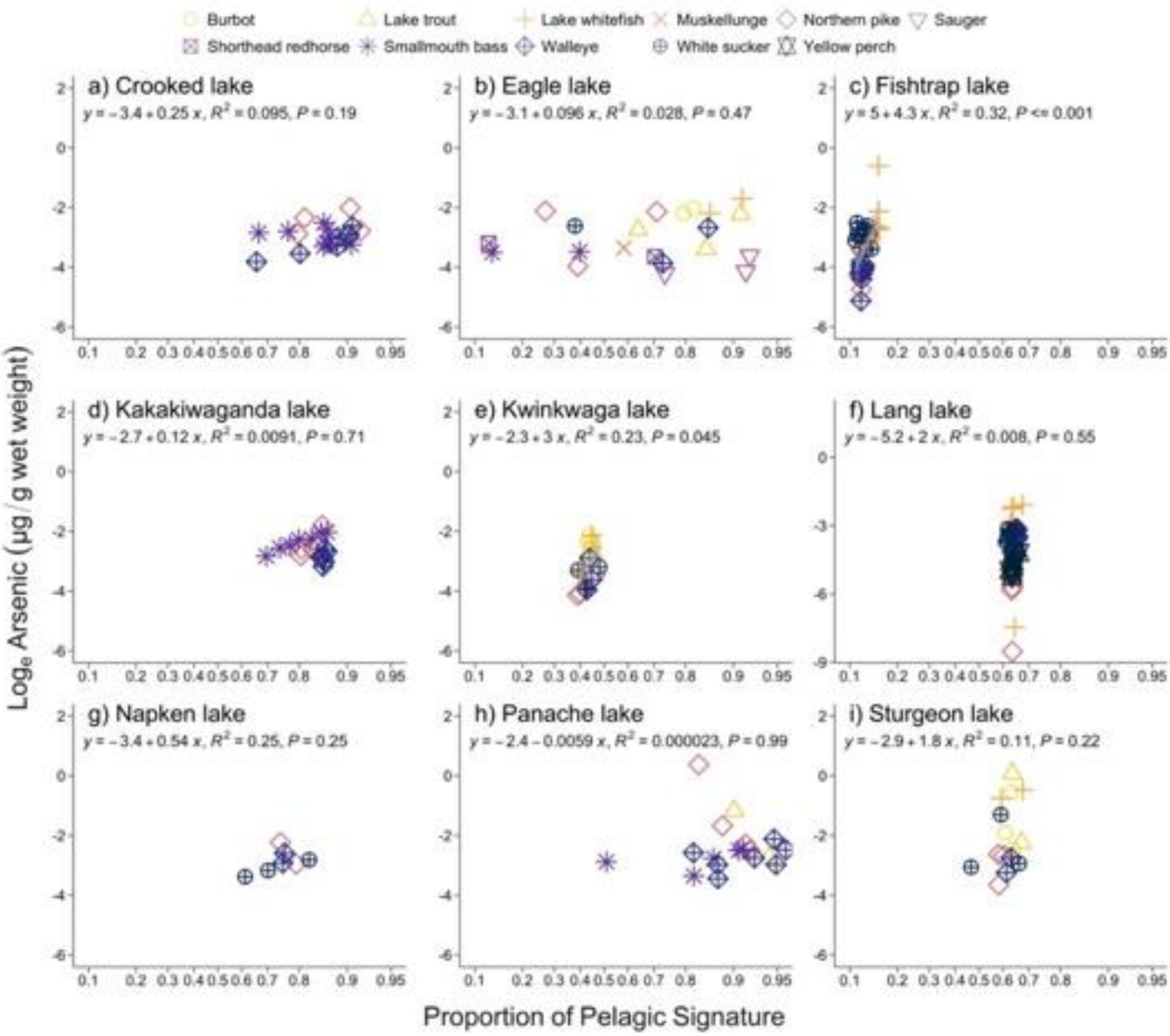

Figure SI-7. Relationships between loge total arsenic in fish muscle tissue and proportion of fish diet from pelagic sources. Proportion of diet from pelagic signature were derived from stable isotope mixing models using R 4.0.5 (R Core Team 2021) and the 'simmr' package (v0.4.5; Parnell 2021) with clams and snails as baseline indicator species. Equations, R2 and p-values are based on ordinary least square regression with logit transformed proportion of pelagic signature; grey lines of best fit are shown for significant regressions (p<0.05). Note: Lang Lake y-axis shows a greater range than other plots due to low loge [As] values.

