Speciation of Arsenic in Freshwater Biota

by

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Abstract

Arsenic can reach potentially concerning levels in fish and other aquatic biota, but the risk posed is strongly dependent on the element's chemical speciation. However, the speciation of arsenic in biotic samples remains analytically challenging and freshwater fish, in particular, have not been extensively studied. The limited information available suggests that freshwater fish can have highly variable arsenic speciation patterns, both within and between populations. Based on these knowledge gaps, my thesis has two main goals: (1) to assess the current state of knowledge on arsenic speciation using a systematic literature review and (2) measure arsenic speciation in biota from boreal lakes to investigate drivers of variation among individual fish and invertebrates.

My literature review focussed on arsenic speciation in freshwater fish muscle. I identified 39 studies that matched predefined criteria for inclusion based on a review of 1096 potential studies. I found considerable variability in the available literature; although less toxic organic species of arsenic typically dominated in fish muscle, there were reports of fish with high concentrations of the most toxic inorganic species. While studies modeling the drivers of this variation were limited, some suggest that waterbody characteristics, fish size, and trophic ecology may contribute.

In my field study, I collected and analyzed fish and invertebrates for two common organic species of arsenic, arsenobetaine (AsB) and dimethylarsinic acid (DMA), in three lakes across a contamination gradient near Sudbury, Ontario. Concentrations of these arsenic species varied widely across fish and invertebrates, generally being found at higher concentrations in the most contaminated system, a lake associated with an abandoned gold mining site. Trophic ecology appeared to be a primary factor affecting arsenic speciation in aquatic food webs, with both AsB

and DMA decreasing in concentration with increasing trophic position, inferred from stable nitrogen isotope values. To my knowledge, this is the first study to apply stable isotope techniques to assess how trophic ecology and diet influence arsenic speciation across whole freshwater food webs; where prior arsenic speciation studies have focused on fish alone and did not observe the same biodilution effect. I also identified other factors that may influence arsenic speciation. These included variation in fish size and age, diet, and interactions with co-occurring chemicals (e.g., selenium). However, considerable unexplained differences in arsenic species among taxa remains for further studies to address.

Future avenues for research on arsenic speciation include continued improvements in analytical techniques and detection levels, deepening our molecular understanding of arsenic biotransformation and accumulation, broadening toxicological testing of various arsenic species, and assessing the behaviour of arsenic species across diverse food webs. Additionally, improving our understanding of arsenic speciation in freshwater environments is essential to accurately assess risk to consumers or the aquatic biota themselves. A refinement of environmental and human health risk assessments based on the results found herein and in future studies are warranted.

Keywords

Arsenobetaine, Dimethylarsinic Acid, Lake, Fish, Invertebrates, IC-ICP-MS

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List of Acronyms

AAS	Atomic Absorption Spectrometry
As(V)	Arsenate
As	Arsenic
As(III)	Arsenite
AsB	Arsenobetaine
BFW	Boreal Food Webs Sample Archive
CRM	Certified reference material
[]	Concentration
CF-IRMS	Continuous flow-isotope ratio mass spectrometry
Cu	Copper
DMA	Dimethylarsinic acid
Hg	Mercury
ICP-MS	Inductively coupled plasma-mass spectrometry
iAs	Inorganic Arsenic
IC	Ion chromatograph
LOQ	limit of quantification
MRL	Maximum Residue Level
MDL	Method detection limit
MMA	Monomethylarsonic acid
NRCC	National Research Council of Canada
Ni	Nickel
NWT	Northwest Territories, Canada
MECP	Ontario Ministry of Environment Conservation and Parks
MNRF	Ontario Ministry of Natural Resources and Forestry
MNDM	Ontario Ministry of Northern Development and Mines
QAQC	Quality assurance and control
Se	Selenium
EPA	United States Environmental Protection Agency

Chapter 1: Thesis introduction

Arsenic (As) is a naturally occurring metalloid that is ubiquitous in the environment (Sadee et al. 2016). It is classified as a Group 1 carcinogen by the International Agency for Research on Cancer and exhibits acute and chronic toxicity at both molecular and organismal levels (IARC 2012; Byeon et al. 2021). The release of arsenic into the environment through anthropogenic activities (e.g., herbicides, pesticides, mining activity) and to a lesser extent, natural processes (e.g., volcanic activity and the weathering of rocks and soils) is, therefore, a major concern (Ruttens et al. 2012). In particular, certain types of mining activities can introduce arsenic to terrestrial and aquatic environments, even after the active mining ends, constituting a potential long-term risk to environmental and human health (Kostarelos et al. 2015). Once in the aquatic environment, arsenic can bioaccumulate within fish and other organisms to levels that can be potentially harmful to consumers, including humans (Rahman et al. 2012; Luvonga et al. 2020).

Although arsenic potentially poses a risk to human and environmental health, the level of risk strongly depends on its chemical speciation; that is, which of the various forms of arsenic, differing in oxidation state or molecular structure, are present (Templeton et al. 2000; Byeon et al. 2021). In surface water and sediments, arsenic mainly exists as the most toxic inorganic species arsenite (As(III)) and arsenate (As(V); Kohlmeyer et al. 2003; Byeon et al. 2021). This arsenic from the abiotic environment can enter biota via direct absorption through gills and skin, as well as through the gastrointestinal tract from the ingestion of sediments, especially in benthic feeding fish (Cui et al. 2021; Lu et al. 2023). Organisms can also uptake arsenic from their prey into the intestines through dietary exposure (Pei et al. 2019). The relative importance of each uptake pathway can vary among taxa with some being more sensitive to waterborne exposure than dietary, or vice versa

(Erickson et al. 2011; 2019). Absorbed arsenic then enters the bloodstream and is distributed through the body. Most of the arsenic absorbed in the GIT enters blood and, due to natural blood transport pathways, first travels through the liver, a detoxification organ and important site for arsenic biotransformation (Pei et al. 2019; Lu et al., 2023).

Although the direct mechanisms of biotransformation are not fully known, and there may be multiple biochemical pathways to form a given species, we do have a general idea of the processes involved. Biotransformation of arsenic typically involves the reduction of pentavalent species (e.g., arsenate) into trivalent species (e.g., arsenite) followed by oxidative methylation by enzymes such as methyltransferases (Byeon et al. 2021; Zhang et al. 2022). These modified species of arsenic often have lower toxicity and/or are easier to move across the cell membrane by transport proteins either alone or as glutathione conjugates, allowing them to be excreted in urine and bile (Leslie, 2012; Byeon et al. 2021; Pei et al. 2021) or distributed to other tissues for storage (Zhang et al. 2016). Overall, these processes contribute to the higher proportions of less toxic organic arsenic species observed in tissues such as the muscle and liver, compared with the intestines where there is sometimes more iAs, especially under high dietary exposure to inorganic species (Pei et al. 2019), suggesting the intestines plays a minimal role in biotransformation and serves mainly as an arsenic uptake site. A variety of factors can influence arsenic uptake, biotransformation, and excretion processes such as exposure duration, with multiple studies noting decreased arsenic uptake as well as increased biotransformation and/or excretion over time with chronic dietborne (Pei et al. 2019, Cui et al. 2021) or waterborne (Chen et al. 2018) exposures. Arsenic uptake can also be influenced by the speciation of arsenic in prey, as some arsenic species may be more bioavailable than others (Zhang et al. 2016) as well as by the subcellular partitioning of these arsenic species within prey, which can also impact their bioavailability (Dutton & Fisher 2011).

While past work on arsenic in the environment has largely been limited to total arsenic measurements, with modern advances in chromatography and mass spectrometry it is now possible to accurately measure the concentrations of individual arsenic species (Reid et al. 2020) and base risk assessment on the most harmful forms (Tanamal et al. 2021). Consumption limits based on arsenic speciation data have already been established for foods like rice and juices (Health Canada 2022). However, research on arsenic speciation in fish is more limited, with most studies focusing on marine fish. It is not clear how well findings in marine fish compare with arsenic speciation profiles in freshwater environments, where arsenic cycling may differ, in part due to more variable water chemistry (Byeon et al. 2021).

The main goals of this thesis are to:

1. Systematically review the current state of knowledge regarding arsenic speciation in freshwater fish;
2. Evaluate the accuracy of assumptions of arsenic speciation used in current risk assessment in freshwater fish when only total arsenic data are available;
3. Conduct a field and analytical study of arsenic speciation in organisms within the food webs of lakes with varying anthropogenic impacts (e.g., mining, urban development) and assess drivers of variation therein.

A systematic review of available literature was used to address thesis goals (1) and (2), while thesis goal (3) was based on experimental study of lakes in the mining region of Sudbury, Ontario, Canada. The Greater Sudbury area has a unique history of mining activity and associated environmental degradation, dating back to the late 1800's. Smelter complexes in Sudbury were one of the largest sources of acid and metal particulate emissions globally until recent decades, leaving local terrestrial and aquatic environments heavily acidified and contaminated with metals (Keller et al. 2019). Emissions have been reduced >95% over the last 40 years, allowing for notable biological and chemical recovery. However, the complex legacy of historical mining practices are

still seen across the region today (Keller et al. 2019). Three lakes with unique characteristics and histories were selected for my thesis project: one with significant arsenic contamination from abandoned mine tailings, one in proximity to smelters and additional urban development, and one that was historically acidified but did not receive large amounts of particulate metal fallout due to its greater distance from the smelters.

This thesis contains two main chapters (Chapter 2 and 3), in addition to a general introduction (Chapter 1) and conclusion (Chapter 4). The structure and purpose of the main chapters are as follows:

Chapter 2: Arsenic speciation in freshwater fish: A systematic review with implications for monitoring and research

This chapter is part of a collaborative systematic review effort in partnership with Camelia Tavakoli, Brian Laird, and Kelly Skinner from The University of Waterloo. The portion of the review included herein, written by myself, systematically assessed the literature available on arsenic speciation in freshwater fish muscle and summarized their results (thesis goal 1). These results were then used to assess the accuracy of assumptions made about arsenic speciation in total arsenic-based risk assessments (i.e., that a small proportion of total arsenic in fish is in the most harmful forms; thesis goal 2). This chapter also discusses patterns in existing arsenic speciation data and potential drivers of variation. Because this chapter represents roughly half of a final planned manuscript, it is notably brief. My collaborators at the University of Waterloo are reviewing the maximum residue limits (MRLs) used for arsenic and its species in foods during public health risk assessments. Additionally, they are discussing information on toxicological reference values and food consumption information that inform these risk assessments. Their summarized assumptions underlying risk assessments will then be compared to my observed trends in arsenic speciation in fish to evaluate their overall accuracy. My co-authors on this work

will include: Gretchen L. Lescord (Vale Living with Lakes Centre, Laurentian University & Wildlife Conservation Society Canada); Camelia Tavakoli (School of Public Health Sciences, University of Waterloo), Brian Laird (School of Public Health Sciences, University of Waterloo), Kelly Skinner (School of Public Health Sciences, University of Waterloo); and John M. Gunn (Vale Living with Lakes Centre, Laurentian University)

Chapter 3: Biodilution of organic species of arsenic in three freshwater food webs

This chapter reports on concentrations of two organic species of arsenic commonly detected in fish and invertebrates (Thesis goal 3). These organic species, AsB and dimethylarsinic acid (DMA), are thought to be less harmful than other forms of As, which has resulted in less focus on their concentrations and behaviour. However, these species are an important part of arsenic biotransformation pathways within fish tissues, making their distribution and proportions important to the overall understanding of arsenic speciation in aquatic ecosystems. The 3 lakes were sampled in collaboration with the Ontario Ministry of Natural Resources and Forestry (MNRF). Samples were analyzed for arsenic speciation on an ion chromatograph paired with an inductively coupled plasma-mass spectrometer (IC-ICP-MS), using methods I helped to validate at Laurentian University and in collaboration with Metrohm®. In addition to raw concentrations, this study also assessed the percentage of total arsenic made up by these two organic species in a subset of samples. Drivers of variability in arsenic speciation including fish size, interactions with co-occurring elements, trophic ecology, diet, and lake specific factors are discussed. This chapter is currently being prepared for submission to *Environmental Pollution*. My co-authors on this work will include: Gretchen L. Lescord (Vale Living with Lakes Centre, Laurentian University & Wildlife Conservation Society Canada); Alan Lock (Laurentian University); Thomas A. Johnston

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Chapter 2: Arsenic speciation in freshwater fish: A systematic review with implications for monitoring and research

1. Abstract

Arsenic can accumulate in fish, sometimes to levels of concern for subsistence and recreational fishers. However, the toxicity of arsenic strongly depends on the chemical forms, or species, that are present. Risk assessments are often based on total arsenic concentrations ([As]), with an adjustment factor applied, assuming a small percentage of total [As] is the most harmful inorganic species. While studies on arsenic speciation in marine fish are widespread, and commonly report non-toxic arsenobetaine (AsB) as the dominant form, fewer studies have been conducted on freshwater fish, where arsenic speciation may be more variable. To amalgamate and assess these findings, we conducted a systematic literature review on arsenic speciation in freshwater fish using Covidence© review management software. From the 1094 studies screened for relevance and quality assurance measures, 39 studies were selected for inclusion based on predefined criteria. These studies reported highly variable arsenic speciation patterns in freshwater fish, calling into question the assumption that AsB is the dominant form present. Sites with suspected or known arsenic contamination issues were prominent, with 50% of data reviewed originating from a contaminated river or lake. Although AsB and other organic forms typically dominated, some fish had elevated concentrations of inorganic arsenic (>0.5 mg/kg dry wt.). Arsenic speciation results rarely accounted for all of the arsenic in fish; a considerable proportion of total [As] was not explained by the measured arsenic species. Given this variability, it appears that total [As] based risk assessment is unlikely to be accurate across diverse locations and taxa. More work is needed to characterize arsenic speciation in freshwater fish and assess the toxicity of various arsenic species to accurately assess the environmental and human health risks associated with arsenic in fish.

2. Introduction

Arsenic is an element of concern that is released to the environment through anthropogenic activities as well as natural processes (Ruttens et al. 2012). In aquatic environments arsenic can accumulate within fish and other biota posing a potential risk to environmental and human health (Luvonga et al. 2020). The toxicity of arsenic in the environment strongly depends on its chemical speciation; that is, the various forms of arsenic differing in oxidation state or molecular structure that are present (Templeton et al. 2000). Of the arsenic species that can be found in the aquatic environment and in fish, the inorganic species arsenite (As(III)) and arsenate (As(V)) are the most toxic and tend to make up the bulk of arsenic present in water and sediments (Kohlmeyer et al. 2003). On the other hand, organic species of arsenic tend to be more prevalent in the biota, though inorganic arsenic (iAs) can also be found in varying concentrations (Zheng and Hintelmann 2004; Miyashita et al. 2009; Ruttens et al. 2012). Of the organic species of arsenic, arsenobetaine (AsB) is considered the least toxic, while methylated arsenic species (e.g., monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) are generally considered more toxic than the other organoarsenicals (Byeon et al. 2021). Arsenosugars, arsenolipids, and arsenocholine appear to be intermediate in toxicity between AsB and MMA, though research on the behaviour and toxicity of all arsenic species is on-going (Byeon et al. 2021).

Despite growing knowledge of the variation in arsenic speciation and associated toxicity, more commonly all species of arsenic are measured together as total arsenic concentrations (total [As]) in fish muscle during monitoring and research studies. This is due, in part, to the relative ease of total arsenic analysis when compared to speciation analysis, particularly when working with complex biological material or matrices like fish tissues that often contain a variety of reducing and oxidizing agents that can alter arsenic speciation during the extraction procedures

(Wolle and Conklin 2018). Although some jurisdictions have recently adopted more specific guidelines in fish, based on inorganic arsenic specifically (e.g., 0.1 mg/kg [iAs] wet wt., Government of China 2017; 2 mg/kg [iAs] wet wt., Food Standards Australia New Zealand 2020), many regions still regulate based on total [As]; this use of a total arsenic guideline includes Canada (e.g., 3.5 mg/kg total [As] wet wt., Health Canada 2022).

When total [As] is used for risk assessment, an adjustment factor is often applied assuming a small percentage of total [As] is the most toxic inorganic species (e.g., <20%), but these assumed percentages can vary between agencies and studies (e.g., <10%, WHO & FAO 2011; 10%, Schoof 2014; 11%, Ai et al. 2022). Additionally, These assumptions are typically based on limited data, much of which comes from marine systems (Lorenzana et al. 2009; Rahman et al. 2012; Luvonga et al. 2020) or has become dated with modern advances in analytical technology (Krachler et al. 2002). Therefore, these assumptions may not be accurate across all fish species and regions, particularly in freshwater systems, which are believed to have more spatial and temporal variation in arsenic speciation compared to marine environments (Byeon et al. 2021).

Previous reviews on arsenic speciation have focused on marine environments primarily due to the larger literature base that exists (Schoof and Yager 2007; Lorenzana et al. 2009; Rahman et al. 2012; Zhang et al. 2022). Although these reviews often mention freshwater fish, this literature review differs in that it specifically examines freshwater fish by using a systematic approach. A total of 39 papers were reviewed herein and their findings were summarized. Using these papers, we evaluated the accuracy of assumptions made about arsenic speciation in total [As]-based environmental monitoring, research, and risk assessments (i.e., that less than ~20% of total arsenic in fish is inorganic arsenic). Patterns in existing arsenic speciation data, including the differences

among taxonomic groupings, contamination level, and with various life history traits were also discussed.

3. Methods

3.1. Database Selection and Search String Development

This review was conducted using Covidence, a web-based collaborative review management software (institutional license: University of Waterloo). To identify papers that reported data on arsenic speciation (i.e., concentrations of arsenic species or percentages of total As) in freshwater fish we designed a search string to target papers that meet those criteria using a combination of "AND" and "OR" operators:

(("Arsenic" AND "speciation") OR "inorganic arsenic" OR "ias" OR "arsenobetaine" OR "AsB" OR "dimethylarsinic acid" OR "DMA" OR "Monomethylarsonic acid" OR "MMA" OR "organic arsenic" OR "organoarsenic") ***AND*** *("freshwater" OR "lake" OR "river" OR "lacustrine" OR "lotic" OR "lentic" OR "riverine")* ***AND*** *("fish" OR "organisms" OR "Biota")*

For broad coverage of the relevant literature, five databases were searched: Web of Science (including all sub-databases), Pubmed, Scopus, Scifinder, and Google Scholar. Because Google Scholar does not support exporting of search results to Covidence, the first 150 results from Google Scholar when sorted by "most relevant" were manually screened at the title and abstract level and then imported to Covidence because it was not practical to screen the over 500,000 results for this search. Additionally, because Scifinder does not support the use of complex search strings, multiple searches were performed using individual keywords to cover the available research as broadly as possible.

3.2. Review of Search Results

The literature searches were current to November 18th, 2021. A total of 1094 unique separate studies were identified and then a two-step screening process was employed. First, an initial screening of the titles and abstracts for relevance to arsenic speciation in freshwater fish was performed by two independent reviewers. When there was uncertainty about including a study at this stage we defaulted to inclusion; 1016 studies were excluded through this process, while 78 studies proceeded to a second review of the full manuscripts. In total, 37 out of 78 studies were selected for inclusion according to the criteria outlined in Table 1. An additional study by Lescord et al. (2022) was added that was published after literature searching, in addition to results from Lepage et al. (in prep) for a total of 39 studies.

Table 2-1. Inclusion criteria applied during the full-text review of 78 peer-reviewed papers, and the number of papers excluded from this study due to each given criterion.

Inclusion / Exclusion Criteria	# of papers removed	# of papers remaining
1. The paper must include data from one or more chemical species of arsenic / studies that only included total arsenic measurements were excluded	5	73
2. The analysis must include measurements from at least one or more known freshwater fish* that is either wild-caught or raised in standard aquaculture conditions / lab-exposure studies and marine studies were excluded	19	54
3. The analysis must have been performed on muscle tissue / results from whole-body homogenates or other tissues were excluded	1	53
4. The paper must include a description of quality assurance and quality control (QAQC) information that supports the accuracy of the data presented; any level of discussion of QAQC measures was considered valid for inclusion.	5	48
5. The methods must include generation of new data / review papers were not included herein	4	44
6. The paper was not a duplicate publication	7	37 + 2

*Anadromous fish, which spend part of their life in freshwater, were included in this review (e.g., Walker et al. 2020; Lescord et al. 2022).

3.3. Data Extraction and Manipulation

Data from the 39 included studies were pooled in Excel, including information on fish source (i.e., waterbody, aquaculture operation, contamination status, etc.), extraction procedure, analytical instrumentation, fish species, sample sizes, and reported concentrations or percentages of various arsenic species and total arsenic in muscle. Percentages of arsenic species represent the relative amount of total [As] made up by each individual species and are often used in the assumptions of total [As] based risk assessment. In some cases, percentage values were calculated based on reported mean concentrations of total arsenic and various species. Reporting of arsenic species concentrations and/or percentages in the surveyed literature included mean values (with or without error estimates) and/or ranges of values, and these were reported either through tables, directly in-text, or as figures. Due to these differences in reporting practices between papers, we are limited in our meta-analysis capabilities. Where concentration values were reported in wet weight, they were converted to dry weight assuming 78% moisture. In rare cases where specific values were reported only in figures, care was taken to estimate the values by measuring figure elements and comparing to the axis scales as noted in tables or text; this process can be prone to error due to figure scaling issues. Additionally, 3 studies reported percentage values calculated from the sum of all measured species, instead of from total [As]; while this is relevant to the methodology used, it is less useful from a risk assessment perspective. We encourage the reporting of percentage values relative to total arsenic, as was more common in the literature, because these values are of higher relevance from a risk assessment perspective. We collected data for 5 arsenic species: the two inorganic species As(III) and As(V), as well as three commonly measured organic species AsB, DMA, and MMA. More novel organic species were reported in 8 studies, but because detection of these species was sporadic their concentrations and percentages were not recorded.

4. Results and Discussion

4.1. General description of the studies included in this review

Thirty-two studies reported concentrations of one or more arsenic species and 32 studies reported either percentages of arsenic species relative to total arsenic, or enough information (i.e., species and total arsenic concentrations) for us to calculate these percentages herein; 29 studies reported both. The surveyed literature included results from over 2200 muscle samples from at least 117 fish species (some studies reported only common names that could refer to several species) and involved 145 sampling locations. These sampling locations included 37 lakes, 67 river sites, 27 aquaculture operations and 14 marketplaces (Figure 2-1). Of the lake and river sites 37.5% (i.e., 39/104) were reported to be contaminated with arsenic while the remaining 65 locations were not (Figure 2-1a). Although contaminated sites made up only just over a third of the sites sampled these fish made up over 50% of the overall number of fish sampled (Figure 2-1b).

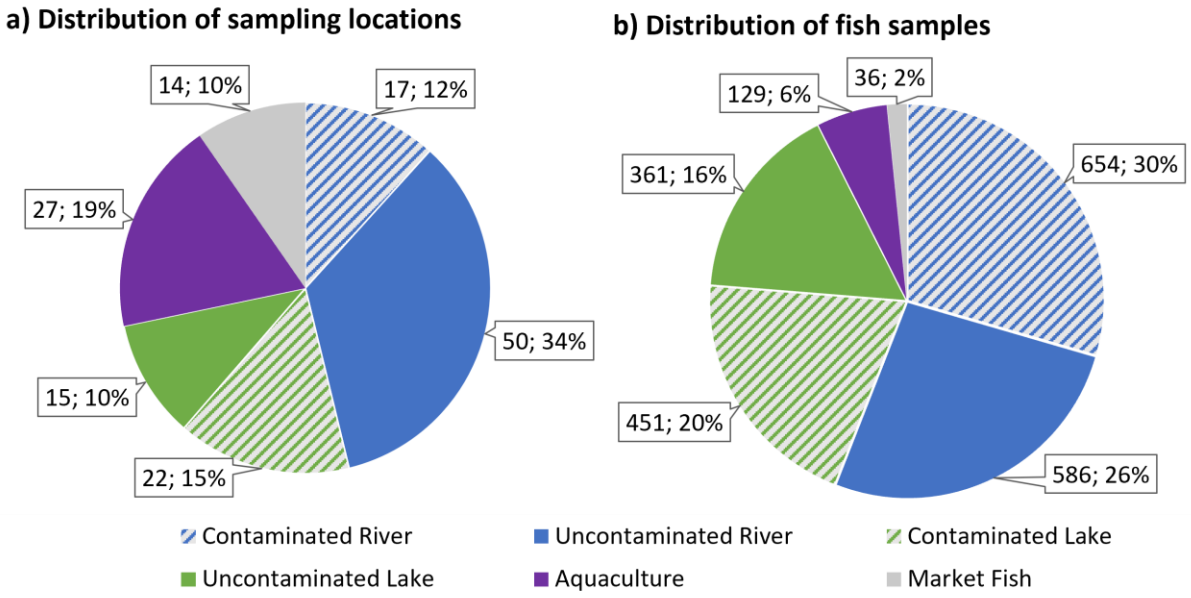

Figure 2-1. Distribution of sampling locations (a) and the number of fish samples collected from different location types (b) in the surveyed literature. Values given are raw count numbers and the percentage of the total number of sampling locations or fish samples.

Arsenic species were most often extracted using a combination of methanol and/or water (i.e., 24/39 studies) but occasionally using acids of varying strength (i.e., 11/39 studies), alkaline solutions (i.e., 1/39 studies, Larsen et al. 2005) or enzymatic extraction (i.e., 2/39 Studies, Zhao et al. 2018; Walker et al. 2020). One study did not report extraction methodology in detail (Karouna-Renier et al. 2011). Separation of extracted arsenic most often used either high performance liquid chromatography (i.e., 28/39 studies) or ion chromatography (i.e., 3/39 studies). Hydride generation was also commonly employed for arsenic speciation (i.e., 8/39 studies). Hydride generation methods are especially useful for isolating hydride generating arsenic species from non-hydride generating species, like AsB (Reid et al 2020). Arsenic species were then typically detected with either ICP-MS (i.e., 28/39 studies) or atomic absorption spectrometry (AAS; i.e., 10/39 studies). One study also used instrumental neutron activation analysis-Compton suppression system based methods (Zwicker et al. 2011). While both ICP-MS and AAS instruments are effective for arsenic speciation, ICP-MS is generally expected to provide lower detection limits, especially in the case of instruments with a quadrupole or hexapole collision cell, while allowing for the analysis of non-hydride generating species like AsB that cannot be easily analyzed by AAS (Krachler et al. 2002). Detection limits for arsenic species in the surveyed literature ranged from 0.00006 – 0.1 mg/kg dry wt., with the majority falling between 0.001 and 0.01 mg/kg dry wt. Although AAS methods did typically have higher detection limits, differences in sample preparation (e.g., mass of sample, volume of digest, or dilution factor) generally had a larger influence on detection limits than differences in the instrumentation used. Occasionally, complementary analyses such as X-ray absorption near-edge structure or electrospray-mass spectrometry were also used to attempt to better characterize unidentified or unextracted arsenic species (e.g., Hong et al. 2014; Stiboller et al. 2015; Yang et al. 2020).