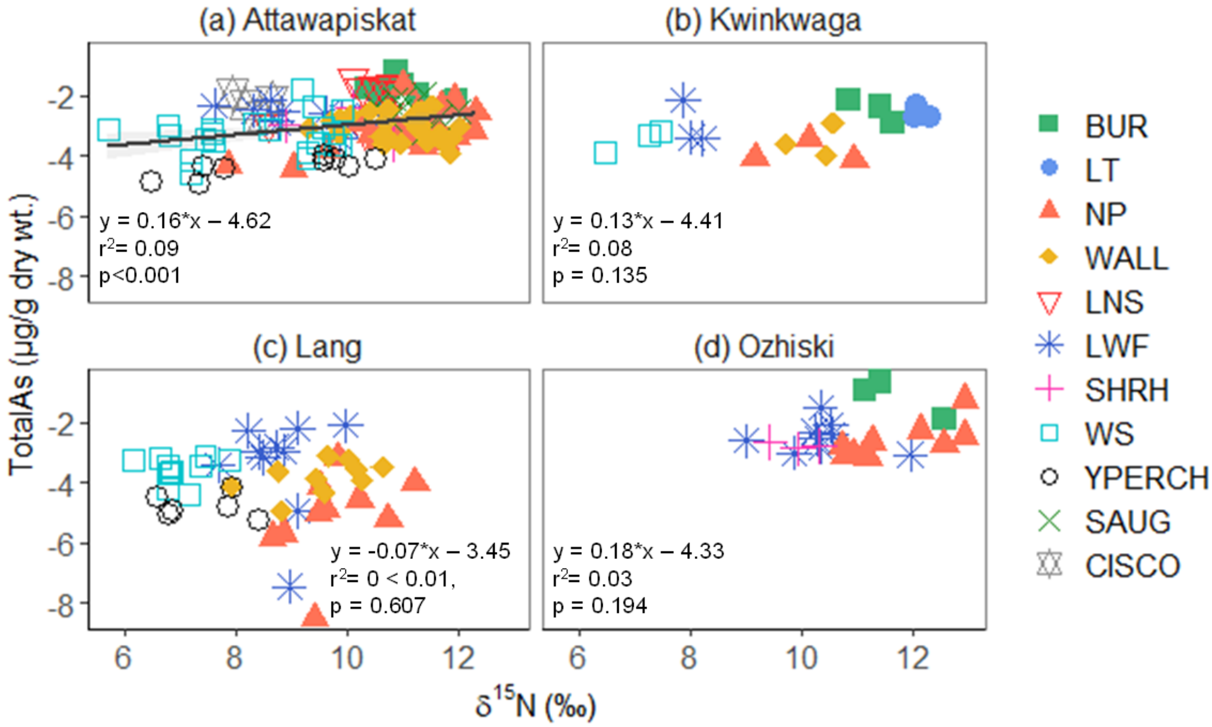

Figure SI-8 - Arsenic biomagnification models through the food webs of four lakes. Note that different fish species are present in each of the four models. Line significance was assessed using a linear regression. Species abbreviations: BUR = burbot, LT = lake trout, NP = northern pike, SMB = smallmouth bass, WALL = walleye, FWDRUM = freshwater drum, GOLDEYE = golden eye, LNS = longnose sucker, LWF = lake whitefish, SHRH = shorthead red horse, WS = white sucker, YPERCH = yellow perch, SAUG = sauger, CISCO = cisco. Four additional biomagnification models are shown in Figure 4 of the main manuscript.

Table SI-2. Matrix of variable loadings of the six principal components extracted from a principal component analysis.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Watershed Area	-0.079	-0.003	0.540	-0.157	0.198	0.083
Lake Area	-0.095	0.007	0.095	-0.105	0.849	0.151
DA:LA	-0.044	-0.004	0.534	-0.188	-0.187	-0.067
DIC	-0.337	0.214	-0.056	-0.047	-0.030	0.076
Median Slope	0.232	0.246	0.077	0.293	-0.006	-0.099
Median Elevation	0.162	0.139	0.184	0.200	-0.017	0.608
Mean CTI	-0.211	-0.218	-0.123	-0.383	-0.036	0.168
Alkalinity	-0.289	0.156	-0.091	-0.025	-0.118	0.143
NH_3/NH_4^+	-0.119	-0.101	0.109	0.539	-0.155	0.309
Ca	-0.334	0.214	-0.071	-0.094	-0.073	0.047
Mg	-0.326	0.229	-0.032	-0.011	-0.043	0.095
Conductivity	-0.310	0.277	-0.043	-0.066	-0.063	0.002
DOC	-0.221	-0.326	0.072	0.085	-0.121	0.093
NO_3/NO_2	0.138	0.117	0.302	-0.308	-0.177	0.288
pH	-0.343	0.045	-0.056	0.080	0.077	-0.022
K	-0.108	0.273	0.263	0.314	0.055	-0.071
Na	-0.037	0.351	0.126	-0.011	-0.081	-0.296
SO_4	0.170	0.315	0.078	-0.188	-0.105	-0.196
TKN	-0.254	-0.229	0.128	0.275	0.034	-0.205
TP	-0.168	-0.191	0.293	0.106	0.081	-0.400
True Colour	-0.114	-0.331	0.186	-0.141	-0.272	0.016
Eigenvalue	6.423	4.498	2.529	1.500	1.111	1.089
Variance Explained (%)	31	21	12	7	5	5
Sum Variance Explained (%)	31	52	64	71	76	82

Bolded values indicate dominant variables (loading of ≥ 0.3)

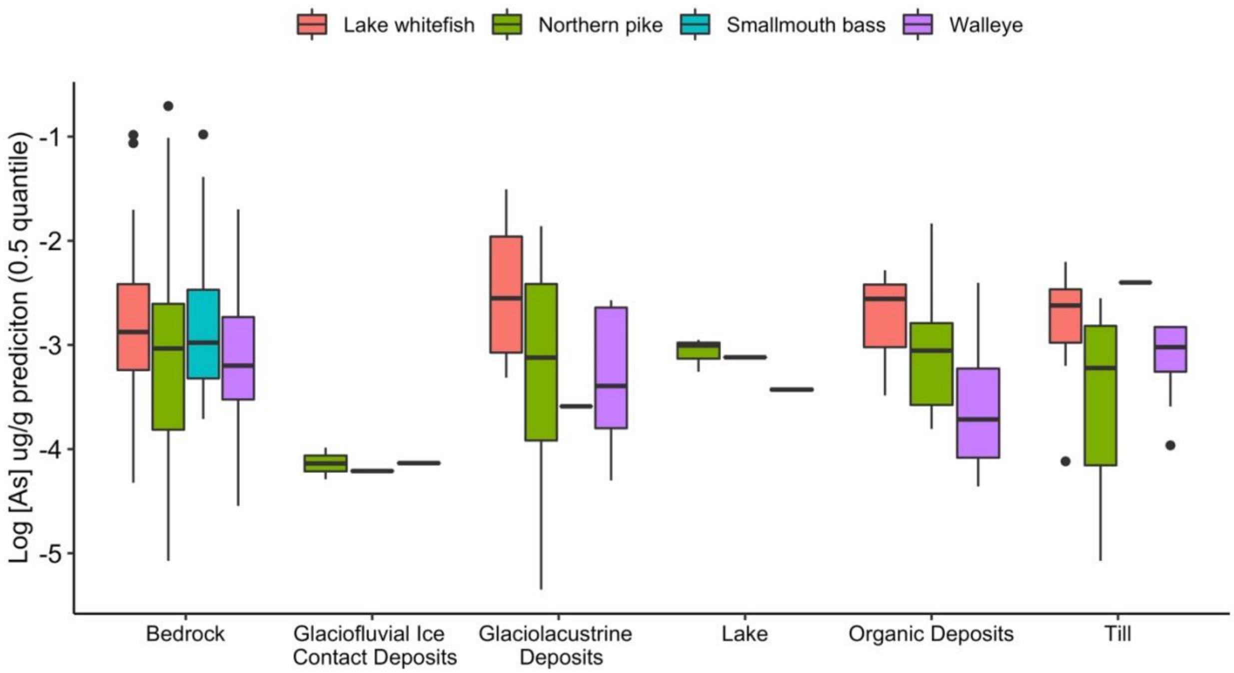

Figure SI-9. Box plots showing arsenic concentrations for four fish species by dominant geology type.