366

367 **4.2. Concentrations of Arsenic Species in Freshwater Fish**

368 The fish in these studies spanned a broad range of total [As] (0.01-168 mg/kg dry wt.), but
369 most were <5 mg/kg. However, 7.7% (i.e., 3/39) of studies did not report total [As] (Table SI-1;
370 Choi et al 2015; Stiboller et al. 2015; Zhao et al. 2018). This range in total [As] in freshwater fish
371 was generally lower than concentrations reported in marine fish (<1.14 - 335 mg/kg dry wt.
372 assuming 78% moisture, Rahman et al. 2012; 0.62 – 74.96 mg/kg dry wt. Lorenzana et al. 2009).
373 In only 6 studies some fish exceeded the limit for total [As] established for fish protein by Health
374 Canada (i.e., 15.9 mg/kg dry wt., assuming 78% moisture; Health Canada, 2022). Fish that
375 exceeded this level were typically from systems contaminated with arsenic from various sources
376 (Pizarro et al. 2003; Jankong et al. 2007; Zwicker et al. 2011; Yang et al. 2020; Lepage et al. in
377 prep). However, in one case elevated total [As] was also observed in fish from an uncontaminated
378 coastal river in the far north of Ontario, Canada. These fish were believed to be anadromous or to
379 feed on anadromous fish that entered the river, suggesting that marine resources increased total As
380 burdens in coastal fish (Lescord et al. 2022). While variation in total [As] in freshwater fish is
381 often considered the result of higher exposure of As in food or water (Huang et al. 2003; Jankong
382 et al. 2007; Hong et al. 2014; Komorowicz et al. 2019; Yang et al. 2020) other factors may
383 influence As bioaccumulation including life history traits or species-specific differences in
384 accumulation or transformation (Chételat et al. 2019; Zhang et al. 2022; Kluke et al. 2023).

385 Generally, organic species of arsenic are reported to be at higher concentrations than
386 inorganic arsenic in freshwater fish (Hong et al. 2014; Jia et al. 2018; Tanamal et al. 2021; Lescord
387 et al. 2022), and the most frequently detected organic species of arsenic were AsB and DMA.
388 However, the concentrations of both AsB and DMA were variable within and across studies. AsB

concentrations were generally higher than other arsenic species, reaching as high as 65 mg/kg dry wt. in trout (species not specified) from the Loa River in Chile, a region with considerable geogenic and anthropogenic arsenic contamination (Pizarro et al. 2003). However, in other studies, AsB has also been reported to be below detection limits in some fish (i.e., in 8/22 studies reviewed herein; Table SI-1). In addition to this variation between study sites, other studies have also reported highly variable [AsB] in freshwater fish from a single study region (<0.01-42.70 mg/kg, Lescord et al. 2022; <0.001-30.144 Lepage et al. *in prep*).

DMA was typically second highest in concentrations in most of the reviewed studies, though in some cases [DMA] actually exceeded [AsB] (e.g., Jankong et al. 2007; Lepage et al. in prep). High [DMA] has been previously reported in multiple studies on northern pike (*Exos lucius*) in both contaminated and uncontaminated regions (de Rosemond et al. 2008; Tanamal et al. 2021; Lepage et al. in prep), and in other species such as smallmouth bass (*Micropterus dolomieu*; Lepage et al. in prep) and striped snakehead (*Channa striata*; Jankong et al. 2007). Concentrations of DMA ranged from below detection limits in some fish (i.e., in 15/21 studies) to as high as 23.1 mg/kg dry wt. in trout (species not specified) from the same region where the highest [AsB] were recorded (Pizarro et al. 2003); most often [DMA] was <1 mg/kg dry wt. (Table SI-1).

The detection of other organoasenicals was more sporadic. In most studies, [MMA] fell below detection limits in some or all fish (i.e., 18/19 studies). Only one study reported a relatively high [MMA], reaching 0.38 mg/kg dry wt. of MMA in a cyprinid, *Puntius orphoides,* from a contaminated pond in Thailand (Jankong et al. 2007; Table SI-1). Other organic species of arsenic reported in freshwater fish muscle include arsenolipids (Arroyo-Abad et al. 2016), arsenosugars (Schaeffer et al. 2006; Miyashita et al. 2009; Saipan et al. 2012; Wolle et al. 2019), arsenocholine (Miyashita et al. 2009; Jia et al. 2018; Wolle et al. 2019), trimethylarsine oxide (Slejkovec 1996;

412 Slejkovec et al. 2004; Jankong et al. 2007; Miyashita et al. 2009; Wolle et al. 2019), trimethylarsine
413 (Slejkovec 1996; Wolle et al. 2019), tetramethylarsonium (Jankong et al. 2007), and
414 trimethylarsoniopropionate (Wolle et al. 2019). These additional organic species of arsenic
415 generally make up a relatively small amount of overall arsenic, but were occasionally observed at
416 higher concentrations, particularly in the case of arsenosugars (Schaeffer et al. 2006; Miyashita et
417 al. 2009). The risks associated with these arsenic species are also expected to be low but there is
418 limited toxicological data available for many of the organic species (Taylor et al. 2017; Wolle et
419 al. 2019). Existing literature on arsenosugars (Andrewes et al. 2004; Ebert et al. 2016) for example
420 suggests that toxicity is limited but newer studies with arsenolipids (Witt et al. 2017; Bornhorst et
421 al. 2020; Chávez-Capilla 2022) have shown some potential for toxic effects, although this can vary
422 among arsenolipid types (Bornhorst et al. 2020). More comprehensive toxicity data are surely
423 needed to assess the risks posed by these species.

424 Of the selected papers, 29 reported on concentrations of inorganic arsenic in freshwater
425 fish muscle, either as As(III) and/or As(V) or as combined inorganic arsenic. Of these studies
426 reviewed herein, 21/29 reported inorganic arsenic concentrations below their respective detection
427 limits in at least some samples; in 7 of these studies, all samples had inorganic arsenic below
428 detection limits (Table SI-1). When inorganic arsenic was detectable it was highly variable among
429 studies, ranging as high as 46 mg/kg dry wt. (Pizarro et al. 2003) in fish with particularly high total
430 [As] (Table SI-1). However, most fish from pristine environments had inorganic arsenic
431 concentrations <0.1 mg/kg dry wt. (Hong et al. 2014; Lescord et al. 2022). Studies show that some
432 fish from more contaminated environments can have higher inorganic arsenic (Jankong et al. 2007,
433 Shah et al. 2010), while other fish have variable and sometimes low concentrations (Cott et al.
434 2016; Jia et al. 2018). For example, striped snakehead (*Channa* striata) from contaminated ponds

435 **Table 2- 2.** Differences in average inorganic arsenic concentrations ([iAs]) and the percentage of total [As] accounted for by [iAs]
436 (i.e., %iAs) in select fish from literature on contaminated and uncontaminated freshwater systems. Concentrations are reported as
437 mg/kg dry wt.; NR = values not reported. n = number of samples analyzed, Contam = contaminated status.

Citation	Sampling Location	Contam	Fish Species	n	Total [As]	[iAs]	%iAs
Jankong et al. 2007	Suphan River, Thailand	No	Striped Snakehead	3	1.9 ± 1.4	0.77 ± 0.73	40.5
	Contaminated Pond A, Thailand	Yes	Striped Snakehead	3	13.1 ± 1.0	0.12 ± 0.08	0.9
	Contaminated Pond B, Thailand	Yes	Striped Snakehead	3	22.2 ± 2.2	0.13 ± 0.04	0.6
Jia et al. 2018	Yueyang, Xiang River, China	Yes	Goldfish	4	0.338±0.176	0.078 ± 0.055	23.1
			Amur Catfish	5	0.195±0.103	0.100 ± 0.051	51.3
	Changsha, Xiang River, China	Yes	Goldfish	3	0.193±0.013	0.080 ± 0.041	41.5
			Amur Catfish	2	0.536±0.602	0.038 ± 0.016	7.1
	Xiangtan, Xiang River, China	Yes	Goldfish	3	0.631±0.277	0.038 ± 0.034	6.0
			Amur Catfish	2	0.293±0.212	0.063 ± 0.061	21.5
	Zhuzhou, Xiang River, China	Yes	Goldfish	3	0.261±0.101	0.044 ± 0.027	16.9
			Amur Catfish	4	1.080±0.386	0.033 ± 0.019	3.1
	Hengyang, Xiang River, China	Yes	Goldfish	4	0.747±0.303	0.063 ± 0.056	8.4
			Amur Catfish	3	2.030±0.766	0.129 ± 0.048	6.4
	Yongzhou, Xiang River, China	Yes	Goldfish	6	0.631±0.340	0.057 ± 0.019	9.0
			Amur Catfish	4	0.063±0.013	0.017 ± 0.004	27.0
Ruangwises et al. 2012	Chao Phra and Tha Chin Rivers, Thailand	Yes	Tilapia	14	0.837 ± 0.154	0.103 ± 0.012	12.5 ± 1.66
			Striped Snakehead	14	1.35 ± 0.331	0.303 ± 0.066	22.9 ± 3.70
	Aquaculture facilities in Thailand	No	Tilapia	14	0.892 ± 0.149	0.111 ± 0.016	12.7 ± 2.61
			Striped Snakehead	10	1.42 ± 0.537	0.280 ± 0.048	21.5 ± 5.99
Tanamal et al. 2021	Yellowknife Bay, NWT, Canada	Yes	Lake whitefish	8	1.82 ± 2.00	0.098 ± 0.035	9.3 ± 6.7
			Northern pike	9	1.59 ± 0.61	0.078 ± 0.015	6.1 ± 3.7
	Great Slave Lake, NWT, Canada	No	Lake whitefish	10	0.65 ± 0.45	0.081 ± 0.016	19.6 ± 14.9
			Northern pike	9	0.60 ± 0.18	0.077 ± 0.013	14.1 ± 5.5
	Lower Martin Lake, NWT, Canada	Yes	Lake whitefish	10	5.97 ± 1.46	0.050 ± 0.025	0.9 ± 0.4
			Northern pike	10	3.67 ± 0.72	0.038 ± 0.016	1.1 ± 0.5

Table 2-2 continued. Differences in average inorganic arsenic concentrations ([iAs]) and the percentage of total [As] accounted for by [iAs] (i.e., %iAs) in select fish from literature on contaminated and uncontaminated freshwater systems.

Citation	Sampling Location	Contam	Fish Species	n	Total [As]	[iAs]	%iAs
Tanamal et al. 2021 (cont.)	Long Lake, NWT, Canada	Yes	Lake Whitefish	10	2.65 ± 1.49	0.061 ± 0.009	3.0 ± 2.0
			Northern pike	10	3.97 ± 1.06	0.064 ± 0.002	1.7 ± 0.5
	Kam Lake, NWT, Canada	Yes	Lake whitefish	10	0.88 ± 0.30	0.131 ± 0.101	15.1 ± 10.3
			Northern pike	10	2.36 ± 0.92	0.077 ± 0.018	3.7 ± 1.5
	Grace Lake, NWT, Canada	Yes	Lake whitefish	10	5.68 ± 5.89	0.107 ± 0.048	3.2 ± 2.7
			Northern pike	8	4.13 ± 1.68	0.079 ± 0.020	2.2 ± 1.0
	Banting Lake, NWT, Canada	Yes	Lake whitefish	10	1.50 ± 0.76	0.087 ± 0.023	6.9 ± 3.6
			Northern pike	10	2.21 ± 0.95	0.061 ± 0.016	3.1 ± 1.4
	Walsh Lake, NWT, Canada	Yes	Lake whitefish	10	1.23 ± 0.56	0.076 ± 0.022	7.7 ± 4.8
			Northern pike	10	1.54 ± 0.55	0.077 ± 0.018	5.6 ± 2.3
	Small Lake, NWT, Canada	No	Lake whitefish	8	0.52 ± 0.20	0.041 ± 0.017	8.9 ± 4.2
			Northern pike	8	0.42 ± 0.11	0.044 ± 0.020	10.4 ± 4.1
Yang et al. 2020	Shimen Realgar Mine, Huangshui River, China	Yes	Goldfish	6	1.36±0.08	NR	<MDL
	1km from central mining area, Huangshui River, China	Yes	Goldfish	5	1.26±0.73	NR	<MDL
	Close to tailings dam, Huangshui River, China	Yes	Goldfish	5	10.48±4.44	NR	16.4 ± 7.3
	Intermediate zone, Huangshui River, China	Yes	Goldfish	7	4.55±3.45	NR	15.1
	Zaoshi reservoir, Huangshui River, China	No	Goldfish	16	1.41±0.72	NR	1.2 ± 2.9
			Amur catfish	4	0.70±0.44	NR	0.9 ± 2.3
Zwicker et al. 2011	Ron Phiboon District, Thailand	Yes	Striped snakehead	NR	23.92 ± 1.08; 11.01 ± 0.16	9.41 ± 0.34; 2.42 ± 0.11	39.4; 21.9
	Talay Noi Sanctuary, Thailand	No	Striped snakehead	NR	0.12	<0.014	<11.6

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in Thailand have been reported to accumulate more As(III) (0.71-2.74 mg/kg dry wt.) and As(V) (1.71-6.67 mg/kg dry wt.) than fish from a nearby uncontaminated pond (<0.007 mg/kg dry wt.; Table 2; Zwicker et al. 2011). In contrast, another study on striped snakehead from Thailand reported that fish from a reference area accumulated lower total [As] (1.9 ± 1.4 mg/kg dry wt.) but higher inorganic arsenic ([As(III)]: 0.04 ± 0.01 mg/kg dry wt., [As(V)]: 0.73 ± 0.73 mg/kg dry wt.) when compared to fish from two nearby contaminated ponds (total [As]: 13.1 ± 1.0 & 22.2 ± 2.2 mg/kg dry wt.; [As(III)]: <0.02 mg/kg dry wt.; [As(V)]: 0.12 ± 0.08 & 0.13 ± 0.04 mg/kg dry wt.; Jankong et al. 2007).

Altogether, these results again demonstrate that there is considerable variability in the concentrations of some arsenic species in freshwater fish, such as AsB and iAs, both within individual studies and across the broader literature. Although less toxic organic species of arsenic tend to be present at higher concentrations than inorganic arsenic (Slejkovec et al. 2004; Wolle et al. 2019; Yang et al. 2020; Lescord et al. 2022), this did not always hold true, with some studies reporting inorganic arsenic accumulating to potentially high levels (>1 mg/kg dry wt.; Jankong et al. 2007; Shah et al. 2010; Zwicker et al. 2011). Additionally, while the biotransformation of inorganic species of arsenic into increasingly complex organic species is generally considered a detoxification process, it can result in the formation of intermediate species of increased toxicity, such as trivalent forms of MMA and DMA (Byeon et al. 2021). It is therefore important to understand the roles of various arsenic species, including the highly toxic inorganic species as well as the moderately toxic and non-toxic organic species to fully understand their risk.

The analytical capacity necessary for arsenic speciation of biotic tissues remains a challenge to future freshwater research. One notable roadblock in the development, validation,

and/or enhancement of these analytical methods is the lack of availability of certified reference materials (CRMs) for many arsenic species. Currently, CRMs are only available for two species of arsenic in fish, AsB and DMA, limiting the certainty with which analytical results for other species can be interpreted. Despite these limitations, modern methods using chromatographic separation and ICP-MS detection have proven effective for the analysis of many arsenic species, with some reporting as many as 16 forms identified in biota (Wolle et al. 2019). However, as noted above, 5 studies were excluded from this review because they lacked any QAQC information. We encourage studies reporting on arsenic speciation analyses to provide an overview of all QAQC procedures and results to bolster confidence in the data produced.

4.3. Percentages of Arsenic Species in Freshwater Fish

Instead of or in addition to concentration values, the speciation of arsenic is reported as the percentage of total [As] made up by individual species in many studies reviewed herein. Here, again, AsB is most often reported to make up the bulk of arsenic in freshwater fish, though %AsB did vary considerably across and within studies (0.19–100% when >MDL; Table SI-2). DMA is generally the second most abundant species of arsenic, with %DMA ranging from 0.07-87% when detected, though most often falling below 30% of total [As] (Table SI-2). The percentage of DMA even surpassed %AsB in some freshwater fish, such as Canadian smallmouth bass (Lepage et al. in prep) and Thai striped snakehead (Jankong et al. 2007). Similarly, high %DMA has been reported in northern pike (de Rosemond et al. 2008), though the mean %DMA in some northern pike varied considerably among sampling locations (15-87%, Tanamal et al. 2021; 9 – 41%, Lescord et al. 2022; 7.8 – 38%, Lepage et al. in prep), and among individual pike collected from the same location (5 – 47% DMA, Lescord et al. 2022; 6.4 – 57.6% DMA, Lepage et al. in prep).

Similarly to results from marine fish (Choi et al. 2015; Wolle et al. 2019), MMA generally makes up a small amount of total [As], with %MMA typically ranging from 0.12 – 15%, though one study reported MMA made up to 57% of total [As] in carp (species not specified) from German markets (Hackethal et al. 2021; Table SI-2). In contrast to the observed variability in freshwater fish, AsB is commonly reported to make up a large percentage of total arsenic in marine fish as it is the end product of marine arsenic metabolism (Zhang et al. 2022). These differences between freshwater and marine systems may be influenced by salinity, with increasing salinity having been reported to increase the retention of AsB in marine fish, which acts as an osmolyte in cells (Zhang et al. 2022). Reduced osmotic stress in freshwater environments may, therefore, contribute to the lower relative abundance of AsB and higher abundance of other organic species like DMA in freshwater fish compared to marine fish. Differences in salinity may also influence observed differences in fish arsenic speciation profiles among freshwater systems, especially with recent trends of salinization of freshwater environments in many regions (Melles et al. 2023).

The percentage of total arsenic made up by inorganic species (As(III) and As(V)) was also highly variable in the surveyed literature (e.g., Table 2-2). In 11/32 studies, the percentages of inorganic arsenic exceeded 20% in some fish (e.g., 54.1% iAs in dark chub (*Zacco temmincki*) from a creek in Pohang City, Korea, Hong et al. 2014), but nearly half of these studies also reported concentrations <MDL in other fish (e.g., <MDL in paradise goby (*Rhinogobius giurinus*) from the same creek, Hong et al. 2014). Similarly, white amur bream (*Parabramis pekinensis*) and goldfish (*Carassius auratus*) from the Changsha river were noted to have high proportions of inorganic arsenic on average (i.e., 34.5% & 41.5% iAs) but yellow catfish (*Pelteobagrus fulvidraco*) and amur catfish (*Silurus asotus*) from the same river did not (i.e., 6.3% & 7.1% iAs; Jia et al. 2018). Although high percentages of inorganic arsenic are sometimes reported, most fish had lower %iAs

(<20%). For example, in 170 fish analyzed from lakes near mining activities in Yellowknife, Northwest Territories (NWT), mean %iAs was always <20% (Tanamal et al. 2021). Likewise, in nearly 500 samples analyzed from the area around a closed realgar mine in China, only one fish species and sampling location had %iAs greater than 20%, with most fish having <5% iAs (Yang et al. 2020). In addition to these mining impacted areas, similar trends have been reported in more pristine boreal waterways, where iAs fell below detection limits in all 300 freshwater and anadromous fish sampled (Lescord et al. 2022).

Overall, organic species of arsenic appear to dominate in most freshwater fish, though this varies and some fish have considerable amounts of inorganic arsenic. Additionally, studies rarely account for the entirety of total arsenic in fish, (Lorenzana et al. 2009; Ciardullo et al. 2010; Reid et al. 2020) often with a considerable residual fraction left in tissues or not able to be separated and detected from extracts. This unmeasured arsenic likely contains arsenolipids that often require dedicated extraction procedures, as well as other arsenic species that are strongly bound within the tissue (Ciardullo et al. 2010; Wolle and Conklin 2018). Taken together, this variability suggests that total [As] based risk assessments, where standardized proportions of iAs relative to total [As] are assumed, may not accurately represent the variation in iAs exposures from the consumption of wild-caught freshwater fish. Although it appears that assuming 20% inorganic arsenic would be adequately protective in most cases, there remain examples where this would underestimate risks or provide overly restrictive consumption guidelines. Additional work is needed on a regional scale to accurately assess risks related to arsenic exposure from freshwater fish, especially in areas with known arsenic contamination.

4.4. Drivers of Variation in Arsenic Speciation in Freshwater Fish

A significant challenge currently faced when developing total [As] based consumption guidelines for freshwater fish is the considerable amount of unexplained variability in the concentrations and relative proportions of inorganic species. One commonly discussed factor influencing arsenic accumulation and speciation in freshwater fish is environmental contamination with arsenic. The effect of contamination level on arsenic speciation varies in the literature, with some studies reporting increased accumulation of iAs in fish from contaminated areas (Huang et al. 2003; Zwicker et al. 2011; Cott et al. 2016; Komorowicz et al. 2019) and others reporting decreased iAs and higher concentrations of organic species (Jankong et al. 2007; Hong et al. 2014; Jia et al. 2019; Tanamal et al. 2021). This variation suggests that while differing levels of contamination with arsenic can influence arsenic speciation patterns, there are still other unidentified factors at play. For example, the biochemical interactions with various co-occurring chemicals such as copper (Huang et al. 2021), selenium (Lepage et al. in prep), or nutrients (Hasegawa et al. 2010) may alter arsenic speciation in fish tissue. Notably, freshwater environments show considerably more variability in water chemistry compared to marine environments due to their smaller volume coupled with diverse local geochemistry and proximity to anthropogenic impacts, and that this increased complexity can be reflected in arsenic speciation (Choi et al. 2015; Jia et al. 2018; Juncos et al. 2019; Byeon et al. 2021).

In addition to local factors driving differences in arsenic speciation between waterbodies or regions, considerable variation also exists between taxonomic groups. For example, freshwater salmonids are commonly reported to have relatively higher concentrations of total arsenic than other freshwater fish, but with a higher proportion of AsB (20-100%, most often >70%; Pizarro et al. 2003; Slejkovec et al. 2004; Choi et al. 2015; Juncos et al. 2019; Komorowicz et al. 2019; Wolle

et al. 2019; Walker et al. 2020; Hackethal et al. 2021; Lepage et al. in prep). Conversely, other fish species are commonly reported to have more variable arsenic speciation with less AsB and more DMA, such as smallmouth bass (Lepage et al. in prep) and northern pike (Zheng and Hintelmann 2004; de Rosemond et al. 2008; Tanamal et al. 2021; Lescord et al. 2022; Lepage et al. in prep). Several potential explanations for differences in As speciation among taxa have been proposed and explored in the literature, including species specific differences in gastrointestinal structure and function (de Rosemond et al. 2008), lipid content (Juncos et al. 2019), and trophic position, diet, or habitat selection (Jankong et al. 2007; de Rosemond et al. 2008; Shah et al. 2010; Choi et al. 2015; Yang et al. 2017; Jia et al. 2018; Juncos et al. 2019; Yang et al. 2020; Tanamal et al. 2021; Lescord et al. 2022; Lepage et al. in prep). Quantitative investigation of relationships with trophic ecology often uses stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) to model the trophic position and dietary carbon sources of aquatic biota; these techniques have been previously applied to freshwater arsenic speciation, but with varying results. Yang et al. (2020) found no relationship between $\delta^{15}N$ and arsenic species concentrations in fish and other aquatic organisms, while Lescord et al. (2022) found a positive relationship between %AsB and $\delta^{15}N$ in some fish species but not in others. The effect of trophic position on arsenic speciation is especially pronounced when invertebrates are considered, because arsenic typically biodilutes across whole food webs (Maeda et al. 1993; Chetelat et al. 2019; Lepage et al. in prep), while patterns within fish communities alone are more variable (Yang et al. 2020; Lescord et al. 2022, Lepage et al. in prep). Overall, more work is needed to properly characterize the influence of trophic ecology and diet, alongside other factors, on the speciation of arsenic in diverse freshwater taxa.

Arsenic speciation can also vary among individual fish, within the same waterbody and species. One commonly discussed factor influencing contaminant levels, including arsenic

speciation, is fish body size and/or age class (Cott et al 2016; Juncos et al. 2019; Komorowicz et al. 2019; Lescord et al. 2022; Lepage et al. in prep). There is some evidence that smaller and younger freshwater fish have more complex arsenic speciation (Cott et al. 2016; Lepage et al. in prep), while larger and older fish contain a higher proportion of organic arsenic, mainly AsB, however, these relationships often vary among taxa (Juncos et al. 2019; Lescord et al. 2022; Lepage et al. in prep). Future work should also consider the role of ontogenetic shifts in trophic level, diet, and habitat use that may also influence the observed relationships with size and age, similar to mercury speciation (Lescord et al. 2018).