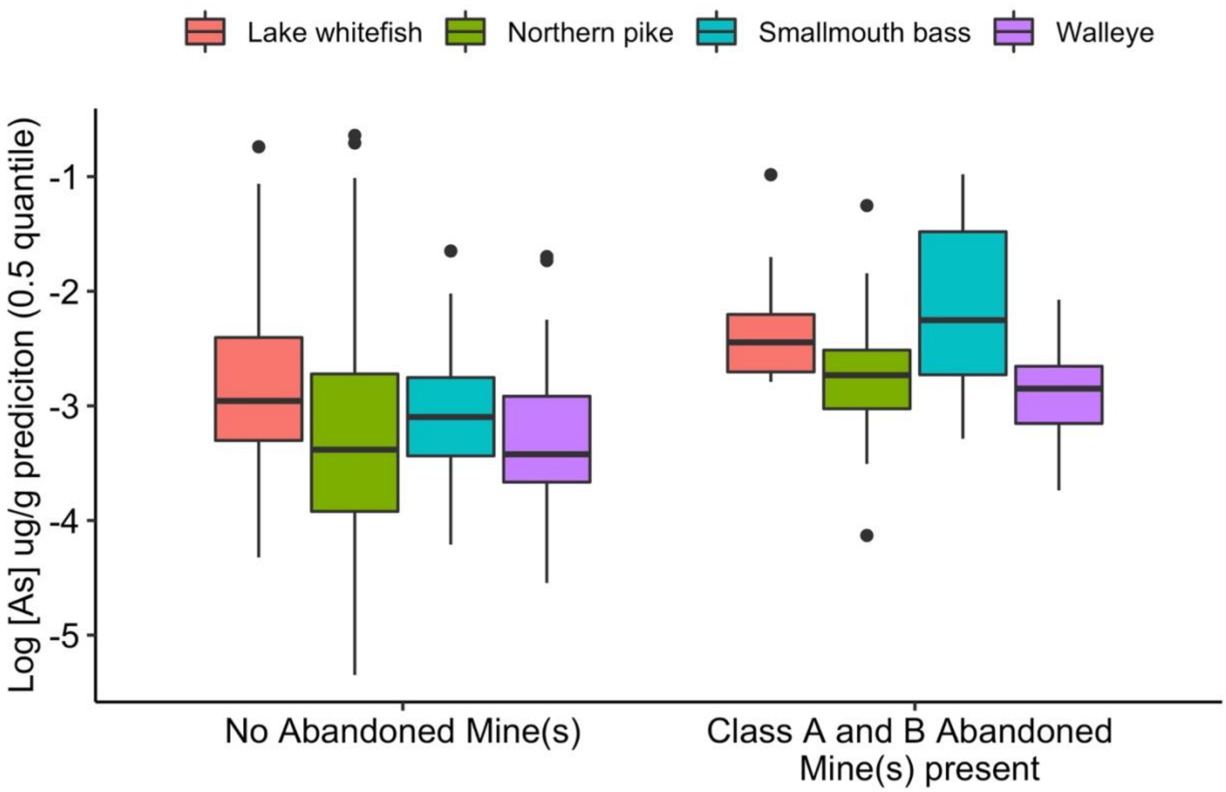

Figure SI-10. Box plots showing [As] for four fish species by presence or absence of abandoned mine site(s).

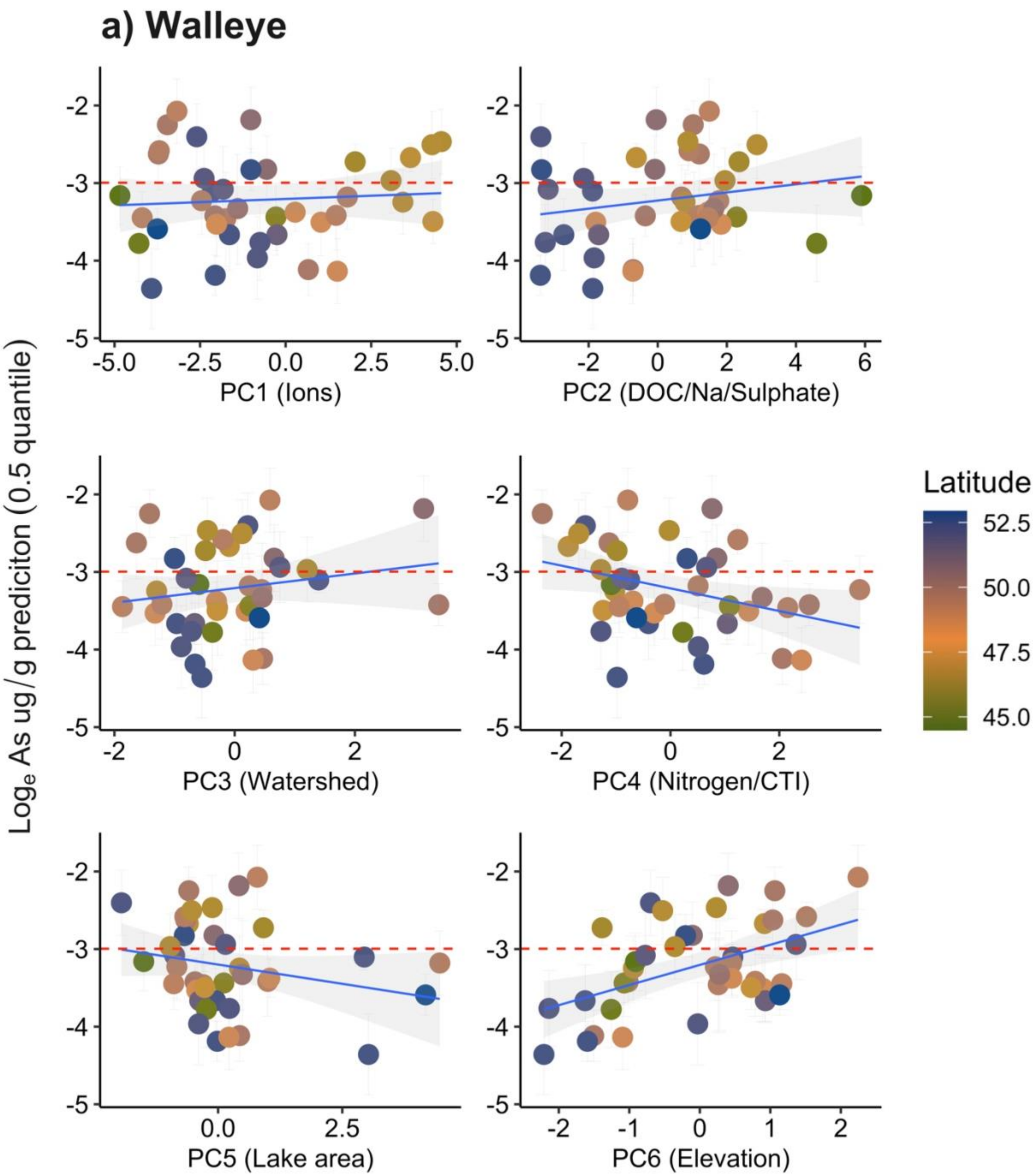

Figure SI-11. Relationships between $\log_e$ size standardized lake-level walleye [As] predictions and PC scores. The red dashed line represents MECP MDL of 0.05 µg/g (log value = -3.0 µg/g). Fitted lines are ordinary least-squares regressions and shading represents 95% confidence interval.