Lastly, there are often differences in arsenic content and speciation between tissues within individual fish (Jankong et al. 2007; de Rosemond et al. 2008; Hong et al. 2014; Cott et al. 2016; Yang et al. 2017; Juncos et al. 2019). It is commonly reported that gastrointestinal, liver, and other organ tissues contain higher total arsenic concentrations, as well as an occasionally higher proportion of inorganic arsenic when compared to muscle. For example, de Rosemond et al. (2008) reported that inorganic arsenic in the muscle of 5 fish species from Yellowknife, NWT averaged 0.5 – 7.5% of total [As], while in liver tissues inorganic arsenic made up 5.5 – 22.3% of total arsenic. The role of the liver as a primary detoxification organ may explain its higher relative amounts of toxic inorganic arsenic compared to other tissues. The same study in Yellowknife also reported high total [As] in gastrointestinal tissues (1.48 – 8.92 mg/kg dry wt.) compared to muscle (0.57-1.15 mg/kg dry wt.) or liver (0.42-2.52 mg/kg dry wt.), but inorganic arsenic did not represent a large fraction of total [As] (<MDL-6% iAs) even though [iAs] were higher (<MDL-0.22 mg/kg dry wt.) than in muscle tissue (<MDL-0.07 mg/kg dry wt.; de Rosemond et al. 2008). Gastrointestinal tissues likely accumulate higher total [As] because they are the primary route of dietary arsenic uptake, as suggested by de Rosemond et al. (2008). Interestingly, the authors also

noted potential differences in total arsenic accumulation based on species-specific differences in gastrointestinal morphology and behaviour. Bottom-feeding suckers (*Catostomus* sp.) accumulated more arsenic in gastrointestinal tissues and were noted to have less developed gastrointestinal systems consisting of only an intestine with no defined stomach or pyloric caeca, which are present in other fish species analyzed (de Rosemond et al. 2008). Tissues other than muscle may be consumed by some fishers, including Indigenous Peoples (McAuley et al. 2018; Chan et al. 2019) and should be incorporated into risk assessments where appropriate and possible.

4.5. Conclusions and Recommendations

We found considerable variability in the literature reporting on arsenic speciation results in freshwater fish. This variability is likely due to several factors, including differences in waterbody contamination level, variation in trophic ecology, and species-specific differences in arsenic accumulation, metabolism, and tissue storage. While organic species of arsenic typically dominated, some fish had elevated inorganic arsenic, particularly in areas with higher exposure to environmental arsenic contamination. Although the use of total [As] in risk assessments, with a default assumption of 20% inorganic arsenic, appears to be generally supported by the literature there are examples where this can underestimate or overestimate risks to consumers. Given this variability in speciation, it appears that inorganic arsenic based limits for fish are a more accurate representation of risk than total [As] based measures. With recent advances in analytical techniques for arsenic speciation, it is prudent that policy makers consider establishing specific limits for inorganic arsenic in fish to better protect human health. Although speciation data is ideal, we also acknowledge the problems that may arise when implementing this in broader monitoring programs (e.g., increased cost and analytical complexity). The National Health Commission and

State Administration of Market Regulation for China have established a unique compromise for this, establishing limits based on [iAs], but allowing the use of total [As] analysis, so long as total [As] falls below the allowable limit for [iAs] (Government of China 2017).

It is also important to take a balanced approach to risk assessment that considers the health benefits of consuming fish (Moriarity et al. 2020) and the potential implications of limiting fish consumption (Harper and Harris 2008). Fish tissue contains a variety of nutrients, proteins, and fatty acids that provide numerous health benefits such as a reduction of cardiovascular disease risk (Chen et al. 2022). Many Indigenous communities rely on the consumption of locally caught freshwater fish as part of their cultural heritage and as a means of feeding themselves (Kuhnlein and Receveur 2007; Hori et al. 2012; Chan et al. 2019; Moriarity et al. 2020). The consumption of traditional foods often improves food security and quality, with noted improvements in the health of individuals who consume fish and other locally caught foods (Dewailly et al. 2002; Kuhnlein and Receveur 2007; Gates et al. 2016). Chemical contamination of these important food sources and inaccurate risk-benefit analysis disproportionally affects Indigenous Peoples, often forcing them to decide between health risks of contaminants and the health and cultural losses associated with limiting the consumption of traditional foods (Harper and Harris 2008; Fernández-Llamazares et al. 2019). It is therefore important to ensure that any assessment of risk from the consumption of these food sources are refined as analytical techniques are advanced and more speciation data are amassed.

With respect to arsenic, we encourage the consideration of arsenic speciation on a local basis to assess human and environmental health risks more accurately across diverse taxa and locations, similar to the work done by Tanamal et al. (2021) in Yellowknife, NWT. Furthermore, additional work is needed to assess how various factors such as water chemistry, trophic ecology,

643 and species-specific factors influence arsenic speciation in freshwater fish. To increase the
644 potential for future meta-analyses that can directly improve risk assessment, we encourage future
645 studies to report results in as much detail as is reasonable, ideally including mean values with
646 measures of variance in addition to ranges of both concentrations and percentages relative to total
647 [As] either in text or in tables. These studies should also strive to fully report QAQC protocols to
648 support their data. We particularly encourage the inclusion of more detailed supplemental
649 information files that proved invaluable in conducting this review (e.g., Yang et al. 2020; Lescord
650 et al. 2022). Consideration of arsenic speciation in addition to other contaminants will help
651 improve risk assessment and mitigation practices and allow individuals to make informed
652 decisions about personal risk from consuming locally caught fish

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Chapter 3: Biodilution of organic species of arsenic in three freshwater food webs

1. Abstract

Arsenic can accumulate in freshwater biota, sometimes reaching potentially harmful levels. However, the toxicity of arsenic strongly depends on which chemical forms, or arsenic species, are present. Although organic species are considered less harmful than inorganic ones, they have not been extensively studied in freshwater environments and drivers of variation in arsenic speciation among sites and taxa remain unclear. We assessed concentrations of two commonly reported organic arsenic species, arsenobetaine (AsB) and dimethylarsinic acid (DMA), in fish and invertebrates from three lakes near Sudbury, Ontario, Canada—a region with widespread mining impacts. Both AsB and DMA were detected in nearly all samples analyzed (n = 212), varying across a wide range of concentrations (<0.001–30.144 and <0.006–5.262 mg/kg dry wt., respectively). The lake with the most severe mining impacts typically had the highest concentrations ([ ]) of AsB and DMA. In contrast, the percentage of total arsenic made up by AsB (%AsB) and DMA (%DMA) did not vary significantly between lakes within a given taxa. Arsenic speciation in fish muscle varied with fish size, selenium concentrations, and trophic ecology (inferred from nitrogen isotopes, $\delta^{15}N$), but relationships with diet (inferred from carbon isotopes, $\delta^{13}C$) were more varied. Within all 3 lake food webs, [AsB] and [DMA] typically underwent biodilution, decreasing with trophic elevation (i.e., $\delta^{15}N$). Although the aforementioned factors explained some variation in arsenic speciation, there remains considerable unexplained variation, particularly among fish and invertebrate taxa. Further studies on arsenic speciation in freshwater biota should target diverse invertebrate and fish taxa to better understand drivers of variation in arsenic speciation. Additionally, work emphasizing the percentage of inorganic arsenic and other organic arsenic species is needed to improve environmental and human health risk assessments.

2. Introduction

Arsenic (As) is a naturally occurring metalloid that can bioaccumulate in aquatic organisms, including fish, and exhibits both acute and chronic toxicity (Byeon et al. 2021). However, the toxicity of arsenic in the environment strongly depends on its chemical speciation; that is, the various forms of arsenic differing in oxidation state or molecular structure (Templeton et al. 2000). Of the arsenic species that exist in aquatic environments, the inorganic species arsenite (As(III)) and arsenate (As(V)) are the most toxic and tend to make up the bulk of arsenic present in water and sediments (Kohlmeyer et al. 2003). In contrast less toxic organic species of arsenic, or organoarsenicals, tend to be more prevalent in fish and other biota, though inorganic arsenic (iAs) can also be found in biota at varying concentrations (Zheng and Hintelmann 2004; Miyashita et al. 2009; Ruttens et al. 2012). Of the organic species, arsenobetaine (AsB) is considered the least toxic (Byeon et al. 2021).

Although not fully understood, the prevalence of organoarsenicals in fish and other biota is due, in part, to complex biotransformation pathways that chemically modify arsenic into less toxic or more easily excreted chemical species (Kumari et al. 2017; Byeon et al. 2021; Cui et al. 2021). In aquatic systems, multiple species of arsenic can enter the food chain at various trophic levels, through absorption or by consumption of water, sediments, and biota (Rahman et al. 2012). Subsequent biotransformation pathways can result in varying arsenic speciation across organisms and systems. For example, research has shown that the capacity to biotransform arsenic can vary among fish species, leading to differences in arsenic speciation in their tissue (Slejkovec et al. 2004; Zhang et al. 2016). Differences in tissue arsenic speciation may also arise from differences in dietary habits (i.e., pelagic vs littoral), which would alter arsenic exposure, or from differences in fish size (de Rosemond et al. 2008). Nevertheless, AsB is the most commonly reported species

of arsenic in fish. Other organoarsenicals that may be detected in fish include dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenosugars, arsenolipids, arsenocholine, and various other methylated forms (Wolle and Conklin 2018).

While the biotransformation of iAs into organoarsenicals is well documented in marine systems, the specific mechanisms and the prevalence of AsB across multiple trophic levels are not well understood in freshwater systems (Caumette et al. 2012; Rahman et al. 2012; Cui et al. 2021; Hussain et al. 2021). Furthermore, much of the available literature on AsB formation in freshwater organisms comes from laboratory exposures rather than natural systems (Caumette et al. 2012). From the existing literature on freshwater environments, there appears to be considerable variation in arsenic speciation profiles in invertebrates and fish, when compared with marine studies (Kaise et al. 1997; Miyashita et al. 2009; Caumette et al. 2012; Caumette et al. 2014; Erikson et al. 2019; Byeon et al. 2021). The drivers of this variation are unclear, but it may be a result of differences in water chemistry, including arsenic concentrations or speciation, which have been shown to alter arsenic speciation in freshwater plankton (Caumette et al. 2014). Additionally, other elements in aquatic environments can alter the bioaccumulation or speciation of arsenic, such as selenium (Belzile et al. 2006) or copper (Huang et al. 2021).

The main goal of this study was to assess arsenic speciation, as AsB and DMA, across whole lake food webs with varying degrees of anthropogenic impacts, including arsenic contamination. This included investigating potential drivers of variation in arsenic speciation among fish (i.e., fish size, total elemental concentrations, trophic elevation and dietary carbon source, taxa, and water body) in addition to investigating how concentrations of arsenic species vary with trophic position and dietary carbon sources within whole food webs. We expect that lakes with more severe arsenic contamination will have higher fish tissue concentrations of total

arsenic, and that a higher proportion of this arsenic will be AsB. We also expect to see differences in arsenic speciation profiles among fish taxa. While concentrations of total arsenic and the various species are generally expected to decline with increasing trophic elevation, we also expect that the proportion of AsB will increase with trophic position and greater reliance on pelagic carbon sources of fish.

3. Methods

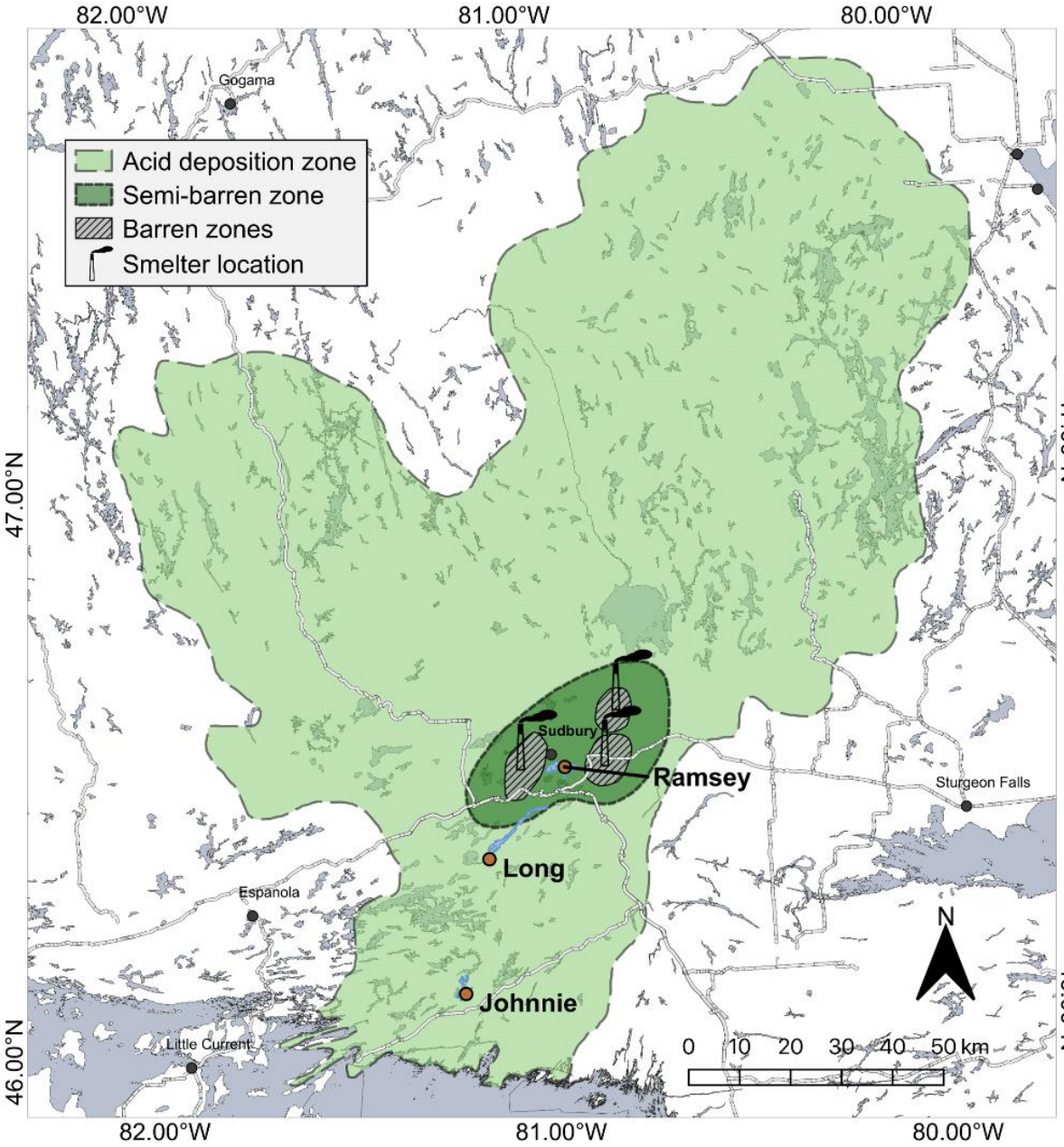
3.1. Study Area and Sampling Sites

This study centers around Sudbury, Ontario, Canada, a region with a mining history dating back to the late 1800s. Sudbury smelters were one of the world's largest emitters of sulfur dioxide and metal particulates until recent decades (Keller et al. 2019). These emissions left local terrestrial and aquatic environments heavily acidified and contaminated with elements, such as Se, Cu, and Ni (Keller et al. 2019). The severity of these impacts across the region are commonly described as barren, semi-barren, and acid deposition zones, based on the extent of damage to terrestrial vegetation (Figure 1; Keller et al. 1999). The barren and semi-barren zones extend around the three main historical smelting operations in Sudbury (Keller et al. 1999). Beyond the barren and semi-barren zones lies a 17,000 km^2 area where more than 7000 lakes were acidified below pH 6.0 (Neary et al. 1990) but were less impacted by metal deposition when compared to lakes closer to the smelter complexes. While there has been a remarkable biological and chemical recovery seen in the Sudbury area with emission reductions over the last 40 years, these complex legacy mining impacts are still seen across the region today (Keller et al. 2019).

Our three study sites (Figure 3-1) were selected based on: proximity to known mining impacts, availability of archived tissues, and previous information on consumption advisories in the Guide to Eating Ontario Fish (MECP, 2017). A summary of water chemistry data for each lake can be found in Table SI-1. Long Lake runs along the boundary between the semi-barren and acid deposition zones just south of Sudbury (Figure 3-1). In addition to historical atmospheric deposition of acid and elements from nearby smelters, Long Lake contains a point source of arsenic from the abandoned Long Lake Gold Mine's unconfined tailings eroding into the lake (MNDM, 2019). This has led to increased As concentrations in surface water in Outlet Bay (26 - 256 ug/L; MNDM, 2019). Elevated total arsenic concentrations in fish tissues have also been reported, with consumption advisories being issued for Smallmouth Bass and Cisco from Outlet Bay (MECP, 2017). For this study, all samples were collected from Outlet Bay.

Ramsey Lake is located approximately in the middle of the three main historic smelters and the semi-barren zone of Sudbury (Figure 3-1). It was heavily impacted by historical atmospheric deposition of acid and elements as well as considerable shoreline and watershed development that has introduced additional stressors (e.g., road salt and nutrient inputs; Gunn and Keller 1995). Fish from Ramsey Lake have elevated mercury and selenium, but not arsenic levels (MECP 2017).

Johnnie Lake is a more remote lake, located in Killarney Provincial Park, roughly 50 km southwest of Sudbury (Figure 3-1). While it did experience acidification because of atmospheric deposition from sulphur sources in Sudbury and elsewhere in North America, it was more isolated from elemental deposition when compared to lakes closer to the smelter complexes. Fish from Johnnie Lake have elevated mercury and organic pollutants, but not arsenic nor selenium (MECP 2017).



1031

1032 **Figure 3-1.** Map of sample lakes around the Sudbury area, showing the approximated boundary
1033 of the historical acid rain deposition (based on Neary et al. 1990). Spatial data for the locations
1034 of the 3 smelters and the extent of their impact on the surrounding vegetation are from the City
1035 of Greater Sudbury (2019). Note: this map was made by Calvin Kluke using ArcGIS ArcMap
1036 10.7 (license: Laurentian University) and QGIS 3.14.16 (open-source) software in 2022.

3.2. Sample Collection and Preparation

Benthic macroinvertebrates were collected in July-September 2021 by kick-sweeping the shoreline at two different sites on each lake as well as by flipping submerged rocks and picking-off invertebrates. Benthic macroinvertebrates were pooled by Order and Suborder (i.e., *Ephemeroptera*, *Megaloptera*, and *Zygoptera*), or Family (i.e., *Gomphidae*, *Macromiidae*, and *Aeshnidae*) and rinsed with lake water to remove debris. Crayfish (*Cambaridae*) were occasionally collected through the kick-sweeps, but more commonly using minnow traps, which were placed along the shoreline in rocky habitat and baited with dry dog food. For crayfish, individual abdominal muscle samples were collected and where necessary pooled with similarly sized individuals to ensure enough biomass was available for all chemical analyses; other benthic invertebrates were pooled whole-body homogenates. No benthic macroinvertebrates except crayfish were sampled from Johnnie Lake. Bulk zooplankton samples were collected from all study lakes in August-September 2021 by towing either an 80 μm or 300 μm Wisconsin net at approximately 3-5 m depth for 10 min; three to six bulk tows were performed per lake. All samples were rinsed with lake water and those collected with the 80 μm net were sieved into two fractions using a 250 μm sieve, with the >250 μm fraction retained for analysis. All samples were then frozen in either whirl-pak bags or 50 mL falcon tubes until further processing.

Where possible, fish muscle samples were selected from the Ontario Ministry of Natural Resources and Forestry (MNRF) Boreal Food Webs (BFW) sample archive, housed at the Vale Living with Lakes Center (VLWLC) at Laurentian University (Sudbury, Ontario). Across the 3 sites, 81 samples of large-bodied fish and 51 samples of forage fish were obtained from this archive. These samples were collected as part of other research projects, from 2019-2022. Additional forage fish were also collected from Long and Ramsey lakes in 2021 using small-mesh

gillnets and minnow traps, and using minnow traps only in Johnnie Lake. Fish were weighed, measured, and dissected for muscle samples. For larger individual fish, samples were taken from dorsal muscle above the lateral line, while whole muscle filet samples were collected from smaller fish (<75 mm total length). In all cases, care was taken to remove all scales, skin, fat, and bone. For some of the smallest forage fish samples (n = 11), muscle tissue from 2 - 5 fish of the same species and similar sizes (± 10 mm) was pooled to ensure enough biomass was available for all chemical analyses; for statistical modelling body size measurements were averaged across pooled individuals. Fish species collected included four large-bodied predators: northern pike (herein referred to as pike; *Esox lucius*), walleye (*Sander vitreus*), smallmouth bass (bass; *Micropterus dolomieu*), and lake charr (charr; *Salvelinus namaycush*); one large-bodied insectivore: white sucker (sucker; *Catostomus commersonii*); three littoral forage fish: yellow perch (perch; *Perca flavescens*), pumpkinseed (*Lepomis gibbosus*), and rock bass (*Ambloplites rupestris*); and one pelagic forage fish: cisco (*Coregonus artedi*).

All samples were freeze-dried using a Labconco FreeZone (Labconco Corporation, Kansas City, Missouri, United States) bulk tray dryer before being homogenized to a powder using a Retsch Mixer Mill MM 400 (Retsch, Haan, Germany) or mortar and pestle. Dried and homogenized tissues were stored in whirl-pak bags or glass scintillation vials and refrigerated at 4°C prior to analysis. A summary of all samples by lake and taxonomic group is available in Table 3-1.

Table 3-1. Sample sizes by lake and taxon for arsenic speciation (Spec) and total elemental (Tot) analyses. All fish and crayfish samples were muscle samples, while other invertebrates were whole-body homogenates.

Taxon	Long		Ramsey		Johnnie		Total	
	Spec	Tot	Spec	Tot	Spec	Tot	Spec	Tot
Large-Bodied Predator	22	22	28	27	17	17	67	66
Northern Pike	9	9	11	10	4	4	24	23
Walleye	9	9	9	9	-	-	18	18
Smallmouth Bass	4	4	8	8	7	7	19	19
Lake Charr	-	-	-	-	6	6	6	6
Large-Bodied Insectivore	3	3	9	7	7	7	19	17
White Sucker	3	3	9	7	7	7	19	17
Pelagic Forage Fish	10	10	0	0	7	7	17	17
Cisco	10	10	-	-	7	7	17	17
Littoral Forage Fish	18	6	22	5	22	4	62	15
Yellow Perch	8	6	6	5	7	4	20	15
Pumpkinseed	10	-	8	-	8	-	26	-
Rock Bass	-	-	8	-	7	-	15	-
Invertebrates	22	-	14	4	11	2	47	6
Zooplankton	2	-	3	3	5	2	1	5
Crayfish	8	-	7	-	6	-	21	-
Aeshnidae	4	-	1	-	-	-	5	-
Macromiidae	3	-	1	-	-	-	4	-
Gomphidae	2	-	1	1	-	-	3	1
Ephemeroptera	2	-	-	-	-	-	2	-
Megaloptera	-	-	1	-	-	-	1	-
Zygoptera	1	-	-	-	-	-	1	-
Total	76	40	74	44	64	37	212	121

3.3. Stable Isotope Analysis

Samples from the BFW archive were previously analyzed for stable isotopes of nitrogen (N) and carbon (C) using continuous flow isotope ratio mass spectrometry (CF-IRMS) at the Stable Isotopes in Nature Lab (SINLAB) at the University of New Brunswick following the method described in Jardine et al. (2003). The invertebrate and additional fish samples collected were also

analyzed at SINLAB for C and N stable isotopes following the same methods. Quality assurance and control (QAQC) measures for stable isotope analysis included analysis of nicotinamide (n = 10; $\delta^{13}C$ = -32.55 ± 0.06‰; $\delta^{15}N$ = 2.14 ± 0.08‰), N_2 (n = 4; $\delta^{15}N$ = 20.28 ± 0.21‰), and CH_7 (n = 3; $\delta^{13}C$ = -32.25 ± 0.01‰) standards. Additionally, 1 in 10 samples were analyzed in duplicate. Stable isotope values are reported relative to Vienna Pee Dee Belemnite for C and atmospheric air for N using delta notation (δ; per mille, ‰). $\delta^{15}N$ can be used to estimate the trophic elevation of an organism within its food chain because it is typically enriched by 3.4‰ with increasing trophic level (Post 2002). In lacustrine systems, $\delta^{13}C$ is used to differentiate between pelagic and littoral energy sources in a fish's diet, with littoral feeding organisms expected to have a less negative $\delta^{13}C$ signature compared to pelagic feeding individuals (Post 2002).

3.4. Total Elemental Analysis

A total of 122 samples were analyzed for concentrations of 9 elements, including total As and Se, at the ISO:17025 accredited Biotron trace-metal laboratory at The University of Western Ontario; the remaining 92 samples did not have sufficient biomass for total elemental analysis (Table 1). Samples were digested using a Milestone ETHOS (Milestone, Sorisole, Italy) microwave digestion system according to EPA3052 method. Briefly, 2 mL of concentrated TraceMetal grade HNO3 was added to approximately 100 mg of freeze-dried tissue in Teflon™ microwave digestion vessels. These were left to off-gas for 30 min before being microwaved at 180°C (10 min ramp + 10 min hold). Sample extracts were then rinsed into 50 mL tubes and filled to volume with ultrapure water. Finally, the extracts were filtered with 0.22 μm syringe filters and analyzed for total elemental concentrations per EPA 200.8 method using an Agilent 7700 inductively coupled plasma-mass spectrometer (ICP-MS; Agilent, Santa Clara, California, United

States). QAQC protocols included analysis of method blanks, spiked method blanks, extraction duplicates, method spikes, duplicate method spikes, internal standard recovery, ongoing performance replicates, and the analysis of a fish protein certified reference material (CRM), DORM-5 (NRCC). Recoveries of As and Se in DORM-5 were 98.8 ± 3.1% and 110.5 ± 4.8%, respectively (n=34). A summary of all QAQC data and detection limits for total elemental analysis can be found in Table SI-2.

3.5. Arsenic Speciation Analysis

3.5.1. Water-Soluble Arsenic Species Extraction

For water-soluble arsenic species extraction, 12 mL of ultrapure water was added to approximately 250 mg of freeze-dried tissue in Teflon™ microwave digestion vessels. Samples were extracted at 75°C (10 min ramp + 15 min hold) using a Questron® QWave microwave digestion system (Questron Technologies, Mississauga, Ontario, Canada) at a maximum power of 750 W. Extracts were rinsed into 50 mL centrifuge tubes with ultrapure water, filled to 35 mL, and centrifuged for 15 min at a power of 9 using a Fisherbrand™ Model 225A centrifuge (Fisher Scientific, Pittsburgh, Pennsylvania, United States). The supernatant was then decanted into clean centrifuge tubes. An additional 12 mL of ultrapure water was then added to the residual tissue and centrifuged at power 9 for another 15 min as a rinse step. The second supernatant was combined with the first and filled to a final volume of 50 mL. Extracts were stored capped and sealed with parafilm at 4°C until analysis. Minimal changes in speciation results for AsB and DMA were observed with increasing lag time for analysis of the same extracts up to 8 weeks (Figure SI-1). Nevertheless, extracts were typically analyzed within 1 week of digestion, with the exception of 12 invertebrate samples that were not able to be analyzed until 4 weeks after digestion and did not

have tissue remaining to re-extract. Directly prior to analysis, extracts were filtered with 0.45 μm PES syringe filters and adjusted to 0.2% H_2O_2 (TraceMetal grade; Fisher Chemical™) to oxidize all inorganic arsenic into As(V) to avoid potential chromatographic interference between the As(III) and AsB peaks. No effect on the stability of AsB and DMA was observed with the addition of H_2O_2.

3.5.2. Quantification of Arsenic Species

Separation and detection of AsB and DMA was performed using a Metrohm® 940 Professional Vario ion chromatograph (IC; Metrohm, Herisau, Switzerland) coupled to a Perkin Elmer© NexIon 1000 (PerkinElmer, Waltham, Massachusetts, United States) ICP-MS for detection. These instruments were integrated using Waters© Empower3 chromatography software; chromatograms were integrated and quantified with the same software using the ApexTrack™ peak integration algorithm (Waters Corporation, Milford, Massachusetts, United States) with a Savitzky-Golay smoothing factor of 9. All instruments are located at the Perdue Central Analytical Facility, Laurentian University. To minimize potential polyatomic interferences with arsenic detection, the ICP-MS was operated in Dynamic Reaction Cell (DRC) mode, with acceptably low method detection limits (AsB: 0.001 mg/kg dry wt.; DMA: 0.006 mg/kg dry wt.). For some invertebrates, less tissue was used due to limited biomass, resulting in higher detection limits (AsB: 0.005 mg/kg dry wt.; DMA: 0.030 mg/kg dry wt.). The chromatographic methods used were adapted from Wolle et al. (2018) and used an anion exchange column with an ammonium carbonate (Certified ACS; Fisher Chemical™) and ammonium bicarbonate (99%; Fisher Chemical™) mobile phase gradient. A constant 5% (v/v) methanol (LC/MS Grade; Fisher Chemical™) was also added to the mobile phase to improve ionization of arsenic in the plasma.

Two slightly different chromatographic methods were employed for fish and invertebrate samples (Figure SI-2). Fish samples, which typically only showed two peaks (AsB and DMA) in their chromatograms, were analyzed using a shorter gradient to decrease run times; while invertebrates were analyzed using an extended gradient to ensure complete chromatographic separation of the target species (AsB and DMA) from other observed peaks not seen in fish samples (e.g., Figure SI-3). These peaks represent other species of arsenic (e.g., iAs, MMA, arsenosugars) that were identified by Wolle & Conklin (2018) but could not be reliably quantified here due to unavailability of CRM's and independent standards or inconsistent recovery of independent standards in the case of iAs. A detailed breakdown of instrument operation parameters for both sample matrices is shown in Table SI-3. Quality assurance and control for arsenic speciation is described in Section SI-1 and results are summarized in Table SI-4.

3.6. Data Handling and Statistical Analyses

All data handling and statistical analyses were completed using RStudio (2022.07.2 Build 576; R Version 4.2.2.). Alpha was set at 0.05 for all analyses. Where [AsB] or [DMA] were <MDL (3 and 7 samples, respectively) a random value between 0 and the MDL was substituted to allow for statistical analyses; total [As] was >MDL in all samples.

Total arsenic concentrations in fish muscle were compared to benchmarks established by MECP for reduced consumption (<8 meals per month) in sensitive and general populations (0.25 and 0.67 mg/kg wet wt., respectively; Gandhi et al. 2017). To enable comparisons with our concentration data, these benchmarks were converted to dry weight basis, assuming 78% moisture (sensitive: 1.14 mg/kg dry wt.; General: 3.05 mg/kg dry wt.).

The percentage of total [As] made up by [AsB] (%AsB) or [DMA] (%DMA) were calculated using equations 1 and 2, respectively. Speciation recovery—the percentage of total [As] accounted for by the sum of [AsB] and [DMA]—was calculated using equation 3.

$$\%AsB = ([AsB] \div total\ [As]) \times 100\% \quad (1)$$

$$\%DMA = ([DMA] \div total\ [As]) \times 100\% \quad (2)$$

$$Speciation\ Recovery\ (\%) = \big(([AsB] + [DMA]) \div total\ [As]\big) \times 100\% \quad (3)$$

One yellow perch from Ramsey Lake was removed from the dataset because speciation recovery was significantly higher than 100% (i.e., 1465%); all other samples were <101.1%.

Percentage values were commonly used in statistical models because they generally improved the normality of model residuals. We acknowledge that the use of ratio data increases the risk of spurious correlations (Kronmal, 1992) and tried to remain cognizant of potential spurious relationships throughout, and account for them where possible. For all parametric models, percentage data were logit transformed while concentrations and fish sizes were $\log_{10}$ transformed. Because %AsB and %DMA were generally consistent between lakes, all lakes were pooled for comparisons of percentage data among taxa to increase sample size. No groups with a sample size <6 were included in the statistical testing described below.

For comparisons of arsenic speciation among lakes and taxa, one way ANOVA was used, and residual normality was assessed with Shapiro-Wilk tests; if residual normality failed a Kruskal-Wallis test was used instead. When these tests indicated significant differences among groups, pairwise post-hoc testing was performed using Tukey's HSD for ANOVA and Dunn's test

for Kruskal-Wallis tests. Dunn's test p-values were Bonferroni corrected for multiple comparisons. Where group sample size was <6 comparisons were made qualitatively.

A modified condition factor (K), calculated using equation 4, was used to represent size as it was more comparable across fish species within functional groups (Figure SI-4c) compared to length or weight alone (Figure SI-4a and b).

$$K = 100{,}000 \times total\ length \times round\ weight^3 \quad (4)$$

To assess relationships between arsenic speciation and size, and to compare these relationships across lakes, ANCOVA models were used. Residual normality was assessed with Shapiro-Wilk tests, and if residuals were non-normally distributed outliers were identified with Cook's Distance and removed; most models passed normality testing using this procedure, exceptions are noted in text or in figures. ANCOVA model structure was lake + log10(K) + lake:log10(K). When there was no significant difference in the slope of relationships among lakes, as indicated by a non-significant interaction term (i.e., lake:log10(K) p-interact > 0.05), the interaction term was removed from the model and the significance of the main effects was assessed with Type III F tests. Additionally, linear regressions were used to assess the strength and slope of relationships between arsenic speciation and fish size within individual lakes, and across all lakes together. These regression models were similarly assessed for normality using Shapiro-Wilk tests, combined with the outlier removal procedure described previously. To increase sample size, these relationships were assessed within fish functional groups as described above (Section 3.2.). Lake charr were excluded from this analysis because they were only collected from Johnnie Lake.

The effect of total selenium concentrations, trophic ecology ($\delta^{15}N$), and diet ($\delta^{13}C$) on arsenic speciation in fish was also assessed using ANCOVA and linear regression models. Stable

isotope values (i.e., $\delta^{15}N$ and $\delta^{13}C$) were baseline corrected to account for variation in isotope baselines between lakes (e.g., Ramsey Lake typically had more negative $\delta^{13}C$ values than the other two lakes; Figure SI-5) by subtracting the average values for crayfish in each lake. Crayfish isotope signatures were selected for baseline correction as they were the most widely distributed invertebrate across lakes and showed a more consistent food web position within lakes relative to zooplankton, the only other invertebrate taxa sampled from all lakes (Figure SI-5). In addition to within fish, the effects of $\delta^{15}N$ and $\delta^{13}C$ on arsenic species concentrations were assessed across taxonomic groups and within individual lake food webs using linear regression and compared across lakes using ANCOVA, similarly to models described above.

4. Results and Discussion

4.1. Arsenic Concentrations in Fish and Invertebrates.

4.1.1. Total Arsenic

Total [As] was detected in all fish muscle samples tested (n = 115), at concentrations ranging from 0.04 – 31.31 mg/kg dry wt., an over 800-fold difference (Figure 3-2a). Total [As] was also detected in all 6 invertebrate samples analyzed (primarily zooplankton) at concentrations from 1.31 - 4.82 mg/kg in Ramsey and Johnnie lakes (Figure SI-6a); no invertebrates from Long Lake had enough biomass for total [As] analysis. As predicted, within individual fish species total [As] was typically highest in Long Lake, while levels in Ramsey Lake and Johnnie Lake fish were lower but similar, though Ramsey Lake generally had slightly higher concentrations (Table SI-5). Total [As] in Long Lake (0.24 – 10.79 mg/kg dry wt.) was generally similar to literature on gold-mine contaminated lakes near Yellowknife NWT (mean total [As] 0.88 - 5.97 mg/kg dry wt.; Tanamal et al. 2021), except in cisco, which had elevated concentrations (0.46 – 31.31 mg/kg dry

wt.) that were more consistent with anadromous cisco from the Far North of Ontario (<0.1 – 47.4 mg/kg dry wt.; Lescord et al. 2022). Concentrations of total arsenic in Ramsey Lake (0.059 – 0.542 mg/kg dry wt.) and Johnnie Lake (0.037 – 0.432 mg/kg dry wt.) were more consistent with the uncontaminated reference lakes used in the Yellowknife lake study (mean total [As] 0.42 – 0.65 mg/kg dry wt.; Tanamal et al. 2021).

Total arsenic concentrations only exceeded consumption benchmarks in fish collected from Long Lake (Figure 3-2a); all fish from both Ramsey and Johnnie Lakes had concentrations that did not pose a risk with more frequent consumption (>8 meals/month; Figure 3-2a). In Long Lake, a high proportion of consumption benchmark exceedances were seen in cisco (7/10 fish; 9.888 – 31.309 mg/kg total [As]), bass (4/4 fish; 3.82 – 10.79 mg/kg total [As]), suckers (3/3 fish; 1.96 – 4.65 mg/kg total [As]), and pike (6/9 fish; 1.36 – 9.34 mg/kg total [As]). Walleye and perch in Long lake showed only a few exceedances in individual fish with the highest total [As] (4/15 fish; 1.18 - 3.43 mg/kg; Figure 3-2a). Based on these total arsenic measures, it appears that arsenic is not as big of a concern in these lakes when compared to other elements like Hg, but risk varies between lakes and fish species.

Within individual lakes, total [As] was typically highest in bass and suckers, followed by pike, then other taxa, though this trend was not seen as strongly in Johnnie Lake (Figure 3-2a; Table SI-6; bass and sucker in Long Lake compared qualitatively). Suckers primarily feed on benthic invertebrates in the sediment on the lake bottom, and smallmouth bass also feed heavily on invertebrates, particularly mayflies, dragonflies, and crayfish—which are noted to make up over half of their diet in lakes (Weidel et al. 2000). It is possible that fish that consume invertebrates may be exposed to higher arsenic burdens, both from prey and consumption of sediment during

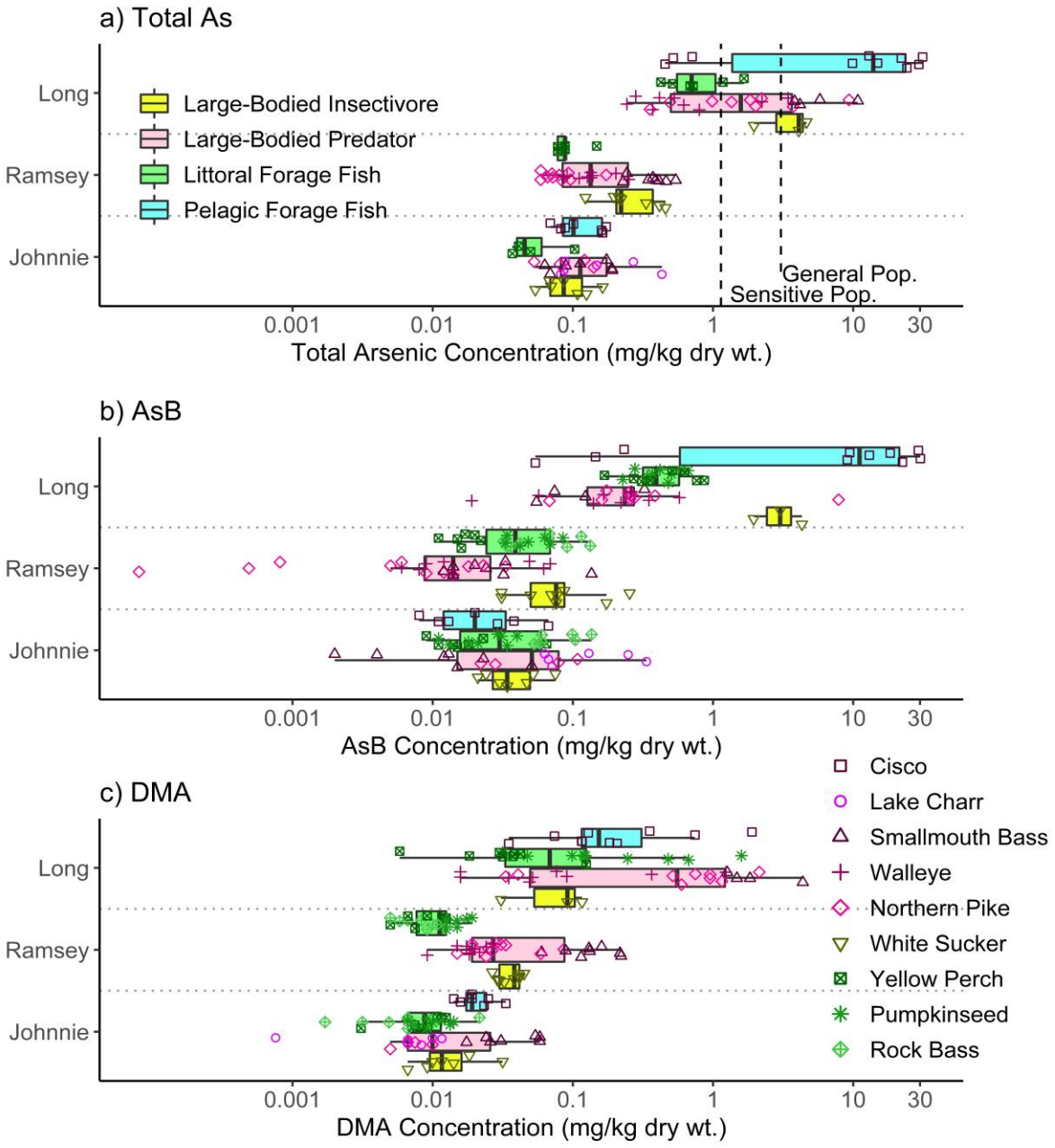


Figure 3-2. Boxplots of $\log_{10}$-transformed total As (a) AsB (b) and DMA (c) concentrations in fish from three lakes across a mining impact gradient near Sudbury, Ontario. Data are grouped by functional groups, with points representing individual fish, and species denoted by colour and shape. Boxes represent the 25th to 75th percentile of the data, the vertical line in each box represents the median, and the horizontal whiskers indicate the spread of the data within 1.5 times the interquartile distance from the 25th and 75th percentile. Vertical dashed lines in panel (a) represent concentration benchmarks for reduced consumption of fish muscle in Ontario (MECP, 2017).

feeding, leading to increased arsenic accumulation. There may also be physiological drivers of variation in arsenic accumulation between fish species, such as differing digestive system morphology. Similar to our results, de Rosemond et al. (2008) found suckers from the Northwest Territories accumulated higher total [As] than other fish species, noting that they have less developed digestive systems than some other fish taxa, which could impact bioaccumulation and transformation of arsenic.

4.1.2. Arsenic Speciation

Arsenobetaine was detected in 98.5% of fish, varying across a wide range of concentrations (0.002 – 30.144 mg/kg dry wt.). In 3 pike from Ramsey Lake, [AsB] was below the MDL (<0.001 mg/kg dry wt.; Figure 3-2b). Similar results have been widely reported, with AsB being frequently detected in marine and freshwater fish, but across a wide range of concentrations (Rahman et al. 2012; Luvonga et al. 2020). AsB was also detected in all 47 invertebrate samples, but across a narrower range of concentrations (0.057 – 1.237 mg/kg dry wt.; Figure SI-6b). This is generally consistent with concentrations reported in freshwater crustaceans from the arsenic contaminated Hayakawa River in Japan (mean [AsB]: 0.280 ± 0.076 mg/kg dry wt.; Miyashita et al. 2009) and two size fractions of zooplankton from uncontaminated Grace Lake, NWT, Canada ([AsB]: 0.335 & 0.990 mg/kg dry wt.; Caumette et al. 2011) but higher than concentrations reported for benthic invertebrates from arsenic contaminated Panther Creek, USA (<0.01 mg/kg dry wt., estimated from figure; Erickson et al. 2019) and zooplankton from arsenic contaminated Long Lake. NWT, Canada (<0.001 mg/kg dry wt.; Caumette et al. 2011).

Dimethylarsinic acid was also detected in 97.6% of fish, across a narrower range of concentrations than AsB and total As (0.006 – 5.262 mg/kg dry wt.; Figure 3-2c). It was <MDL (<0.006 mg/kg dry wt.) in 6 fish (Johnnie Lake: 1 charr, 1 perch, 3 rock bass; Ramsey Lake: 1 rock bass). Overall detection rates of DMA in this study were similar to those observed in fish near gold mining impacts by de Rosemond et al. (2008) but were much higher than detection rates in fish from more pristine boreal systems (38%; Lescord et al. 2022). Similar to [AsB], [DMA] in invertebrates ranged from 0.006 – 1.266 mg/kg dry wt. (Figure SI-6c) and were <MDL in 2 samples (2 crayfish: 1 Ramsey, 1 Johnnie). These concentrations are generally higher than those previously reported in benthic invertebrates (0.06 mg/kg dry wt., Erickson et al 2019) and zooplankton (0.08 – 0.15 mg/kg dry wt. Caumette et al. 2011) from mining impacted areas.

As with total [As], [AsB] and [DMA] were typically highest in Long Lake, followed by Ramsey and Johnnie Lakes within a given taxon (Table SI-5). The main deviation from this trend were zooplankton from Long Lake, that generally had concentrations less than half of [AsB] and [DMA] in Ramsey and Johnnie Lakes (Figure SI-6; qualitative comparison). In Long Lake, saturation of biotransformation pathways within zooplankton by high arsenic exposure (Caumette et al. 2014) may be leading to increased accumulation of less modified arsenic species, such as inorganic arsenic. Chromatographic evidence of this was seen in invertebrates in this study (Figure SI-3) but these other arsenic species could not be quantified herein (see Section 3.5). Similar trends have also been noted in laboratory exposures of freshwater zooplankton, where zooplankton exposed to lower levels of arsenic accumulated a higher proportion of organic arsenic species, while those exposed to high levels of arsenic in sediment or water accumulated more inorganic arsenic and less organic arsenic (Caumette et al. 2014). Alternatively, these differences between

lakes could also be explained by differences in phytoplankton and/or zooplankton community composition with unexplained differences in accumulation patterns between taxa.

Within lakes, differences in [AsB] and [DMA] among taxa were more varied (Table SI-6). Generally, invertebrates had higher [AsB] and [DMA] than fish (Figure 3-2b and c; Figure SI-6b and c). Within the invertebrates, benthic macroinvertebrates generally had higher [AsB], and sometimes [DMA], than crayfish, while zooplankton were highly variable across lakes, though generally having higher [DMA] than other invertebrates (Figure SI-6; qualitative comparisons). It is noteworthy that crayfish samples were tail muscle and thus may be more comparable to concentrations in fish muscle than in the whole-body invertebrate and zooplankton samples.

4.2. Percentage of Arsenobetaine (%AsB) and Dimethylarsinic Acid (%DMA) in Fish

On average, the sum of [AsB] and [DMA] accounted for 57.7 ± 20.9% of total [As] in fish, with values ranging from 17.1 – 99.6% (Figure SI-B). On average [AsB] made up 34.2 ± 27.7% of arsenic in fish, but this varied considerably (0.8 – 99.0%; Figure 3-3a). Dimethylarsinic acid was less variable, making up 19.5 ± 14.5% of the total arsenic in fish (0.5 - 69.3%; Figure 3-3b). Similarly broad ranges in the percentage of total arsenic accounted for by AsB and DMA have been previously reported in freshwater fish (18 – 42 %AsB, 4-9 %DMA, Juncos et al. 2019; <5 - >95 %AsB, <5 - >85 %DMA, estimated from figure, Tanamal et al 2021; 2.4 – 99.2 %AsB, 0.5 – 106 %DMA, Lescord et al 2022).

Conversely from arsenic concentrations, %AsB and %DMA typically did not differ significantly between lakes within a given fish species—except in the case of cisco, which had higher %AsB and lower %DMA in Long Lake than in Johnnie Lake (Figure 3-3; Table SI-7). This

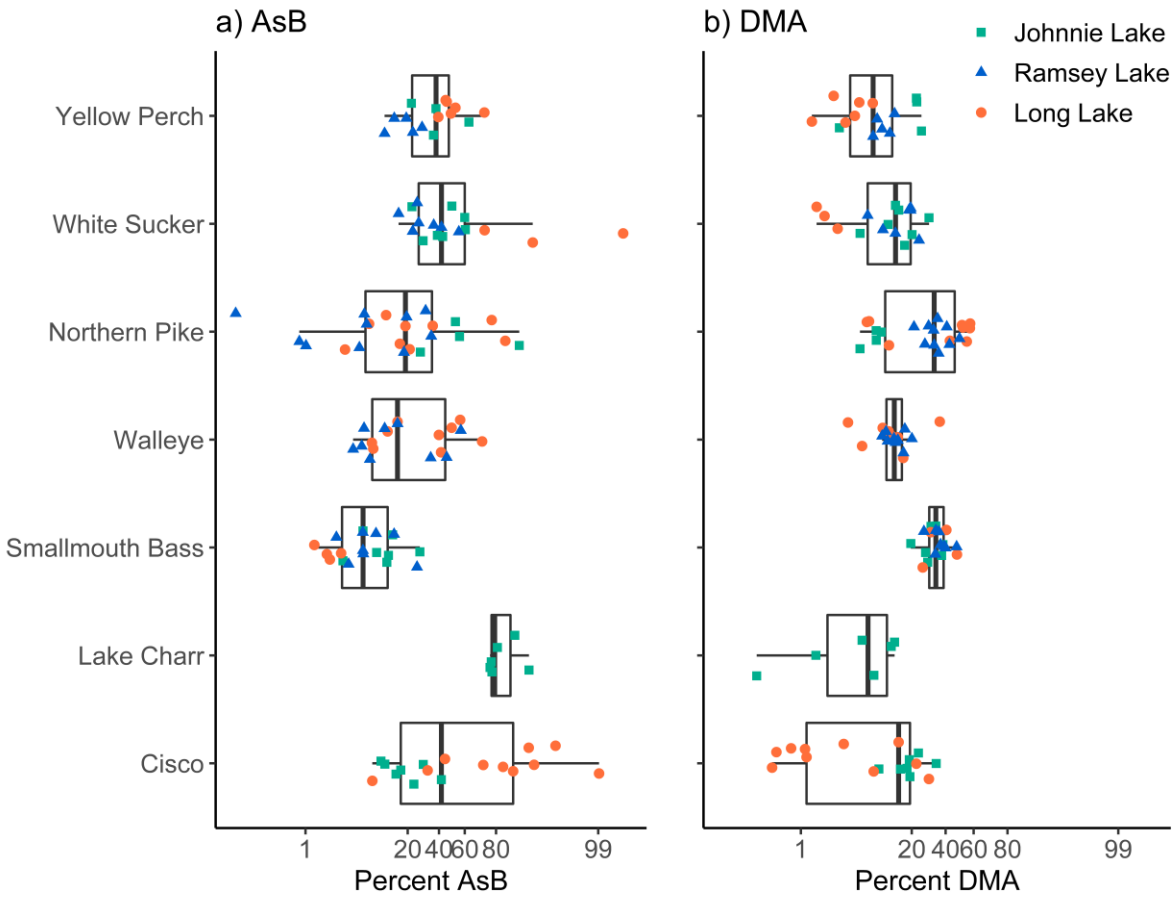


Figure 3-3. Boxplots of logit transformed percentages of total As detected as AsB (a) and DMA (b) in fish from three lakes near Sudbury, Ontario. Points are individual fish, with lake denoted by colour and shape. Boxes represent the 25th to 75th percentile of the data, the vertical line in each box represents the median, and the horizontal whiskers indicate the spread of the data within 1.5 times the interquartile distance from the 25th and 75th percentile.

was contrary to our predictions that %AsB would be higher in more contaminated lakes. Previous laboratory studies have reported chronic exposure to elevated iAs, such as in Long Lake, increased %AsB in freshwater fish muscle over time (Cui et al. 2021). Altogether, this suggests that within the limited arsenic contamination gradient present across our study lakes, there is not a considerable effect of contamination level (i.e., lake) on the relative proportions of these organic arsenic species within fish taxa, as opposed to the differences in concentrations discussed above

(Section 4.1). There were, however, significant differences in arsenic speciation among fish species (Table SI-8; Figure 3-3). Lake charr, although only collected from Johnnie Lake, had consistently higher %AsB (82.1 ± 6.3%) than most other taxa, together with relatively low %DMA (6.8 ± 5.2%), and overall high speciation recovery (90.3 ± 6.2%). This is consistent with prior literature on arsenic speciation in freshwater salmonids, where AsB makes up the majority of total [As] (58 – 90% AsB, Slejkovec et al. 2004; 0.270 – 1.490 mg/kg [AsB], 0.645 – 1.700 mg/kg total [As] dry wt., Ruttens et al. 2012; 86% AsB, Hackethal et al. 2021). Cisco from Long Lake also generally had higher %AsB (70.8 ± 31.6%) and lower %DMA (8.1 ± 10.4%), with high overall speciation recovery (80.5 ± 21.8%). Interestingly, this trend was not seen in cisco from the less contaminated Johnnie Lake (%AsB: 20.9 ± 11.5%; %DMA: 19.6 ± 7.6%; Recovery: 44.5 ± 12.6%).