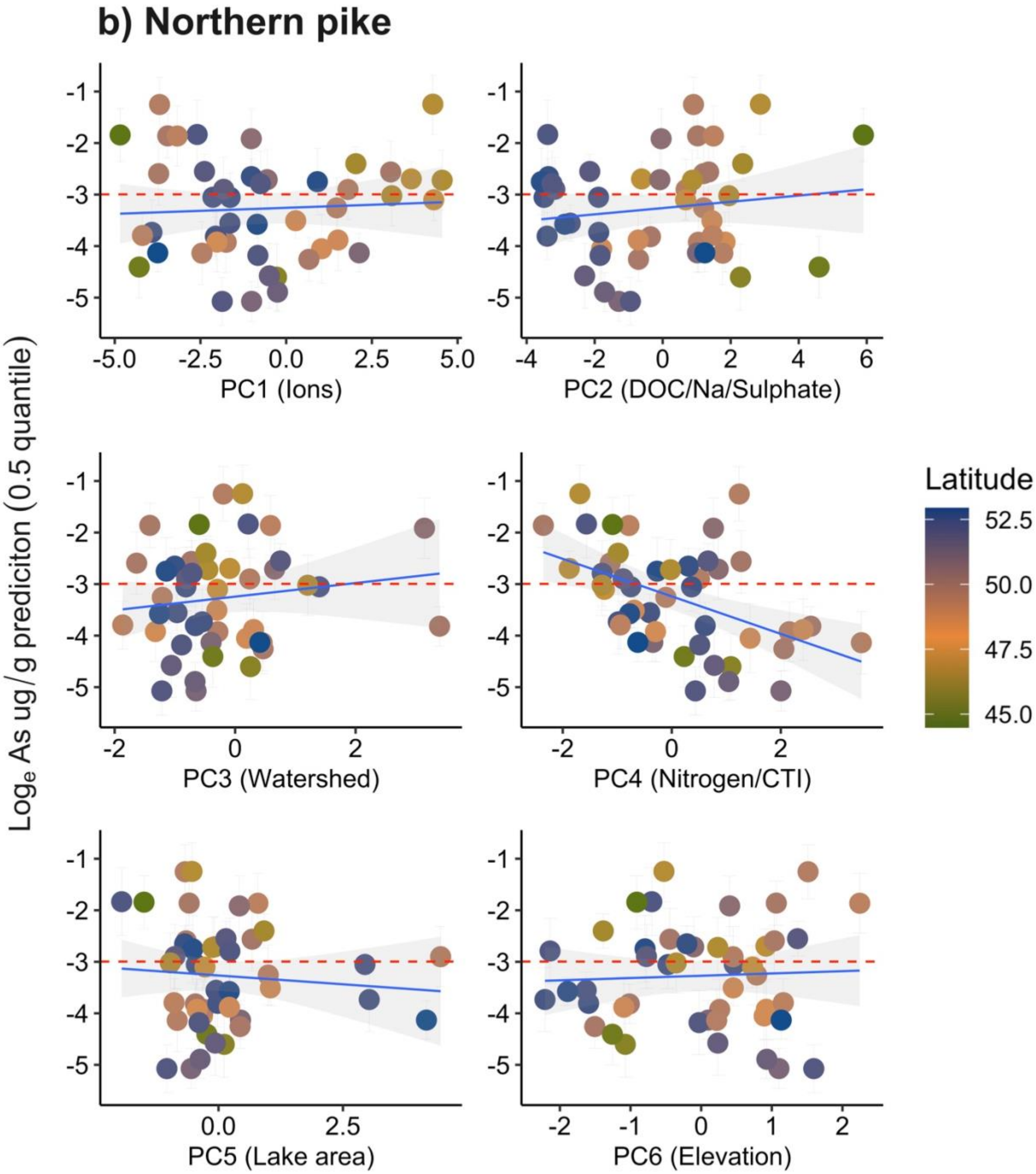

Figure SI-12. Relationships between $\log_e$ size standardized lake-level northern pike [As] predictions and PC scores. The red dashed line represents MECP MDL of 0.05 µg/g (log value = -3.0 µg/g). Fitted lines are ordinary least-squares regressions and shading represents 95% confidence interval.