Although AsB dominates in some fish taxa, other taxa show differing arsenic speciation patterns. For example, both bass and, less consistently, pike generally had higher %DMA (34.2 ± 7.4% and 31.2 ± 18.5%, respectively) than %AsB (8.5 ± 7.3% and 27.1 ± 27.3%, respectively). Relatively high DMA has been previously reported in several other studies on pike (46% of extracted arsenic, Zheng and Hintelmann, 2003; 23 ± 18%, de Rosemond et al. 2008; approx. 15 – 85%, estimated from a figure, Tanamal et al. 2021). To the best of our knowledge, no previous studies have reported on arsenic speciation in smallmouth bass. However, in largemouth bass, AsB and DMA both made up around 15% of extractable arsenic (Zheng and Hintelmann, 2003). Overall, these results suggest that although AsB dominates in muscle tissue of some fish, this pattern varies between species. This variability in the dominant organic species of arsenic among fish species may have a variety of underlying causes, including differences in: diet (Dutton and Fisher 2011; Zhang et al. 2016), habitat selection (pelagic vs. littoral), sensitivity to various

exposure pathways (Lu et al. 2023), gastrointestinal morphology (de Rosemond et al. 2008) and biotransformation capacity or pathways (Slejkovec et al. 2004; de Rosemond et al. 2008; Foust et al. 2016; Zhang et al. 2016). More work is needed to understand variation in arsenic speciation patterns across diverse taxonomic groups and the mechanisms driving this variation.

The percentage of AsB and DMA also varied among invertebrate taxa, although sample sizes were limited due to low biomass availability for total [As] analysis. Zooplankton from Ramsey Lake (n = 3) averaged 14.3 ± 2.2% AsB and 18.8 ± 0.7% DMA. While those from Johnnie Lake (n = 2) had similar %AsB (12.1 & 14.0%) but over double %DMA (39.3 & 38.0%). Gomphidae, on the other hand, contained relatively more AsB (29.5%) and less DMA (4.8%) in a single sample from Ramsey Lake. These are higher than values seen by Erikson et al. (2019) in mining-contaminated Panther Creek, Idaho, USA (<5% AsB and DMA). Overall speciation recovery in these invertebrates (34 – 60%) was lower than in fish, suggesting the presence of other species of arsenic, such as arsenosugars and inorganic arsenic. Chromatography further supported this theory, with evidence of other arsenic species in chromatograms of invertebrate samples (Figure SI-3). This is consistent with prior literature on arsenic speciation in freshwater invertebrates, where AsB and DMA make up a smaller proportion of total arsenic, with other species playing a larger role (Caumette et al. 2014; Erickson et al. 2019) although there is considerable unexplained variation among taxa, potentially due to differing enzymatic or physiological capabilities among taxa.

4.3. Drivers of Variation in %AsB and %DMA in Freshwater Fish

4.3.1. Fish Size

Relationships with size varied between fish functional groups. For predatory fish and cisco, larger fish generally had higher %AsB and lower %DMA ($p = <0.001 – 0.002$; Figure 3-4a, 4d), but no significant relationships were observed in suckers or perch (Figure 3-4b & 4c). Notably, cisco were larger in Long Lake and had higher %AsB but lower %DMA than cisco from Johnnie Lake; these factors influenced the observed relationship (Figure 3-4d). Overall, these results are similar to those previously reported for %AsB in northern boreal lakes, where %AsB showed significant positive relationships with fish weight in two species of predators (pike and walleye), but not in two groups of insectivores (suckers and whitefish; Lescord et al. 2022). The slope of relationships between K and %AsB and %DMA varied between lakes in large-bodied predators (*p*-interact = 0.022 & 0.027, respectively; Table SI-10), but this could not be assessed in other groups due to low sample sizes. Similar variability has also been seen in relationships between total [As] and fish size (Culioli et al. 2009; Chételat et al. 2019; Juncos et al. 2019).

One potential explanation for differences among taxa are the varying degree of ontogenetic niche shifts experienced by different taxa as they grow. It has been previously demonstrated that mercury speciation varies with body size and age in freshwater fish, with the smallest and youngest fish specifically deviating from trends widely observed in larger fish (Lescord et al. 2018). It is possible that similar trends might exist for arsenic. For example, although sample size was limited in our study, in Long Lake, the smallest walleye (n = 2; 126 & 195 mm; likely age = 0-1, Simoneau et al. 2005) had considerably lower %AsB (12 & 8%, respectively) and higher %DMA (16 & 36%, respectively) compared to larger walleye (n = 7; total length = 529 ± 136 mm; %AsB = 40.5 ± 22.5%; %DMA = 10.1 ± 4.0%). This also may be related to ontogenetic shifts from planktivory to

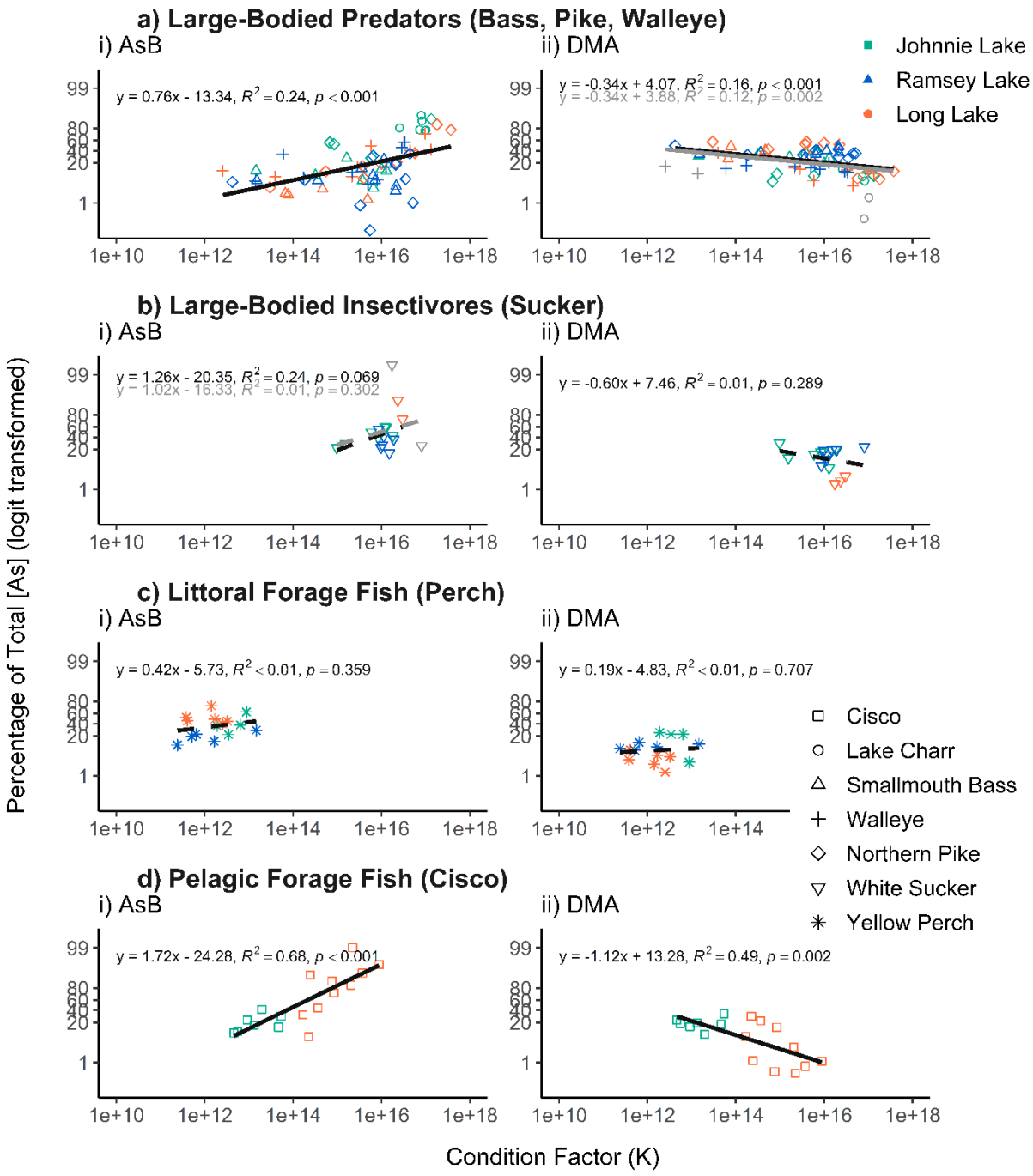


Figure 3-4. Relationships between logit-transformed %AsB/%DMA and log_{10}-transformed modified condition factor (K) in fish pooled from 3 lakes in a mining impacted region. Data are grouped by functional feeding groups (a-d). Points are individual fish, with species and catch location denoted by shape and colour, respectively. Solid lines indicate statistically significant relationships; dashed lines indicate statistically non-significant relationships. Model stats shown in grey in panel a-ii) include outliers removed to pass normality assumptions

piscivory in maturing walleye (Uphoff et al. 2019). Previous studies have also reported relationships between fish age and total [As] accumulation, with older lake whitefish having lower concentrations of arsenic (Cott et al. 2016). Additional work is needed, with particular emphasis on early life stages, to better characterize how arsenic speciation in freshwater fish varies with size and age across diverse taxa.

4.3.2. Total Selenium Concentrations

Selenium was detected in all fish tested (n = 115), at concentrations from 2.15-11.91 mg/kg dry wt. (Figure SI-8a). Concentrations of selenium in fish were generally more similar between Long Lake and Ramsey Lake, and slightly lower in Johnnie Lake (Long: 5.50 ± 1.66; Ramsey: 5.19 ± 0.90; Johnnie: 4.26 ± 1.59), except for suckers, which had elevated selenium concentrations in all lakes (qualitative comparisons; Figure SI-8a). Overall, selenium concentrations were similar to those seen in a review of various anthropogenically impacted areas across North America (Gilron et al. 2021). In most fish selenium was present at higher concentrations than arsenic, except fish with the highest total [As] (7 Cisco & 1 Pike from Long Lake). This is reflected in the As:Se molar ratio, which was <1 in most fish samples (n = 115; Figure SI-8b). It has been previously noted that arsenic (particularly inorganic arsenic) readily binds to Se in cells (Korbas et al., 2008). This binding could negatively impact the biological activity of cellular selenium, potentially impacting the toxicity of arsenic and other elements, like mercury (Ponton et al. 2022).

Muscle selenium concentrations were generally a strong predictor of arsenic speciation in fish. Both across all lakes (Figure 3-5) and within each lake (Figure SI-9), %AsB increased and

%DMA decreased with increasing total [Se]. The slope of the relationship between [Se] and %AsB did not vary between lakes (*p*-interaction = 0.112), but it did for %DMA (*p*-interaction = 0.008; Table SI-11). Selenium concentrations were also positively related to total [As] (Figure SI-10) and [AsB] (Figure SI-10), but not [DMA] (Figure SI-12). As previously mentioned, the use of ratio data runs an increased risk of spurious correlations. In this case the relationship with %DMA appears to potentially be spurious, as there is no relationship with the numerator ([DMA]) but there is with the denominator (total [As]). This does not appear to be a concern for AsB, where the trend is primarily driven by increasing [AsB] with a steep slope and increasing total [As] with a less steep slope. Similarly, it has previously been reported that exposure to selenium, as selenomethionine, increased accumulation of total [As] in a model freshwater fish (Jamwal et al. 2018), but we are unaware of similar studies on arsenic speciation.

While the mechanisms underlying relationships between selenium and arsenic speciation are unclear, they could be related to the presence of selenium at the reactive sites of many antioxidant proteins, including glutathione peroxidase (Arteel and Sies, 2001), an important protein that plays a dual role in cellular responses to arsenic (Figure SI-13; Byeon et al 2021). Additionally, although there may be potential for protective effects of selenium against arsenic toxicity, it has also been noted in humans that beneficial effects of selenium are dose dependent, and that excessive Se can negatively impact arsenic biotransformation and excretion (Sun et al. 2014). Further work is needed to fully understand how a wide range of co-occurring chemicals, including but not limited to selenium, influence arsenic speciation. Specifically, effort is needed to understand how complex chemical mixtures of varying concentrations interact with the cellular processes underpinning arsenic speciation.

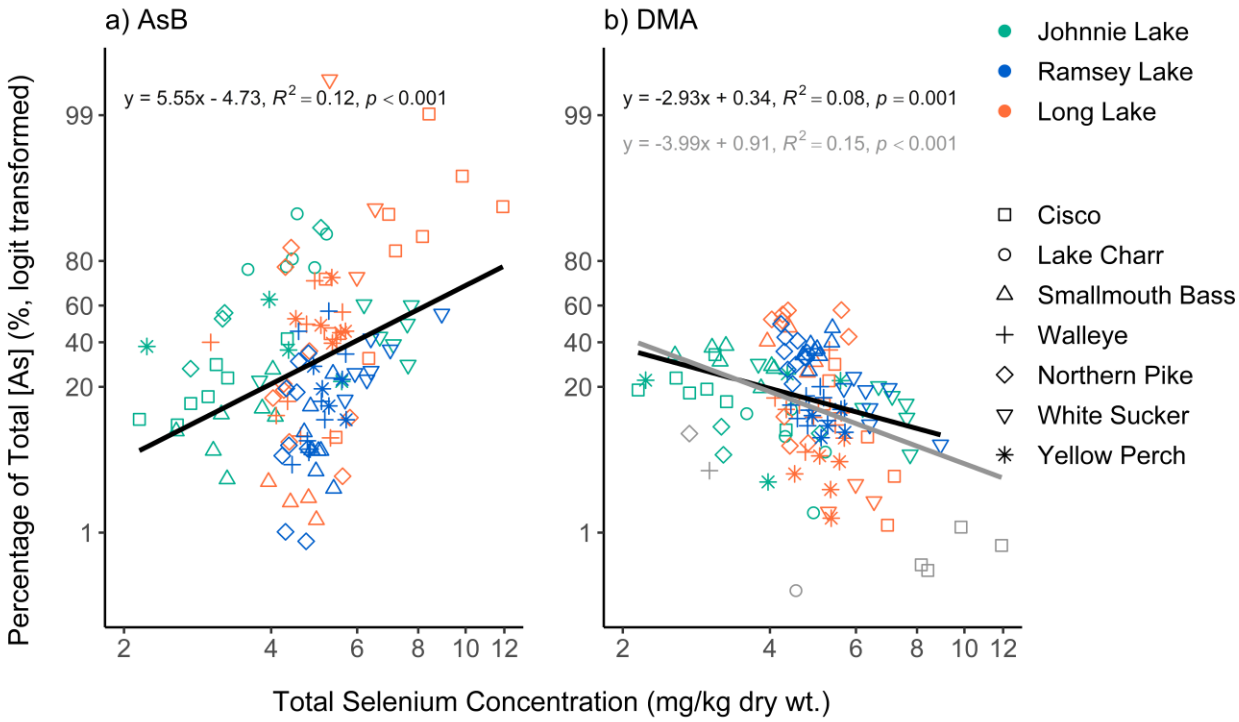


Figure 3-5. Relationships between logit-transformed %AsB/%DMA and $\log_{10}$-transformed total Selenium concentrations in fish pooled from 3 lakes in a mining impacted region. Points are individual fish, with species and catch location denoted by shape and colour, respectively. Solid lines indicate statistically significant relationships. Model stats shown in grey in panel b) include outliers removed to pass normality assumptions.

4.3.3. Trophic Ecology ($\delta^{15}N$) and Diet ($\delta^{13}C$)

Trophic ecology is another potential driver of arsenic speciation in freshwater fish (Rahman et al. 2012). Across all lakes and fish species, %AsB increased and %DMA decreased with increasing $\delta^{15}N$ (Figure 3-6). The slope of this relationship varied significantly between lakes for %AsB but not for %DMA (p-interaction = 0.041 & 0.113, respectively; Table SI-11). However, despite the non-significant interaction term, there were visual differences in regression slope and significance between lakes; in Ramsey Lake %AsB decreased and %DMA increased with $\delta^{15}N$ (Figure SI-14b), while in Long Lake and Johnnie Lake (Figure SI-14a,c) the opposite trend was

observed. Similar trends have also been observed in northern boreal lakes, where %AsB increased with increasing $\delta^{15}N$ across multiple fish species (Lescord et al. 2022). Relationships between $\delta^{15}N$ and concentrations of AsB, DMA, and total As in fish were also assessed, generally being non-significant, weak, negative relationships (p = 0.052 – 0.720; R^2 = <0.01 – 0.09; data not shown), except a positive relationship with [AsB] in Johnnie Lake (p = 0.004, R^2 = 0.15) and a negative relationship with [AsB] in Ramsey Lake (p = 0.004, R^2 = 0.13). Thus, unlike relationships with [Se], spurious correlations due to concentrations are less likely to be a concern herein.

Interestingly, cisco differed in their relative $\delta^{15}N$ values between lakes. Although cisco from Johnnie Lake generally had baseline corrected $\delta^{15}N$ values in line with other forage fish (4.29 ± 1.00‰), cisco from Long Lake generally had elevated $\delta^{15}N$ signatures (5.39 ± 1.17‰), which were more consistent with predatory fish across the dataset (5.10 ± 1.51‰). This could be related to differences in fish size between lakes; cisco from Johnnie Lake were notably smaller (20.5 - 62.4 g) than those from Long Lake (126 - 1027 g; Figure SI-4). Larger cisco also tended to have elevated $\delta^{15}N$ signatures when compared to smaller cisco both across the two lakes and within Long Lake, but no significant effect of size on $\delta^{15}N$ was observed in Johnnie Lake (Figure SI-15d). A similar relationship was also observed for large-bodied predators, but the significance varied for suckers and littoral forage fish (Figure SI-15a-c). Similar positive relationships between fish size and $\delta^{15}N$ have been previously reported, with larger fish generally having elevated $\delta^{15}N$ (Johnston et al. 2021). The previously discussed increases in %AsB and decreases in %DMA with increasing size for predators and cisco (Section 4.3.1.; Figure 3-4) may be driven by relationships with trophic position—which had more consistent relationships with arsenic speciation—rather than relationships with size itself which were generally less consistent.

Altogether, our results indicate that larger fish feeding at a higher trophic position generally had more AsB relative to total [As] in their muscle. Similar trends have previously been reported in marine systems, commonly attributed to shifts in diet composition from mainly invertebrates—with more complex arsenic speciation—towards mainly fish with much higher %AsB (Maher et al. 2011). Conversely, other studies of marine fish have found that although AsB still dominated at high trophic level, the retention of AsB from diet was relatively low, suggesting that accumulated AsB was primarily a product of internal biotransformation of other more bioavailable arsenic species (Zhang et al. 2016). While we cannot determine the mechanism behind the higher %AsB in fish with elevated $\delta^{15}N$ values observed herein, tropic ecology clearly had an impact on arsenic speciation in these fish.

The effects of fish diet on As speciation were not as clear as those of trophic level. Across all lakes, no relationship was seen with %AsB or %DMA in fish (Figure 3-6b). Likewise, $\delta^{13}C$ was not related with %AsB or %DMA within individual lakes, with the exception of a slight positive relationship between %DMA and $\delta^{13}C$ in Ramsey Lake (Figure SI-16). The slope of these relationships did not vary significantly between lakes for %AsB but did for %DMA (p-interact = 0.126 and 0.008, respectively; Table SI-12). Cisco from Long Lake were again unique in these models, with more negative $\delta^{13}C$ values than any other fish taxa, coupled with high %AsB as previously discussed (Figure SI-16a).

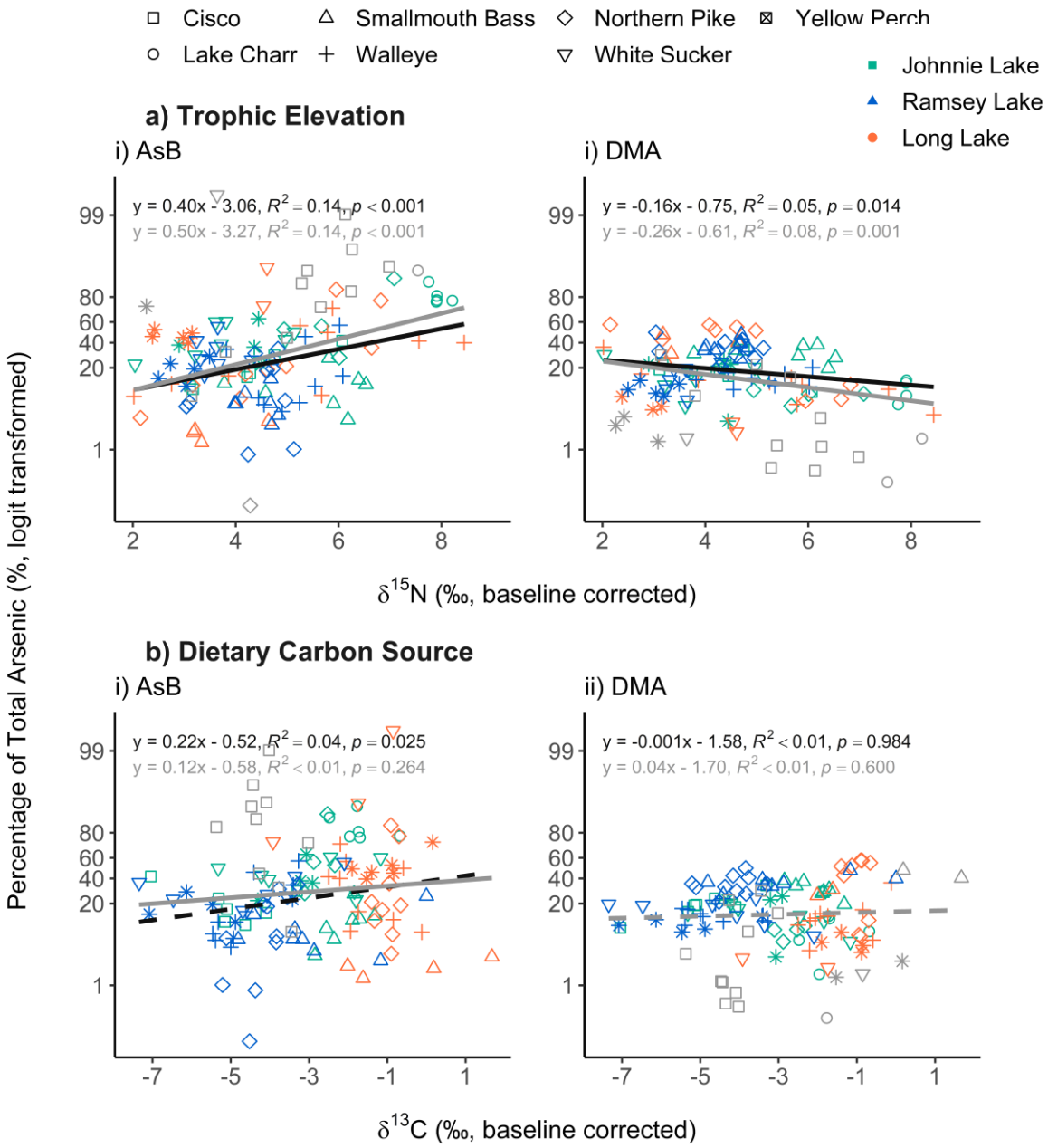


Figure 3-6. Relationships between %AsB (i) or %DMA (ii) and baseline corrected $\delta^{15}N$ (a) or $\delta^{13}C$ (b) values in freshwater fish pooled from 3 lakes in a mining impacted region. Points represent individual fish, with species and catch location denoted by shape and colour, respectively. Solid lines indicate statistically significant relationships; dashed lines indicate statistically non-significant relationships. Models shown in grey include cisco from Long Lake, which were removed due to their separation in $\delta^{13}C$ from other taxa indicating they are not being consumed in large quantities by other taxa, as well as any outliers that were removed to pass normality assumptions.

Overall, it appears that although variation in arsenic speciation among fish is related to trophic position (i.e., $\delta^{15}N$), it is not strongly related to dietary carbon source ($\delta^{13}C$). This is contrary to relationships previously reported, where $\delta^{13}C$ had a negative relationship with total [As] (Chételat et al. 2019). It is possible that $\delta^{13}C$, which primarily differentiates pelagic and littoral carbon sources in lakes, may not effectively account for the variation in arsenic speciation driven by differences in diet. Future studies could incorporate varied and complementary measures of fish diet—such as stomach contents, DNA metabarcoding, additional isotope tracers, and fatty acids—and consider sampling the entire food web more completely to better understand the role of diet in freshwater arsenic cycling

4.4. Biodilution of AsB and DMA Across Freshwater Food Webs

As predicted, across individual lake food webs [AsB] and [DMA] generally biodiluted, decreasing in concentration with increasing $\delta^{15}N$, though significance varied for [DMA] (Figure 3-7). The slope of these relationships also varied between lakes for [AsB] (*p*-interact = <0.001) but not for [DMA] (*p*-interact = 0.958; Table SI-13), implying potential interactions between relationships with [AsB] and lake-specific characteristics. Again, cisco from Long Lake deviated from other fish, typically having higher [AsB] (13.2 ± 11.5 mg/kg dry wt.) and $\delta^{15}N$ (5.39 ± 1.37‰; baseline corrected). Overall, these results are similar to previous reports of biodilution of total [As] (Chételat et al. 2019; Maeda et al. 1993) and inorganic arsenic (Maeda et al. 1993) in freshwater food webs. Contrarily, other studies have reported more variable relationships between $\delta^{15}N$ and arsenic concentrations, particularly when the lower trophic levels (benthic invertebrates and zooplankton) are not well represented in the sample set (Yang et al. 2020), as seen in the previously discussed insignificant relationships between arsenic concentrations and $\delta^{15}N$ in fish only (Section

4.3.3). Thus, it appears that linkages between the lowest trophic levels may play a key role in biodilution of arsenic in freshwater environments. Further work is needed to determine the mechanisms behind arsenic biodilution in freshwater food webs, which are likely tied to biotransformation in fish and invertebrates.

Relationships between $\delta^{13}C$ and [AsB] or [DMA] across food webs were more varied. Generally, most taxa with lower [AsB] also had more negative $\delta^{13}C$, except for zooplankton and cisco (Figure SI-17). No relationship was observed between [DMA] and $\delta^{13}C$ across full food webs (Figure SI-17). The slope of relationships with $\delta^{13}C$ did not vary significantly among lakes for [AsB] or [DMA] (*p*-interact = 0.168 and 0.192, respectively; Table SI-14). However, the observed trends with $\delta^{13}C$ and both [AsB] and [DMA] may be related to invertebrate sample availability. Generally, the organisms with the least negative $\delta^{13}C$ signatures (littoral) were benthic invertebrates, with fish having more negative signatures (more pelagic). No profundal invertebrates (e.g., clams, chironomids) were collected in this study, which may influence observed relationships. It is possible that observed trends with $\delta^{13}C$ might be related to generally decreasing trophic level with more pelagic carbon sources in this sample set. This is particularly evident in Long Lake where benthic invertebrates were most well represented (Figure SI-17a). Future work should seek to more fully characterize both pelagic and littoral invertebrate communities to be better able to identify linkages between carbon sources and arsenic speciation independent of trophic elevation.

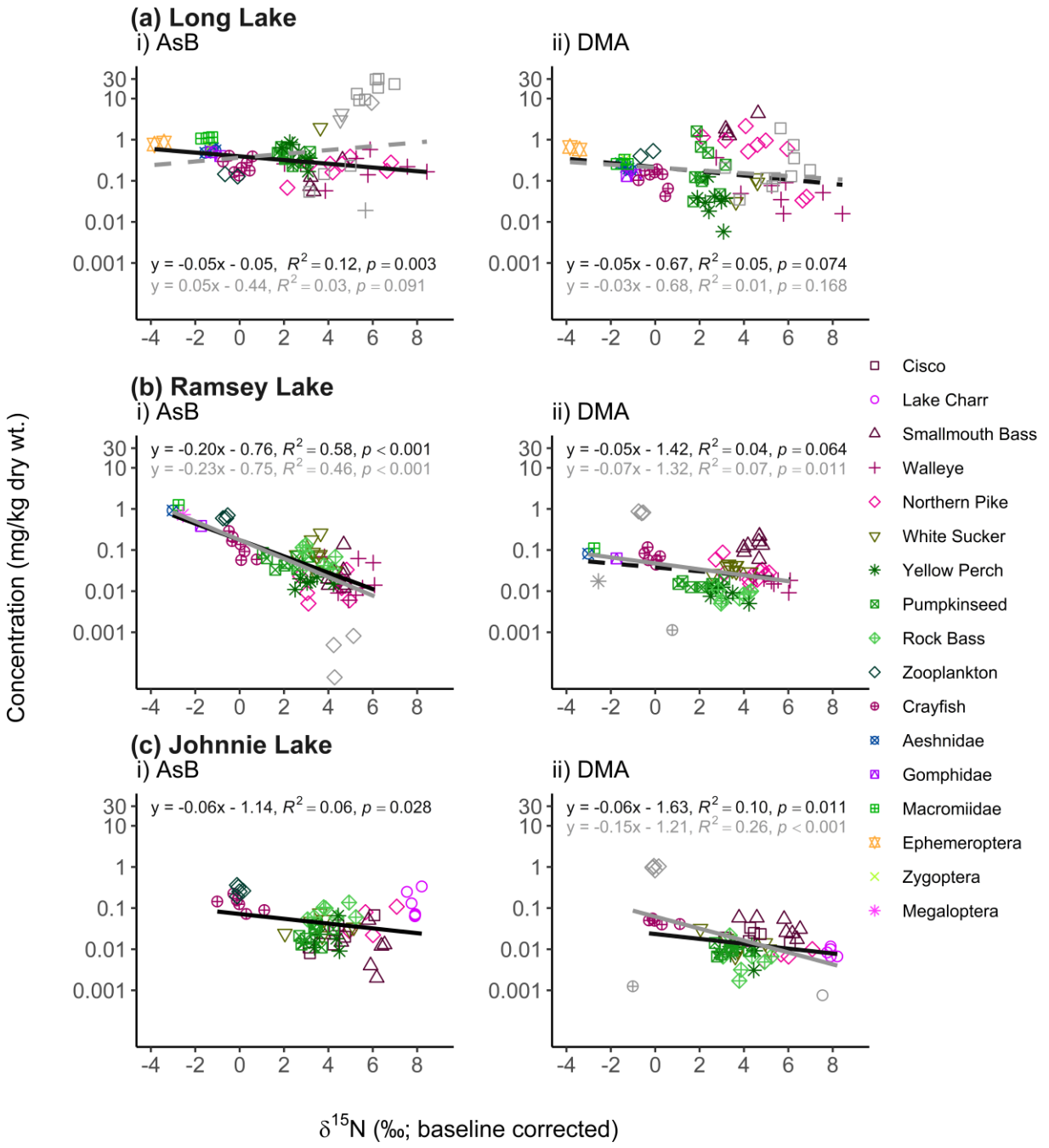


Figure 3-7. Relationships between AsB (i) and DMA (ii) concentrations and baseline corrected $\delta^{15}N$ values in freshwater fish and invertebrates in 3 lakes in a mining impacted region. Points are individual fish and invertebrates, with species denoted by shape and colour. Solid lines indicate statistically significant relationships; dashed lines indicate statistically non-significant relationships. Models shown in grey include cisco from Long Lake, which were removed due to their separation in $\delta^{13}C$ from other taxa indicating they are not being consumed in large quantities by other taxa, as well as any outliers identified by Cook's Distance which were removed to pass normality assumptions.

Overall, trophic ecology seems to be a primary driver of arsenic speciation patterns in freshwater food webs. However, other factors also appear to be at play, such as fish size and diet, as well as complex interactions with co-occurring chemicals. Additionally, there is also considerable unexplained variation in arsenic speciation among taxa, which may be a result of underlying physiological or metabolic differences. Future studies should further target diverse invertebrate and fish to better understand the mechanisms underlying arsenic speciation across naturally occurring freshwater food webs and quantify the differences among taxa and systems. Additional studies are also needed to identify the biological mechanisms underlying both the dietary accumulation and internal biotransformation of a variety of arsenic species, as well as the relative importance of each.

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Chapter 4: Thesis conclusions and directions for future work

Although our knowledge of arsenic speciation in freshwater environments is developing, there is still much we do not know. In my systematic review chapter, I found considerable variability in previous studies on arsenic speciation in freshwater fish. In my experimental data chapter, I found that trophic ecology appears to be a primary factor driving variability in two organic species of arsenic, because they biodiluted across food webs; fish size and interactions with other chemicals also accounted for additional variability therein. I also found unexplained differences among taxa, which warrants further study. One major knowledge gap that remains is our lack of understanding of how inorganic arsenic behaves in diverse freshwater taxa and systems

Through my research, I have also identified five major directions that future work should consider. First, work is needed to refine analytical techniques for separation, detection, and identification of arsenic and to develop reference materials and standards to support analyses for a wider variety of arsenic species. Secondly, biochemical work is also needed to understand the mechanisms underlying uptake, biotransformation, and accumulation of arsenic species at both the cellular and organismal levels. Thirdly, toxicological studies are needed to assess the potential toxicity of a variety of arsenic species including less toxic organic species to determine if they should also be incorporated into risk assessment, in addition to highly toxic inorganic arsenic. Fourthly, these laboratory-based developments can be applied in environmental studies to determine arsenic speciation profiles in various biota and determine the associated risks to both environmental and human health. In particular, human health risk assessments incorporating arsenic speciation data in addition to other contaminants are needed, especially in areas with known anthropogenic or geogenic contamination or where subsistence fishing is practiced. Ideally,

this would be community-based research, incorporating relevant contaminants, harvesting areas, fish species, and tissues identified in partnership with those whom the risk assessment is intended to benefit. Finally, work is needed to identify what drives the observed variability in arsenic speciation in freshwater environments, including but not limited to: size, age, taxa, trophic position, diet, and interactions with other chemicals. A strong understanding of the distribution of arsenic species and the mechanisms underlying these patterns is critical for accurate assessment of environmental and human health risks posed by arsenic.

Supplemental Information for Chapter 2

Table SI-1. Summary of concentrations of total arsenic and arsenic species in freshwater fish reported in the literature. ROM = range of reported means, TR = true range of values.

Citation	n	Total [As]	As(III)	As(V)	iAs	AsB	DMA	MMA	Other species	Notes
Arroyo-Abad et al. 2016		0.082-1.236							Arsenolipids	Limited quantification
Batista et al. 2012	20	0.247-0.353	<MDL-0.087	0.028-0.039		0.056-0.283	<MDL-0.027	<MDL-0.026		ROM
Choi et al. 2015	18	-	<MDL-0.023	<MDL-0.141		0.082-0.982	<MDL-0.032	<MDL		ROM
Chung et al. 2014	NR	0.943								ROM
Ciardullo et al. 2010	16	0.354-1.804								ROM
Cott et al. 2016	57	0.05-2.80	<0.01	<0.04			<0.01-0.09	<0.02		TRs
de Rosemond et al. 2008	34	0.57-1.15	<0.01-0.05	<0.01-0.02		0.05-0.13	0.02-0.18	<0.08		ROM
Hackethal et al. 2021	11	0.010-0.770			<MDL-0.024	0.008-0.724	<MDL-0.072	<MDL-0.095		TRs, Composite samples
Hong et al. 2014	160	0.64-5.4	<MDL-0.66	<MDL-0.53		0.18-4.7	<MDL-0.099	<MDL-0.021		ROM
Huang et al. 2003	68	0.184-3.291	<MDL-0.169	0.003-0.092		0.078-1.691	0.052-0.340	<MDL-0.047		ROM
Jankong et al. 2007	12	1.9-22.2	<0.02-0.91	0.12-1.72		trace-0.49	0.07-13.9	trace-0.38	TMAO, TETRA	ROM
Jia et al. 2018	120	0.063-2.844	0.004-0.144	0.010-0.289		0.029-1.864	<MDL-0.269	<MDL-0.081	AsC	ROM
Juncos et al. 2019	20	0.33-0.81			<0.020	0.06-0.28	0.02-0.05	<0.020		ROM

Table SI-1 Continued. Summary of concentrations of total arsenic and arsenic species in freshwater fish reported in the literature. ROM = range of reported means, TR = true range of values.

Citation	n	Total [As]	As(III)	As(V)	iAs	AsB	DMA	MMA	Other species	Notes
Karouna-Renier et al. 2011	23	0.09-3.27			<0.05					ROM, Converted from wet wt.
Komorowicz et al. 2019	8	0.066-5.932		<MDL-0.1337		0.060-5.23				TR
Larsen et al. 2005	10	0.55±0.11		<0.003-0.0077						Mean and TR
Lawrence et al. 1985	9	0.032-1.091				<MDL				TR
Lescord et al. 2022	300/297	<0.1-47.4	<0.01	<0.01		<0.01-42.70	<0.01-3.38	<0.01		TR, sample sizes are species/totAs
Lepage et al. in prep	165/115	0.037-31.309				<0.001-30.144	<0.006-4.37			TR, sample sizes are species/totAs
Miyashita et al. 2009	>17	0.150-2.100	<MDL	<0.00025		0.0078-0.290	<0.00025-0.044	<0.00025-0.023	TMAO, TMA, AsC, Glycerol & phosphate Sugars,	ROM
Norin et al. 1985	6	0.05-0.24			0.01-0.03					TR
Pizarro et al. 2003	5	168	30	16		65	23.1	<MDL		Means
Ruangwises et al. 2012	108	0.556-2.35			0.064-0.367					TRs
Ruttens et al. 2012	12	0.136-7.727	<0.005	<0.009-0.009		<0.005-6.773	<0.005-0.177	<0.005-0.014		TRs, converted from wet wt.
Saipan et al. 2012	105	0.582-2.55			0.053-0.764					TRs
Schaeffer et al. 2006	5	1.16-1.35	<0.02	<0.03-0.1		<0.02-0.03	<0.03	<0.03	phosphate arsenosugar dominant	ROM

Table SI-1 Continued. Summary of concentrations of total arsenic and arsenic species in freshwater fish reported in the literature. ROM = range of reported means, TR = true range of values.

Citation	n	Total [As]	As(III)	As(V)	iAs	AsB	DMA	MMA	Other species	Notes
Schoof et al. 1999	4	0.025-0.555			<MDL		<MDL	<MDL		TRs
Shah et al. 2010	100	6.11-11.8	1.38-2.05	0.17-0.46						ROM
Slejkovec 1996	1	0.667			0.045	0.059	0.069	0.014	TMA, TMAO	ROM, AsB/TMAO co-eluted
Slejkovec et al. 2004	43	0.08-1.235	<MDL-0.0046			<MDL-0.815	<MDL-0.0565		TMAO	ROM
Stiboller et al. 2015	1	-				0.62 umol/kg				Single sample
Tanamal et al. 2021	170	0.42-5.97			0.038-0.131					ROM
Wolle et al. 2019	15	0.018-0.377	<MDL	<MDL		<MDL-0.347	<MDL-0.005	<MDL	AsC, TMA, TMAO, TMAP	TR
Yang et al. 2017	>50	0.91-0.97								ROM
Yang et al. 2020	>477	0.60-21.53								ROM
Zhao et al. 2018	21	-	<MDL-0.021	<MDL-0.016		0.021-6.909	<MDL-0.062	<MDL		TR
Zheng and Hintelmann 2004	11	0.23-2.05								TR
Zwicker et al. 2011	NR	0.05-23.92	<0.007-2.74	<0.007-6.67						ROM

1831

Table SI-2. Summary of percentages of arsenic species in freshwater fish reported in the literature. ROM = range of reported means, TR = true range of values.

Citation	n	%As(III)	%As(V)	%iAs	%AsB	%DMA	%MMA	Notes
Batista et al. 2012	20	<MDL-35.2%	7.9%-15.8%	7.9%-51.0%	22.7%-80.2%	<MDL-10.9%	<MDL-10.5%	ROM, calculated from means
Choi et al. 2015	18	<MDL-5%	<MDL-25%	<MDL-29%	69%-100%	<MDL-11%	<MDL	ROM, calculated with sum of species
Chung et al. 2014	NR			0.5%-1.3%				TR
Ciardullo et al. 2010	16	0.02%-1.07%	<MDL-0.34%	0.12%-1.41%	58.35%-95.80%	0.07%-7.64%		ROM, calculated with sum of species
Cott et al. 2016	57	<MDL	<MDL	<MDL		<MDL-3.4%	<MDL	ROM, calculated from mean
de Rosemond et al. 2008	34	<0.01%-7.5%	<0.01%-1.6%	<MDL-7.5%	6.0%-16.5%	3.4%-23.3%	<0.01%	ROM
Hackethal et al. 2021	11			<MDL-60%	4%-104%	<MDL-13%	<MDL-57%	TR, calculated
Hong et al. 2014	160	<MDL-30.0%	<MDL-24.1%	<MDL-54.1%	4.9%-100.0%	<MDL-9.7%	<MDL-0.5%	ROM, calculated from means
Huang et al. 2003	68	<MDL-9.1%	0.1%-12.5%	1.0%-15.4%	13.9%-80.2%	3.5%-52.8%	<MDL-9.3%	ROM, calculated from means
Jankong et al. 2007	12	<MDL-8.1%	0.9%-38.4%	0.6%-40.5%	<MDL-3.1%	3.7%-62.6%	<MDL-3.4%	ROM, calculated from means
Jia et al. 2018	120	0.6%-31.8%	0.9%-40.3%	2.6%-51.3%	5.1%-91.3%	<MDL-27.6%	<MDL-11.3%	ROM, calculated from means
Juncos et al. 2019	20			<MDL	18%-42%	4%-9%	<MDL	ROM
Karouna-Renier et al. 2011	23			<2%-<55%				ROM, calculated <MDL, converted wet wt.
Komorowicz et al. 2019	8		<MDL-3.0%		45.9%-91.5%			TR
Larsen et al. 2005	10			<0.4%-1.0%				TR
Lawrence et al. 1985	9				<MDL			TR
Lescord et al. 2022	177	<MDL	<MDL	<MDL	<MDL-99%	<MDL-106%	<MDL	TR
Lepage et al. in prep	115				<MDL-99.5%	<MDL-57.6%		TR
Miyashita et al. 2009	>17	<MDL	<MDL-8.7%	<MDL-8.7%	3.1%-24.3%	<MDL-19.3%	<MDL-3.5%	ROM

Table SI-2 Continued. Summary of percentages of arsenic species in freshwater fish reported in the literature. ROM = range of reported means, TR = true range of values.

Citation	n	%As(III)	%As(V)	%iAs	%AsB	%DMA	%MMA	Notes
Norin et al. 1985	6			2.5%-30.0%				TR
Pizarro et al. 2003	5	17.9%	9.5%		38.7%	13.8%	<MDL	Calculated from means
Ruangwises et al. 2012	108			8.56%-31.6%				TR
Saipan et al. 2012	105			6.62%-37.2%				TR
Schaeffer et al. 2006	5	<1.5%-<1.7%	<2.5%-7.4%		<1.5%-2.6%			ROM, calculated from means/MDL
Schoof et al. 1999	4			<MDL		<MDL	<MDL	TR
Shah et al. 2010	100			17.3%-31.9%				ROM
Slejkovec 1996	1			6.7%	8.8%	10.3%	2.5%	ROM, calculated from means, AsB/TMAO coelute
Slejkovec et al. 2004	43	<MDL-5.8%			<MDL-133.6%	<MDL-58.2%		ROM, calculated from means
Tanamal et al. 2021	170			0.9%-19.6%	6%-98%	<MDL-87%	<MDL-1%	ROM, organic% estimated from figure 3
Walker et al. 2020	4				67%-97%			TR
Wolle et al. 2019	15	<MDL	<MDL	<MDL	16.2-87.0	0.5-5.4	<MDL	TR
Yang et al. 2017	>50	1.8%-7.7%	<MDL	1.81%-7.68%		74.8%-76.0%	12.4%-15.3%	ROM
Yang et al. 2020	>477	<MDL-6.3%	<MDL-15.2%	<MDL-20.63%	<MDL-26.4%	<MDL-39.7%	<MDL-15.1%	ROM
Zheng and Hintelmann 2004	11	9.3%-39%	1.3%-56.3%		0.6%-29.1%	1.3%-49.6%	<MDL-2.2%	TR, calculated from sum of species, not totAs
Zwicker et al. 2011	NR	<MDL-11.5%	<MDL-27.9%	<MDL-39.4%				ROM

1834

Supplemental Information for Chapter 3

SI-1. Arsenic Speciation Quality Assurance and Control

Quality assurance and control for arsenic speciation analysis included the analysis of instrument blanks, method blanks, digestion method duplicates, method spikes with duplicates, instrument spikes, spiked method blanks, ongoing performance replicates, calibration linearity, CRMs, and an intra-lab standard material. For arsenic speciation analysis, three CRMs were selected: fish protein, DORM-5 (NRCC); and tuna fish tissue, BCR-627 (IRMM), both of which are fish matrix reference materials; as well as lobster hepatopancreas, TORT-3 (NRCC), that more closely approximates a crayfish sample matrix. All three CRMs are certified for concentrations of AsB, and BCR-627 is also certified for concentration of DMA. Recoveries of AsB in DORM-5 (95.9 ± 6.7%; n = 5), BCR-627 (87.6 ± 3.8%; n = 22), and TORT-3 (98.1 ± 3.6%; n = 5) were within acceptance criteria. Recoveries of DMA in BCR-627 were consistently around 20% higher than expected (120.4 ± 5.9%; n = 22), suggesting overestimation of DMA concentrations. Accordingly, detected DMA concentrations were systematically reduced by a factor of 1.204 across all samples. In addition to CRMs, we also used an intra-lab standard material of burbot (*Lota lota*) muscle, herein referred to as BO2, that was also analyzed for total arsenic per the methods in Section 3.4. (total [As] = 2.276 ± 0.048 mg/kg dry wt.). While BO2 does not have certified concentrations of any As species, it was separately digested and analyzed repeatedly (n = 17) to test the consistency of analytical results over time in a freshwater fish matrix. In BO2, both AsB (1.957 ± 0.159 mg/kg dry wt.) and DMA (0.258 ± 0.018 mg/kg dry wt.) were detected consistently across repeated analyses with relative standard deviation (RSD) of 8.1% and 6.9%, respectively. A detailed breakdown of QAQC data for arsenic speciation is give in Table SI-4.

1856 Supplemental Tables and Figures

1857 **Table SI-1.** Water chemistry data from 3 lakes near Sudbury, Ontario. Three sets of values are reported for Long Lake: the relatively uncontaminated northern
1858 arm of the lake (Baseline), the creek outlet where arsenic-containing tailings entered the lake (Luke Creek) and the main outlet of the lake (Round Lake
1859 Outflow). N/A = not analyzed

Water Chem Measure	Johnnie Lake[1]	Ramsey Lake[2]	Long Lake (Baseline[§])[2]	Long Lake (Luke Creek)[3]	Long Lake (Round Lake Outflow)[3]
Sampling Year	2019	2017	2017	2012-2018	2013-2018
Alkalinity; Gran (mg/L CaCO3)	1.91	37.4	16.3	N/A	N/A
Alkalinity; TFE (mg/L CaCO3)	3.19	38.5	17.8	N/A	N/A
M-Alkalinity (pH 4.5; mg/L CaCO3)	N/A	N/A	N/A	8.16	16.4
Calcium (mg/L)	1.16	15.9	7.54	3.375	6.03
Carbon; Dissolved Organic (mg/L)	4.9	3.6	4.1	N/A	N/A
Chloride (mg/L)	0.21	85.2	27.4	3.5	20.8
Copper (mg/L)	<MDL	0.0099	0.0101	0.0212	0.0098
Nickel (mg/L)	0.0055	0.0337	0.035	0.0427	0.0289
Nitrogen: Ammonia + Ammonium (mg/L)	0.028	0.02	0.018	0.061	N/A
Nitrogen: Nitrate + Nitrite (mg/L)	0.018	0.006	0.004	0.088	N/A
Nitrogen; Total (mg/L)	0.24	0.27	0.24	N/A	N/A
pH	6.31	7.6	7.17	6.01	7.15
Phosphorus; Total (mg/L)	0.0048	0.0058	0.0058	N/A	N/A
Sulphate (mg/L)	3.65	14.7	9.7	14.7	8.7
Arsenic (mg/L)	N/A	0.0013	0.0009	0.3194[†]	0.0251[†]
Selenium (mg/L)	N/A	0.0005	0.0003	<0.0010	<0.0010

[1]Data from the Ontario Ministry of Environment, Conservation, and Parks (MECP) inland waters lakes and streams water chemistry dataset used under the Open Government License – Ontario. [2]Data from water samples previously collected and analyzed by MECP (MECP, 2017). [3]Data from Ontario Ministry of Northern Development and Mines (MNDM) Long Lake Gold Mine Rehabilitation Project Category C Environmental Assessment, data averaged across multiple years (MNDM, 2019). [§]Due to natural site hydrology, elevated arsenic levels in water are not observed further north in Long Lake, with arsenic concentrations reaching background levels approximately 6 km from the outlet of Luke Creek (MNDM, 2019). [†]As(V) has been reported as the dominant form of arsenic in surface water at Long Lake (MNDM, 2019).

1860

Table SI-2. Quality assurance and control data for total arsenic and selenium analysis. Note: Samples were analyzed as part of a larger dataset (n = 330), and the QAQC data presented spans that broader whole dataset.

	As	Se
Ongoing Performance Replicate Recovery[1] (%; n=55)	95.2 - 104.8 (100.0 ± 2.4)	98.2 - 108.3 (102.3 ± 2.6)
Fortified Method Blank Recovery[1] (%; n=16)	95.1 - 101.6 (97.1 ± 1.8)	95.5 - 102.5 (98.3 ± 2.0)
Certified Reference Material Recovery DORM-5[1] (%; n=34)	92.8 - 109.8 (98.8 ± 3.1)	96.6 - 124.4 (110.5 ± 4.8)
Method Spike Recovery[1] (%; n=68)	93.4 - 104.9 (99.9 ± 2.5)	97.5 - 109.3 (103.7 ± 2.5)
Calibration Point Recovery[1] (0.1-2000 ppb; %)	84.8 - 123 (99.5 ± 5.4)	98.0 - 105.2 (100.3 ± 1.5)
Duplicate Relative Percent Difference[1] (%; n=33-36)[2]	0.0 - 20.1 (4.4 ± 5.1; n=33)	0.0 - 10.8 (2.8 ± 3.0; n=36)
Method Spike Duplicate Relative Percent Difference (%; n=34)	0.0 - 4.7 (1.2 ± 1.4)	0.0 - 6.0 (1.4 ± 1.3)
Number of Method Blanks > 2.2 * MDL (n=35)	0	0
Tissue MDL (mg/kg dry wt.)[3]	0.02	0.23

[1]Values presented as min - max (average ± SD); [2]Three duplicate samples from the larger dataset had [As] <MDL, these samples were not included in the subset of observations discussed here. [3]Based on average sample weight of 0.1082 g.