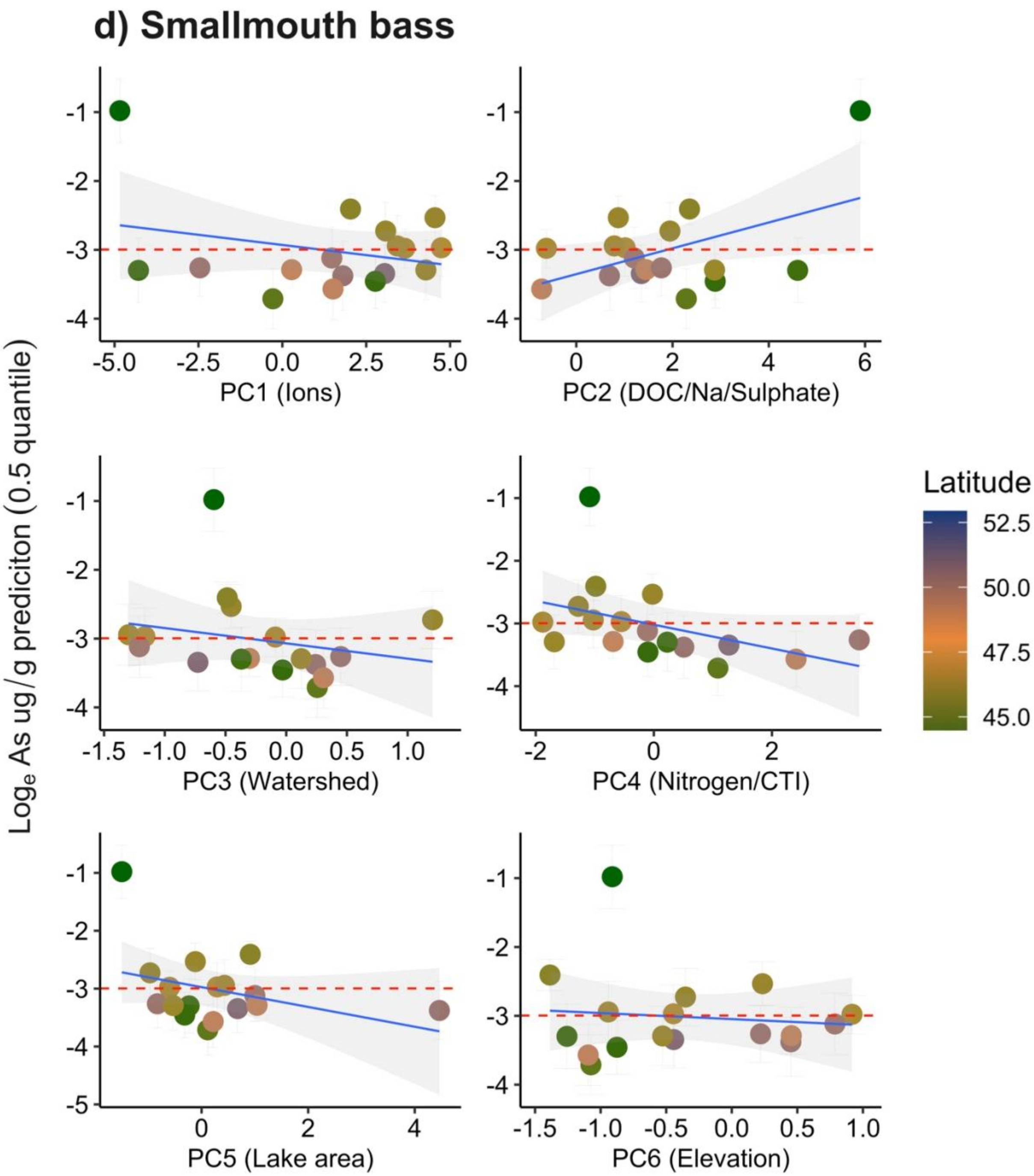

Figure SI-13. Relationships between $\log_e$ size standardized lake-level smallmouth bass [As] predictions and PC scores. The red dashed line represents MECP MDL of 0.05 µg/g (log value = -3.0 µg/g). Fitted lines are ordinary least-squares regressions and shading represents 95% confidence interval

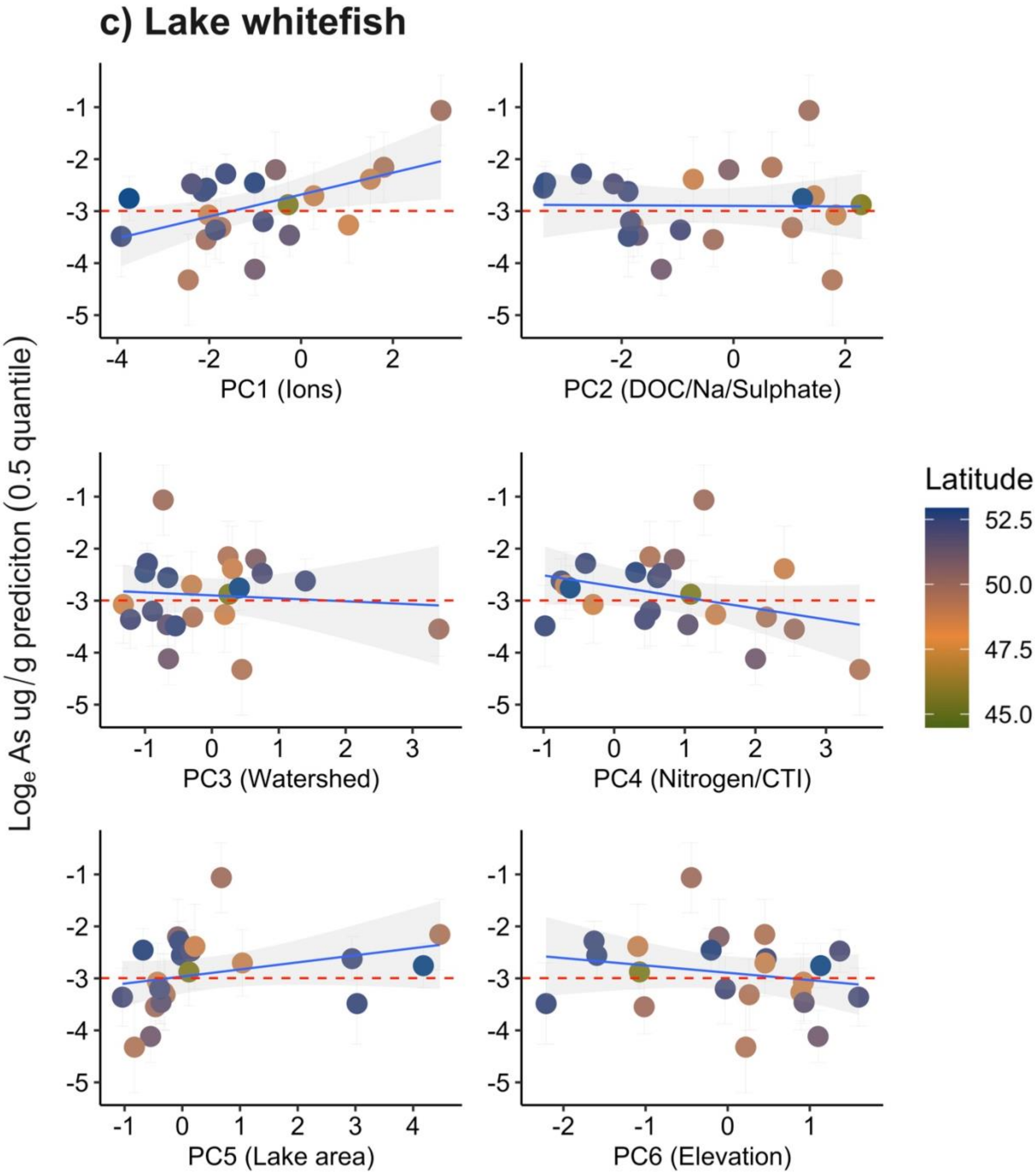

Figure SI-14. Relationships between $\log_e$ size standardized lake-level lake whitefish [As] predictions and PC scores. The red dashed line represents MECP MDL of 0.05 µg/g (log value = -3.0 µg/g). Fitted lines are ordinary least-squares regressions and shading represents 95% confidence interval.

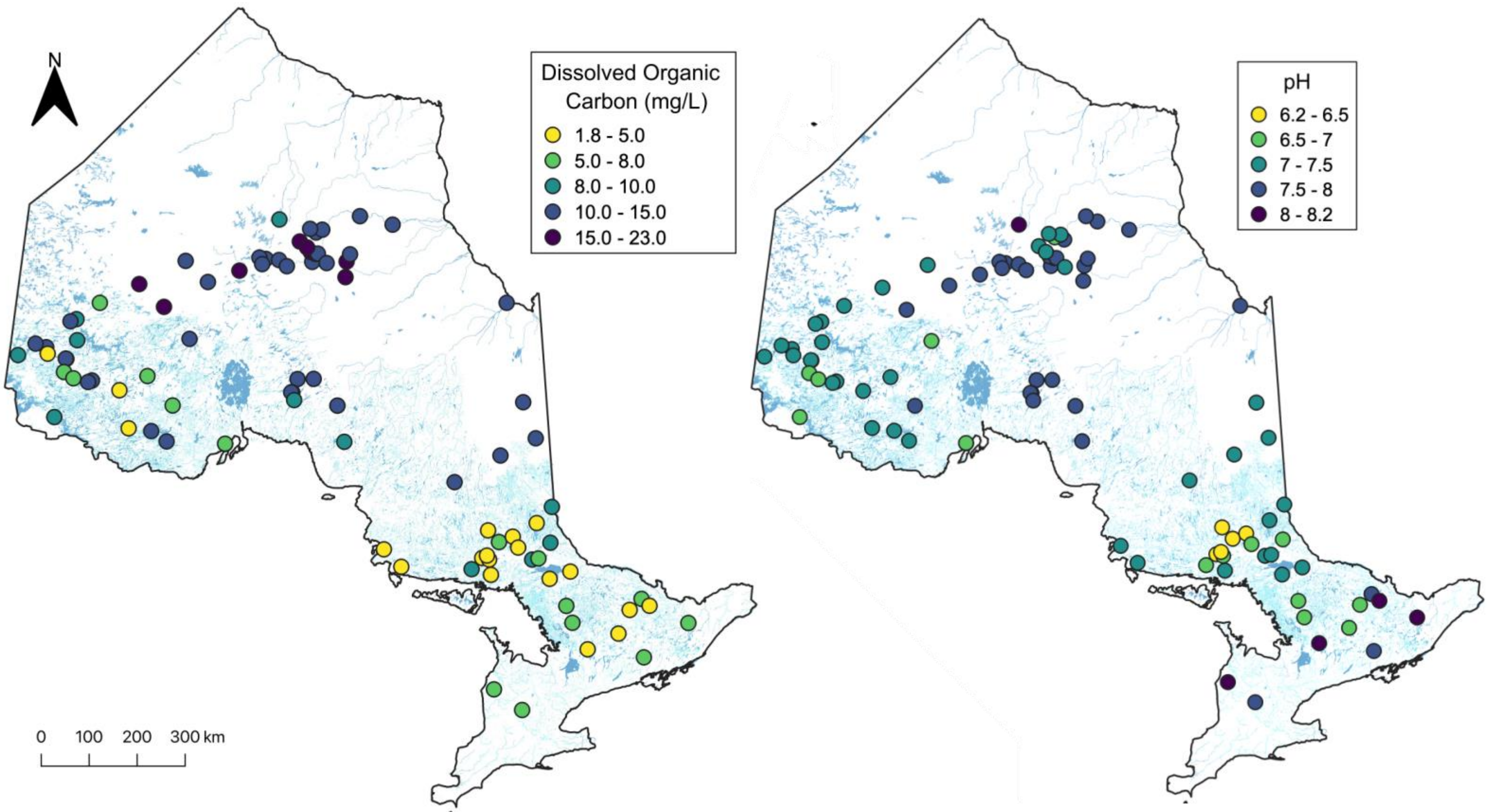

Figure SI-15. Maps showing DOC and pH concentrations in water from lakes and river sites across Ontario, Canada.