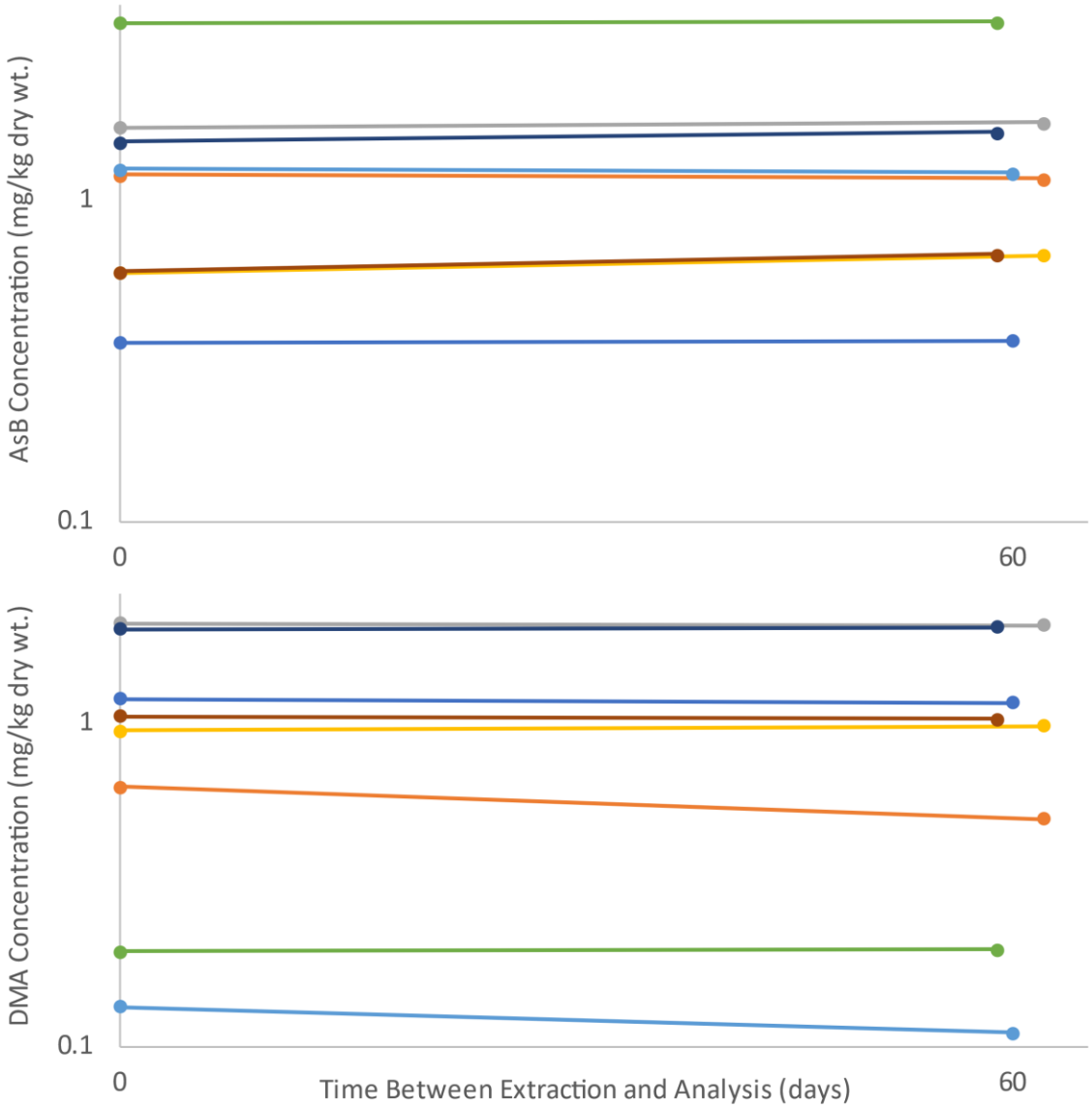


Figure SI-1. Minimal changes in concentrations of AsB and DMA with increasing lag time between extraction of samples and analysis of extracts from 0 - 62 days. Colours represent individual fish or invertebrate sample extracts.

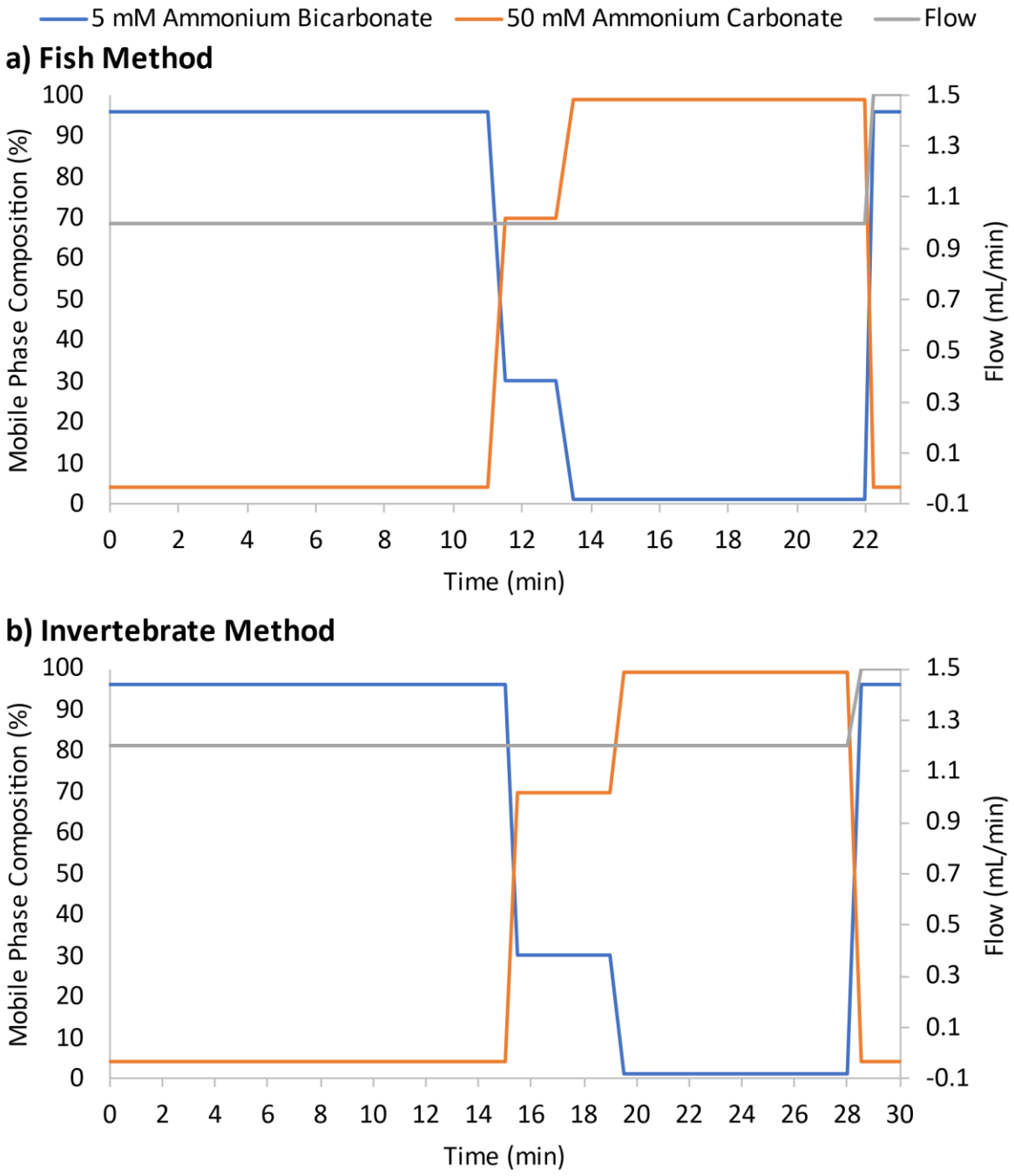


Figure SI-2. Eluent gradient schedules for IC-ICP-MS analysis of fish (a) and invertebrate (b) samples for arsenic speciation analysis. Changes in mobile phase composition and flow between gradient steps occurred linearly over 30 seconds.

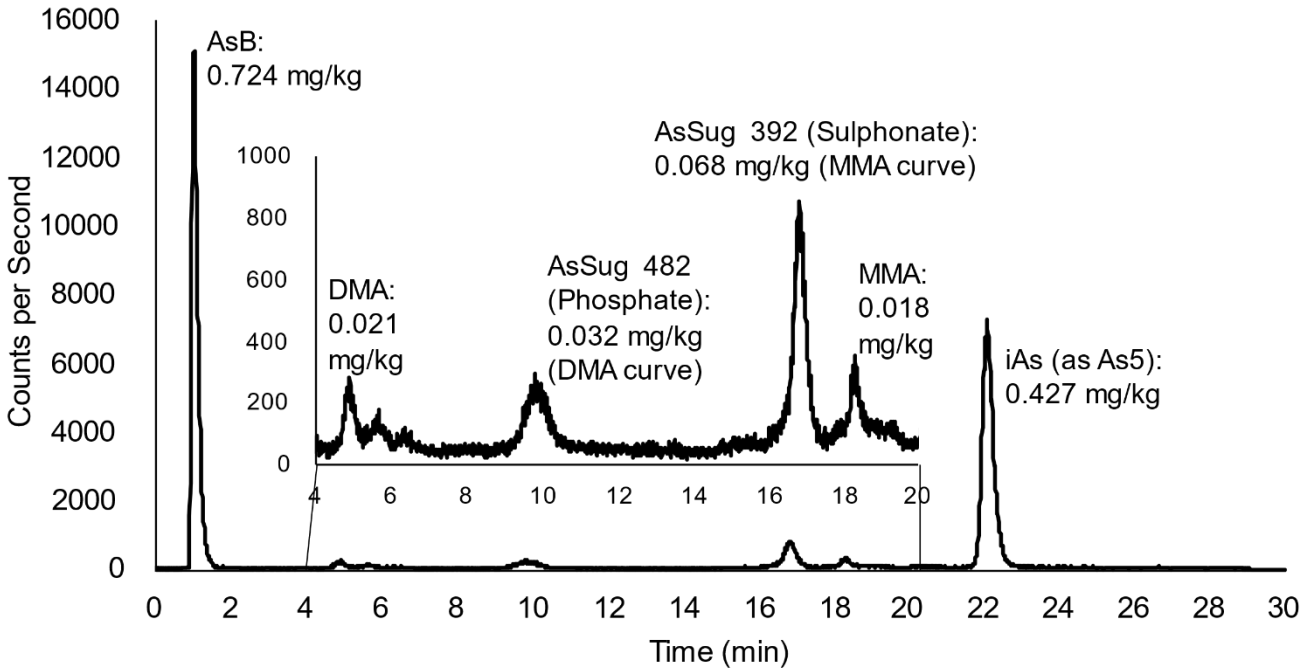


Figure SI-3. Representative IC-ICP-MS chromatograph of an invertebrate sample (Ramsey Lake Megaloptera) showing the presence of arsenobetaine (AsB), dimethylarsinic acid (DMA), two arsenosugars (AsSug; identified by relative retention time based on Wolle and Conklin 2018), monomethylarsonic acid (MMA), and inorganic arsenic (iAs). Estimated concentrations of MMA, iAs, and AsSug are provided for information, but were not able to be reliably quantified; interpret with caution.

Table SI-3. IC-ICP-MS instrument operation parameters for arsenic speciation in fish and invertebrate samples.

Injection Volume	50 µL
Analytical Column	Hamilton PRP-X100 (4.0 mm x 125 mm x 10 µm) anion exchange column
Guard Column	Hamilton PRP-X100 guard cartridge in PEEK holder, connected to analytical column with 0.01 mm x 1/16" PEEK tubing
Column Temperature	27°C
Autosampler Temperature	Ambient
Mobile Phase A	5 mM NH_4HCO_3, 5% methanol (v/v)
Mobile Phase B	50 mM $(NH_4)_2CO_3$. 5% methanol (v/v)
Fish Mobile Phase Gradient	0-11 min (96% A, 1 mL/min), 11.5-13 min (30% A, 1 mL/min), 13.5-22 min (1% A, 1 mL/min), 22.5-23 min (96%A, 1.5 mL/min)
Invertebrate Mobile Phase Gradient	0-15 min (96% A, 1.2 mL/min), 15.5-19 min (30% A, 1.2 mL/min), 19.5-28 min (1% A, 1.2 mL/min, 28.5-30 min (96%A, 1.5 mL/min)

Table SI-4. Quality assurance and control data for arsenic speciation analysis by IC-ICP-MS. QAQC Data for iAs is shown even though results were not reported because of variable recoveries. OPR = Ongoing performance replicate; MS = Method Spike; IS = Instrument Spike; RPD = Relative percent difference; RSD = Relative Standard deviation

	AsB	DMA	iAs
BCR-627 CRM recovery (n = 22)	87.6 ± 3.8% (78.8 – 95.4%)	120.4 ± 5.9% (110.7 – 130.7%)	N/A
DORM-5 CRM recovery (n = 5)	95.9 ± 8.2% (88.7 104.8%)	N/A	N/A
TORT-3 CRM recovery (n = 5)	98.1 ± 3.6% (94.0 102.3%)	N/A	N/A
5 ppb OPR recovery (n = 62)	97.8 ± 4.9% (85.1 – 111.1%)	96.8 ± 4.5% (84.5 – 104.7%)	95.2 ± 7.1% (77.4 – 114.3%)
1 ppb independent iAs OPR recovery (n = 42)			95.5 ± 26.2% (56.4 – 155.5%)
0.2 – 0.5 ppb OPR recovery (n = 21)	97.1 ± 4.6% (87.0 – 104.5%)	99.3 ± 6.2% (89.0 – 113.5%)	83.8 ± 16.5% (62.5 – 119.6%)
Spiked method blank recovery (n = 12)	93.0 ± 2.9% (85.7 – 96.7%)	93.0 ± 3.8% (84.8 – 98.5%)	(94.8 ± 7.2% (79.5 – 106.3%)
0.5 ppb fish IS recovery (n = 14)	95.4 ± 7.7% (86.8 – 106.2%)	98.0 ± 17.2% (83.8 – 136.2%	80.6 ± 18.6% (46.3 – 124.8%)
0.5 – 1 ppb Invertebrate IS recovery (n = 9)	108.4 ± 9.6% (93.8 – 126.8%)	102.1 ± 9.1% (82.9 – 115.8%)	99.5 ± 14.0% (74.9 – 127.7%)
2 ppm independent iAs IS recovery (n = 6)			77.7 ± 4.1% (70.1 – 82%)
5 ppb Fish MS recovery (n = 35)	94.4 ± 4.5% (87.4 – 113.3%)	90.0 ± 4.1% (82.8 – 97.0%)	81.7 ± 6.6% (59.0 – 91.6%)
5 ppb Invertebrate MS recovery (n = 7)	92.2 ± 5.5% (85.5 – 99.3%)	93.3 ± 5.8% (84.7 – 98.4%)	25.1 ± 13.5% (0.0 – 43.9%)
Method spike duplicate RPD (n = 7)	1.80 ± 1.3% (0.50 – 4.60%)	2.27 ± 1.4% (0.80 – 4.20%)	1.0 ± 0.7% (0.2 – 2.1%)
Digestion duplicate RPD (n = 22)[3]	5.3 ± 4.7% (0.2 – 17.0%)	8.9 ± 6.9% (1.6 – 33.6%)[1]	16.7 ± 8.4% (5.2-62.6%)
BO2 intra-lab standard duplicate relative standard deviation (n = 7)	8.1% (at 1.957 ± 0.159 mg/kg dry wt.)	6.9% (at 0.258 ± 0.018 mg/kg dry wt.)	
Calibration curve R^2 (n = 9)	0.9997 – 1.0000	0.9992 – 1.0000	0.9993-1.0000

[1]Elevated relative percent differences were seen in two samples where DMA concentrations were between MDL and LOQ. [3]Only 10 samples had [iAs] > MDL. N/A = Not applicable because no certified concentrations available

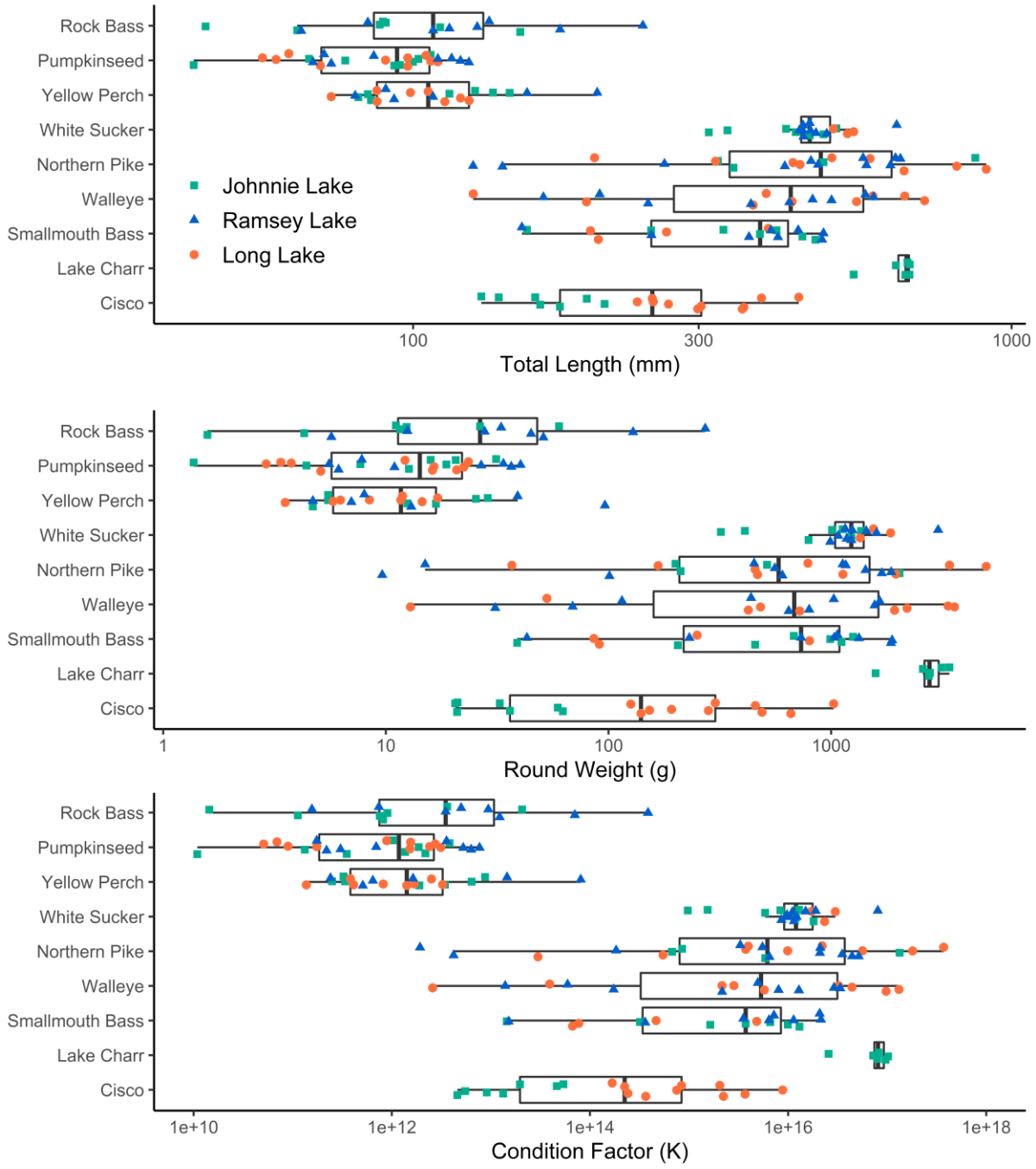


Figure SI-4. Total length (a), round weight (b) and condition factor (c) in fish from 3 lakes near Sudbury, Ontario. Data are grouped by fish species, with points representing individual fish and lake denoted by colour and shape. Boxes represent the 25th to 75th percentile of the data, the vertical line in each box represents the median, and the horizontal whiskers indicate the spread of the data within 1.5 times the interquartile distance from the 25th and 75th percentile.

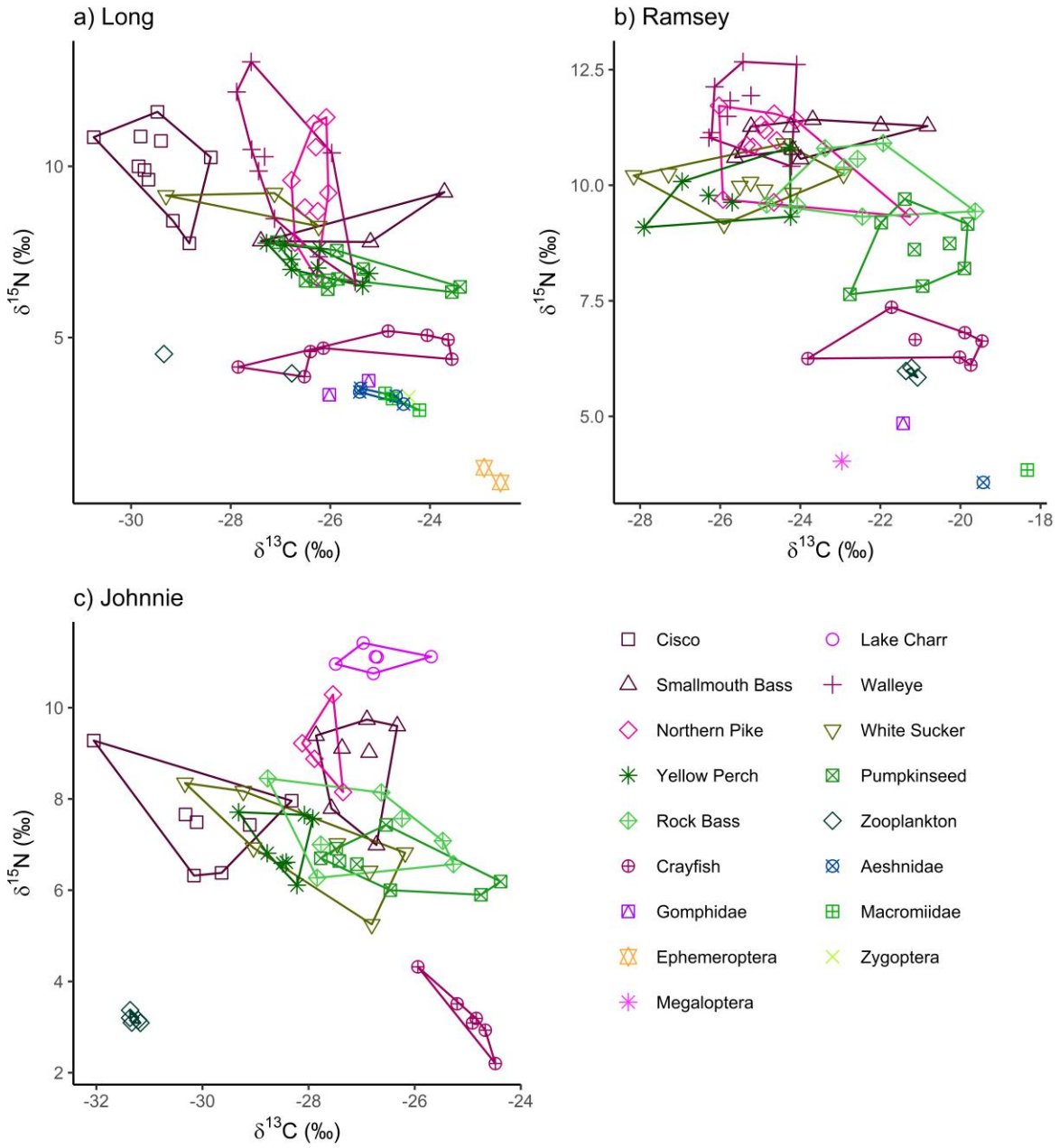


Figure SI-5. Isoscape plots ($\delta^{13}C$ vs $\delta^{15}N$) for fish and invertebrates from 3 lakes near Sudbury, Ontario (a-c). Points are individual fish and invertebrates with taxa represented by shape and colour.

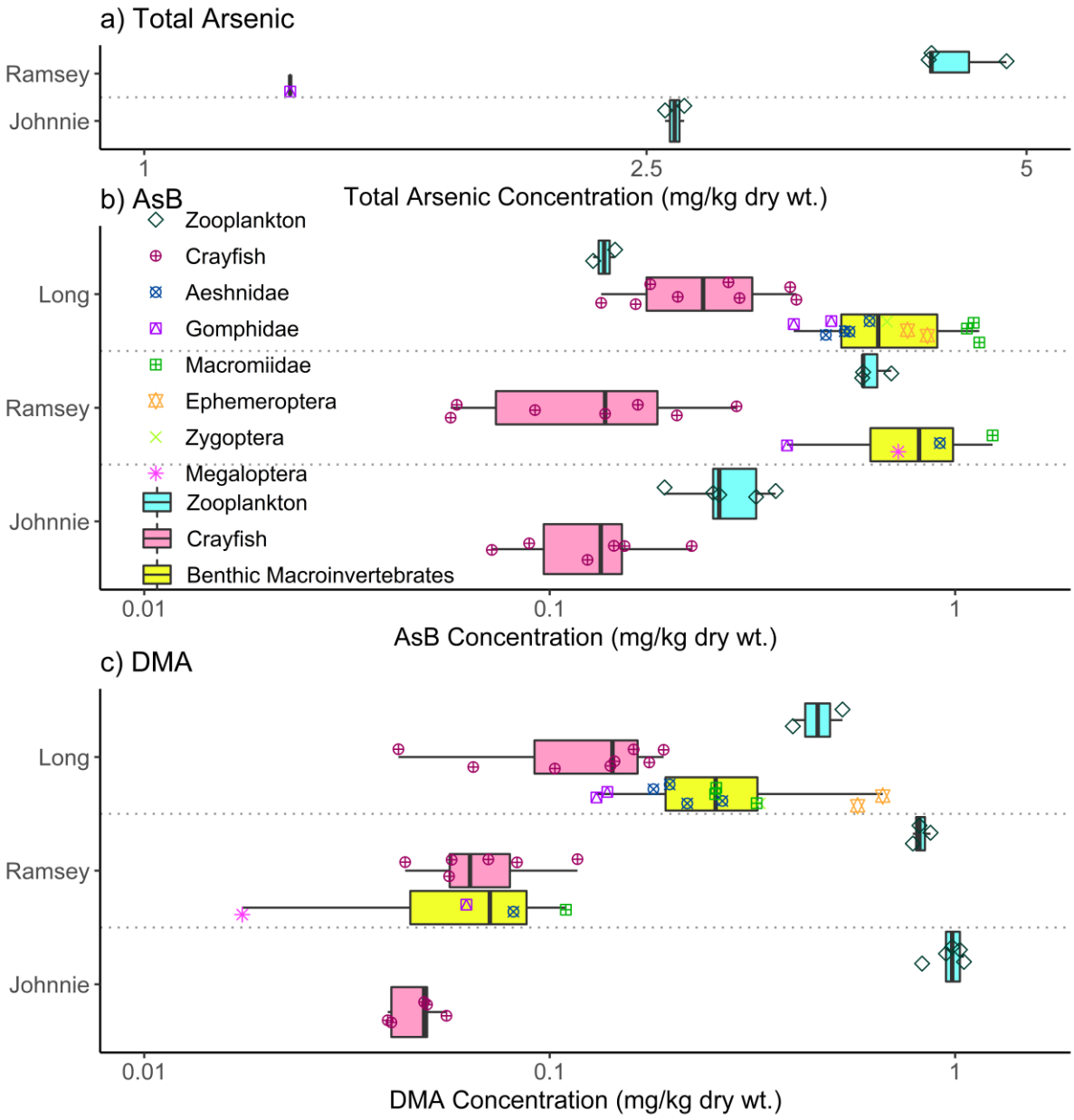


Figure SI-6. Boxplots of $\log_{10}$ transformed AsB (a) and DMA (b) concentrations in invertebrates from three lakes across a mining impact gradient near Sudbury, Ontario. Data are grouped by functional groups, with points representing individual fish, with taxon denoted by colour and shape. Boxes represent the 25th to 75th percentile of the data, the vertical line in each box represents the median, and the horizontal whiskers indicate the spread of the data within 1.5 times the interquartile distance from the 25th and 75th percentile. Note: 2 crayfish with concentrations <MDL are not plotted in panel (b). No invertebrates from Long Lake were analyzed for total As]

Table SI-5. Parametric (ANOVA and Tukey HSD) or nonparametric (Kruskal-Wallis and Dunn's tests) comparison tests of total arsenic (Total [As]), arsenobetaine ([AsB]), and dimethylarsinic acid [DMA] among fish and invertebrate taxa within 3 lakes near Sudbury, Ontario. DFn = F ratio numerator degrees of freedom for ANOVA, or Kruskal-Wallis degrees of freedom; DFd = F ratio denominator degrees of freedom. Significant differences are bolded.

Lake	Analyte	Statistic	Taxon 1	Taxon 2	DFn	DFd	F	p	Diff.
Long	**Total [As]**	**ANOVA**			**3**	**30**	**7.011**	**0.001**	
		Tukey HSD	**Cisco**	**Walleye**				**0.001**	**-1.006**
			Cisco	Pike				0.069	-0.617
			Cisco	**Perch**				**0.009**	**-0.932**
			Walleye	Pike				0.406	0.389
			Walleye	Perch				0.993	0.074
			Pike	Perch				0.667	-0.315
Long	**[AsB]**	**Kruskal-Wallis**			**5**			**0.011**	
		Dunn	**Cisco**	**Walleye**				**0.04**	**-3.001**
			Cisco	Pike				0.215	-2.424
			Cisco	Perch				1	-0.643
			Cisco	Pumpkinseed				1	-0.696
			Cisco	Crayfish				0.217	-2.394
			Walleye	Pike				1	0.562
			Walleye	Perch				0.299	2.209
			Walleye	Pumpkinseed				0.242	2.323
			Walleye	Crayfish				1	0.501
			Pike	Perch				0.808	1.664
			Pike	Pumpkinseed				0.808	1.746
			Pike	Crayfish				1	-0.045
			Perch	Pumpkinseed				1	-0.013
			Perch	Crayfish				0.808	-1.661
			Pumpkinseed	Crayfish				0.808	-1.737
Long	**[DMA]**	**ANOVA**			**5**	**48**	**5.264**	**0.001**	
		Tukey HSD	Cisco	Walleye				0.473	-0.422
			Cisco	Pike				0.648	0.357
			Cisco	**Perch**				**0.019**	**-0.808**
			Cisco	Pumpkinseed				1	-0.04
			Cisco	Crayfish				0.93	-0.23
			Walleye	**Pike**				**0.024**	**0.779**
			Walleye	Perch				0.627	-0.386
			Walleye	Pumpkinseed				0.582	0.381
			Walleye	Crayfish				0.971	0.191
			Pike	**Perch**				**<0.001**	**-1.165**
			Pike	Pumpkinseed				0.538	-0.397
			Pike	Crayfish				0.185	-0.588
			Perch	**Pumpkinseed**				**0.029**	**0.767**
			Perch	Crayfish				0.227	0.577
			Pumpkinseed	Crayfish				0.968	-0.19
Ramsey	**Total [As]**	**ANOVA**			**4**	**34**	**27.592**	**<0.001**	
		Tukey HSD	**Bass**	**Walleye**				**<0.001**	**-0.429**
			Bass	**Pike**				**<0.001**	**-0.649**
			Bass	Sucker				0.327	-0.149
			Bass	**Perch**				**<0.001**	**-0.588**
			Walleye	**Pike**				**0.024**	**-0.22**
			Walleye	**Sucker**				**0.006**	**0.28**
			Walleye	Perch				0.336	-0.159
			Pike	**Sucker**				**<0.001**	**0.5**
			Pike	Perch				0.946	0.061
			Sucker	**Perch**				**<0.001**	**-0.439**

Table SI-5 continued. Parametric (ANOVA and Tukey HSD) or nonparametric (Kruskal-Wallis and Dunn's tests) comparison tests of total arsenic arsenobetaine, and dimethylarsinic acid concentrations among Fish and invertebrate taxa within 3 lakes near Sudbury, Ontario. DFn = F ratio numerator degrees of freedom for ANOVA, or Kruskal-Wallis degrees of freedom; DFd = F ratio denominator degrees of freedom.

Lake	Analyte	Statistic	Taxon 1	Taxon 2	DFn	DFd	F	p	Diff.
Ramsey	**[AsB]**	**Kruskal-Wallis**			**7**			**<0.001**	
		Dunn	Bass	Walleye				1	-0.539
			Bass	Pike				1	-1.7
			Bass	Sucker				0.368	2.297
			Bass	Perch				1	-0.724
			Bass	Pumpkinseed				1	1.655
			Bass	Rock Bass				0.862	1.87
			Bass	Crayfish				0.066	2.967
			Walleye	Pike				1	-1.175
			Walleye	Sucker				0.073	2.923
			Walleye	Perch				1	-0.244
			Walleye	Pumpkinseed				0.386	2.242
			Walleye	Rock Bass				0.262	2.463
			Walleye	**Crayfish**				**0.009**	**3.567**
			Pike	**Sucker**				**0.001**	**4.241**
			Pike	Perch				1	0.786
			Pike	**Pumpkinseed**				**0.012**	**3.481**
			Pike	**Rock Bass**				**0.005**	**3.712**
			Pike	**Crayfish**				**<0.001**	**4.81**
			Sucker	Perch				0.085	-2.859
			Sucker	Pumpkinseed				1	-0.594
			Sucker	Rock Bass				1	-0.373
			Sucker	Crayfish				1	0.832
			Perch	Pumpkinseed				0.386	2.256
			Perch	Rock Bass				0.262	2.455
			Perch	**Crayfish**				**0.012**	**3.463**
			Pumpkinseed	Rock Bass				1	0.215
			Pumpkinseed	Crayfish				1	1.368
			Rock Bass	Crayfish				1	1.161
Ramsey	[DMA]	Kruskal-Wallis			7			**<0.001**	
		Dunn	**Bass**	**Walleye**				**0.007**	**-3.626**
			Bass	Pike				0.179	-2.536
			Bass	Sucker				0.75	-1.79
			Bass	**Perch**				**<0.001**	**-5.042**
			Bass	**Pumpkinseed**				**0.001**	**-4.254**
			Bass	**Rock Bass**				**<0.001**	**-5.459**
			Bass	Crayfish				1	-1.471
			Walleye	Pike				1	1.298
			Walleye	Sucker				0.703	1.892
			Walleye	Perch				0.75	-1.824
			Walleye	Pumpkinseed				1	-0.751
			Walleye	Rock Bass				0.65	-1.992
			Walleye	Crayfish				0.65	1.985
			Pike	Sucker				1	0.686
			Pike	**Perch**				**0.044**	**-3.043**
			Pike	Pumpkinseed				0.619	-2.041
			Pike	**Rock Bass**				**0.017**	**-3.338**
			Pike	Crayfish				1	0.863
			Sucker	**Perch**				**0.009**	**-3.516**
			Sucker	Pumpkinseed				0.165	-2.587
			Sucker	**Rock Bass**				**0.003**	**-3.827**

Table SI-5 continued. Parametric (ANOVA and Tukey HSD) or nonparametric (Kruskal-Wallis and Dunn's tests) comparison tests of total arsenic, arsenobetaine, and dimethylarsinic acid concentrations among Fish and invertebrate taxa within 3 lakes near Sudbury, Ontario. DFn = F ratio numerator degrees of freedom for ANOVA, or Kruskal-Wallis degrees of freedom; DFd = F ratio denominator degrees of freedom.

Lake	Analyte	Statistic	Taxon 1	Taxon 2	DFn	DFd	F	p	Diff.
Ramsey	[DMA]	Dunn	Sucker	Crayfish				1	0.216
(cont.)	(cont.)	(cont.)	Perch	Pumpkinseed				1	1.104
			Perch	Rock Bass				1	-0.012
			Perch	**Crayfish**				**0.009**	**3.526**
			Pumpkinseed	Rock Bass				1	-1.205
			Pumpkinseed	Crayfish				0.15	2.639
			Rock Bass	**Crayfish**				**0.003**	**3.803**
Johnnie	**Total [As]**	**ANOVA**			**4**	**26**	**2.995**	**0.037**	
		Tukey HSD	Cisco	Charr				0.842	0.121
			Cisco	Bass				1	0.012
			Cisco	Sucker				0.914	-0.096
			Cisco	Perch				0.13	-0.328
			Charr	Bass				0.886	-0.109
			Charr	Sucker				0.376	-0.216
			Charr	**Perch**				**0.023**	**-0.448**
			Bass	Sucker				0.874	-0.108
			Bass	Perch				0.108	-0.34
			Sucker	Perch				0.424	-0.232
Johnnie	**[AsB]**	**ANOVA**			**7**	**47**	**13.219**	**<0.001**	
		Tukey HSD	**Cisco**	**Charr**				**<0.001**	**0.767**
			Cisco	Bass				0.633	-0.269
			Cisco	Sucker				0.717	0.249
			Cisco	Perch				1	-0.069
			Cisco	Pumpkinseed				1	0.034
			Cisco	**Rock Bass**				**0.011**	**0.566**
			Cisco	**Crayfish**				**<0.001**	**0.781**
			Charr	**Bass**				**<0.001**	**-1.036**
			Charr	**Sucker**				**0.037**	**-0.517**
			Charr	**Perch**				**<0.001**	**-0.835**
			Charr	**Pumpkinseed**				**<0.001**	**-0.733**
			Charr	Rock Bass				0.903	-0.201
			Charr	Crayfish				1	0.014
			Bass	**Sucker**				**0.025**	**0.519**
			Bass	Perch				0.883	0.201
			Bass	Pumpkinseed				0.444	0.304
			Bass	**Rock Bass**				**<0.001**	**0.836**
			Bass	**Crayfish**				**<0.001**	**1.05**
			Sucker	Perch				0.426	-0.318
			Sucker	Pumpkinseed				0.818	-0.215
			Sucker	Rock Bass				0.432	0.317
			Sucker	**Crayfish**				**0.029**	**0.531**
			Perch	Pumpkinseed				0.997	0.103
			Perch	**Rock Bass**				**0.003**	**0.635**
			Perch	**Crayfish**				**<0.001**	**0.849**
			Pumpkinseed	**Rock Bass**				**0.015**	**0.532**
			Pumpkinseed	**Crayfish**				**<0.001**	**0.746**
			Rock Bass	Crayfish				0.868	0.215
Johnnie	[DMA]	Kruskal-Wallis			7			**<0.001**	
		Dunn	Cisco	Charr				0.113	-2.783
			Cisco	Bass				1	0.869
			Cisco	Sucker				1	-1.337

Table SI-5 continued. Parametric (ANOVA and Tukey HSD) or nonparametric (Kruskal-Wallis and Dunn's tests) comparison tests of total arsenic, arsenobetaine, and dimethylarsinic acid concentrations among Fish and invertebrate taxa within 3 lakes near Sudbury, Ontario. DFn = F ratio numerator degrees of freedom for ANOVA, or Kruskal-Wallis degrees of freedom; DFd = F ratio denominator degrees of freedom.

Lake	Analyte	Statistic	Taxon 1	Taxon 2	DFn	DFd	F	p	Diff.
Johnnie	[DMA]	Dunn	Cisco	Perch				0.384	-2.323
(cont.)	(cont.)	(cont.)	Cisco	Pumpkinseed				0.608	-2.075
			Cisco	**Rock Bass**				**0.033**	**-3.208**
			Cisco	Crayfish				1	0.308
			Charr	**Bass**				**0.008**	**3.617**
			Charr	Sucker				1	1.498
			Charr	Perch				1	0.551
			Charr	Pumpkinseed				1	0.878
			Charr	Rock Bass				1	-0.3
			Charr	Crayfish				0.067	2.978
			Bass	Sucker				0.466	-2.206
			Bass	**Perch**				**0.034**	**-3.191**
			Bass	Pumpkinseed				0.067	-2.972
			Bass	**Rock Bass**				**0.001**	**-4.077**
			Bass	Crayfish				1	-0.527
			Sucker	Perch				1	-0.986
			Sucker	Pumpkinseed				1	-0.695
			Sucker	Rock Bass				0.919	-1.871
			Sucker	Crayfish				1	1.592
			Perch	Pumpkinseed				1	0.324
			Perch	Rock Bass				1	-0.886
			Perch	Crayfish				0.222	2.539
			Pumpkinseed	Rock Bass				1	-1.238
			Pumpkinseed	Crayfish				0.384	2.306
			Rock Bass	**Crayfish**				**0.018**	**3.39**

Table SI-6. Results of parametric (ANOVA and Tukey HSD) or nonparametric (Kruskal-Wallis and Dunn's tests) comparison tests of total arsenic, arsenobetaine , and dimethylarsinic acid concentrations among 3 lakes near Sudbury, Ontario within fish and invertebrate taxa. Some taxa were not represented in all 3 lakes (n>6), for three taxa total [As] was not measured. DFn = F ratio numerator degrees of freedom for ANOVA, or Kruskal-Wallis degrees of freedom; DFd = F ratio denominator degrees of freedom. Significant differences are bolded.

Taxon	Analyte	Test	Lake1	Lake2	DFn	DFd	F	p	Difference
Perch	**[AsB]**	**ANOVA**			**2**	**18**	**90.992**	**<0.001**	
		Tukey HSD	Johnnie	Ramsey				0.962	-0.034
		Tukey HSD	**Johnnie**	**Long**				**<0.001**	**1.379**
		Tukey HSD	**Ramsey**	**Long**				**<0.001**	**1.413**
Perch	**[DMA]**	**Kruskal-Wallis**			**2**			**0.012**	
		Dunn	Johnnie	Ramsey				0.852	-0.186
		Dunn	**Johnnie**	**Long**				**0.03**	**2.489**
		Dunn	**Ramsey**	**Long**				**0.03**	**2.577**
Pumpkinseed	**[AsB]**	**ANOVA**			**2**	**23**	**134**	**<0.001**	
		Tukey HSD	**Johnnie**	**Ramsey**				**0.001**	**0.368**
		Tukey HSD	**Johnnie**	**Long**				**<0.001**	**1.25**
		Tukey HSD	**Ramsey**	**Long**				**<0.001**	**0.883**
Pumpkinseed	**[DMA]**	**Kruskal-Wallis**			**2**			**<0.001**	
		Dunn	Johnnie	Ramsey				0.124	1.538
		Dunn	**Johnnie**	**Long**				**<0.001**	**4.398**
		Dunn	**Ramsey**	**Long**				**0.011**	**2.777**
Crayfish	**[AsB]**	**ANOVA**			**2**	**18**	**4.409**	**0.028**	
		Tukey HSD	Johnnie	Ramsey				0.996	-0.01
		Tukey HSD	Johnnie	Long				0.065	0.278
		Tukey HSD	**Ramsey**	**Long**				**0.044**	**0.288**
Crayfish	**[DMA]**	**Kruskal-Wallis**			**2**			**0.007**	
		Dunn	Johnnie	Ramsey				0.179	1.345
		Dunn	**Johnnie**	**Long**				**0.006**	**3.096**
		Dunn	Ramsey	Long				0.149	1.785
Pike	**Total [As]**	**ANOVA**	**Long**	**Ramsey**	**1**	**17**	**81.032**	**<0.001**	
	[AsB]	**ANOVA**			**1**	**18**	**32.118**	**<0.001**	
	[DMA]	**Kruskal-Wallis**			**1**			**0.001**	
Walleye	**Total [As]**	**ANOVA**	**Long**	**Ramsey**	**1**	**16**	**23.881**	**<0.001**	
	[AsB]	**ANOVA**			**1**	**16**	**22.334**	**<0.001**	
	[DMA]	**Kruskal-Wallis**			**1**			**0.019**	
Bass	**Total [As]**	**ANOVA**	**Ramsey**	**Johnnie**	**1**	**13**	**31.579**	**<0.001**	
	[AsB]	ANOVA			1	13	2.847	0.115	
	[DMA]	**ANOVA**			**1**	**13**	**30.278**	**<0.001**	
Sucker	**Total [As]**	**ANOVA**	**Ramsey**	**Johnnie**	**1**	**12**	**20.715**	**0.001**	
	[AsB]	**ANOVA**			**1**	**14**	**5.341**	**0.037**	
	[DMA]	**ANOVA**			**1**	**14**	**30.794**	**<0.001**	
Cisco	**Total [As]**	**ANOVA**	**Long**	**Johnnie**	**1**	**15**	**35.928**	**<0.001**	
	[AsB]	**Kruskal-Wallis**			**1**			**0.001**	
	[DMA]	**ANOVA**			**1**	**15**	**25.214**	**<0.001**	
Rock Bass	[AsB]	ANOVA	Ramsey	Johnnie	1	13	0.986	0.339	
	[DMA]	ANOVA			1	13	1.659	0.22	

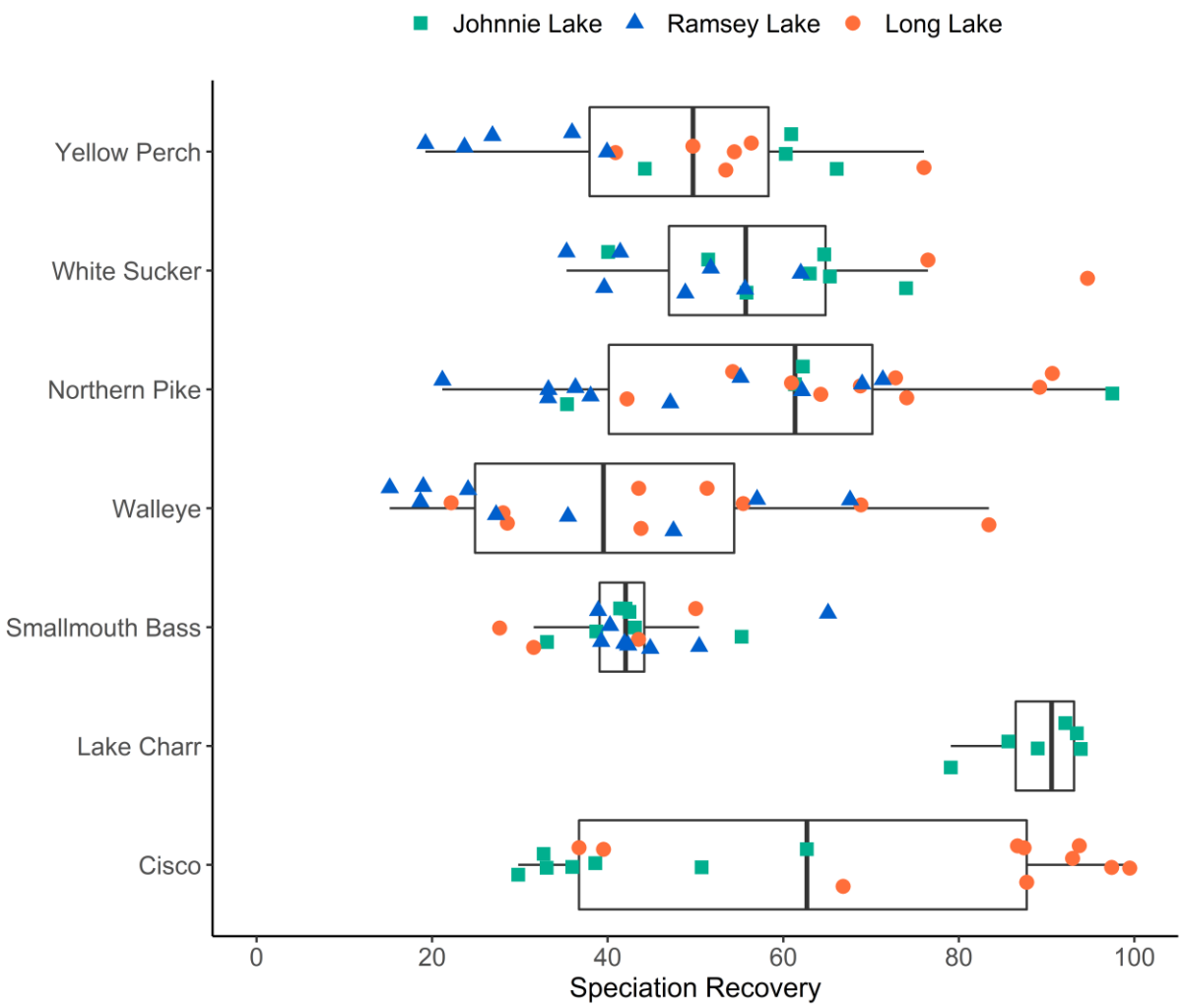


Figure SI-7. The percentage of total [As] accounted for by [AsB] and [DMA] (speciation recovery) in freshwater fish from three lakes near Sudbury Ontario. Data are grouped by species, with points representing individual fish and lake denoted by colour and shape. Boxes represent the 25th to 75th percentile of the data, the vertical line in each box represents the median, and the horizontal whiskers indicate the spread of the data within 1.5 times the interquartile distance from the 25th and 75th percentile.

Table SI-7. Results of parametric (ANOVA) or nonparametric (Kruskal-Wallis) comparisons of %AsB, and %DMA between lakes near Sudbury, Ontario within fish taxa. Sample sizes were only large enough (n>6) to allow for statistical comparisons between 2/3 lakes for each taxa. DFn = F ratio numerator degrees of freedom for ANOVA, or Kruskal-Wallis degrees of freedom; DFd = F ratio denominator degrees of freedom. Significant differences are bolded.

Taxon	Analyte	Test	Lake1	Lake2	DFn	DFd	F	p
Pike	%AsB	ANOVA	Long	Ramsey	1	17	4.377	0.052
	%DMA	Kruskal-Wallis			1			0.253
Walleye	%AsB	ANOVA	Long	Ramsey	1	16	1.881	0.189
	%DMA	ANOVA			1	16	0.434	0.519
Bass	%AsB	ANOVA	Ramsey	Johnnie	1	13	0.775	0.395
	%DMA	ANOVA			1	13	3.108	0.101
Sucker	%AsB	ANOVA	Ramsey	Johnnie	1	12	2.36	0.15
	%DMA	ANOVA			1	12	0.009	0.926
Cisco	**%AsB**	**ANOVA**	**Long**	**Johnnie**	**1**	**15**	**11.82**	**0.004**
	%DMA	**ANOVA**			**1**	**15**	**8.379**	**0.011**

Table SI-8. Results of parametric (ANOVA and Tukey HSD) or nonparametric (Kruskal-Wallis and Dunn's tests) comparison tests of the percentage of total arsenic made up by arsenobetaine (%AsB), and dimethylarsinic acid (%DMA) among fish pooled from 3 lakes near Sudbury, Ontario. Significant differences are bolded. DF = degrees of freedom.

Analyte	Statistic	Taxon 1	Taxon 2	DF	p	Diff.
%AsB	**Kruskal-Wallis**			**6**	**<0.001**	
	Dunn	Cisco	Charr		0.521	1.845
		Cisco	**Bass**		**<0.001**	**-4.637**
		Cisco	Walleye		0.369	-2.044
		Cisco	Pike		0.176	-2.441
		Cisco	Sucker		1	0.211
		Cisco	Perch		1	-0.541
		Charr	**Bass**		**<0.001**	**-5.176**
		Charr	**Walleye**		**0.014**	**-3.324**
		Charr	**Pike**		**0.005**	**-3.614**
		Charr	Sucker		0.543	-1.692
		Charr	Perch		0.271	-2.21
		Bass	Walleye		0.129	2.605
		Bass	Pike		0.173	2.474
		Bass	**Sucker**		**<0.001**	**4.853**
		Bass	**Perch**		**0.002**	**3.926**
		Walleye	Pike		1	-0.285
		Walleye	Sucker		0.264	2.258
		Walleye	Perch		0.766	1.428
		Pike	Sucker		0.115	2.667
		Pike	Perch		0.531	1.775
		Sucker	Perch		1	-0.746
%DMA	**Kruskal-Wallis**			**6**	**<0.001**	
	Dunn	Cisco	Charr		1	-1.1
		Cisco	**Bass**		**<0.001**	**4.492**
		Cisco	Walleye		1	0.537
		Cisco	**Pike**		**0.012**	**3.327**
		Cisco	Sucker		1	0.401
		Cisco	Perch		1	-0.697
		Charr	**Bass**		**<0.001**	**4.318**
		Charr	Walleye		1	1.493
		Charr	**Pike**		**0.008**	**3.461**
		Charr	Sucker		1	1.39
		Charr	Perch		1	0.57
		Bass	**Walleye**		**0.001**	**-4.007**
		Bass	Pike		1	-1.405
		Bass	**Sucker**		**0.001**	**-4.08**
		Bass	**Perch**		**<0.001**	**-5.057**
		Walleye	Pike		0.061	2.804
		Walleye	Sucker		1	-0.13
		Walleye	Perch		1	-1.226
		Pike	**Sucker**		**0.049**	**-2.897**
		Pike	**Perch**		**0.001**	**-3.95**
		Sucker	Perch		1	-1.086

Table SI-5. Results of analysis of covariance models assessing the effect of log10-transformed condition factor (K) on logit-transformed %AsB and %DMA in large bodied predators pooled from 3 lakes with lake as a class variable and K as the covariate. Bolded lines are significant.

a) %AsB

	Sum Sq.	Df	F-value	p-value
Lake	**35.453**	**2**	**8.868**	**<0.001**
K	**43.646**	**1**	**21.835**	**<0.001**
Interaction	**16.244**	**2**	**4.063**	**0.022**
Residuals	119.934	60		

b) %DMA

	Sum Sq.	Df	F-value	p-value
Lake	**8.726**	**2**	**4.668**	**0.013**
K	**8.238**	**1**	**8.814**	**0.004**
Interaction	**7.146**	**2**	**3.823**	**0.027**
Residuals	56.082	60		

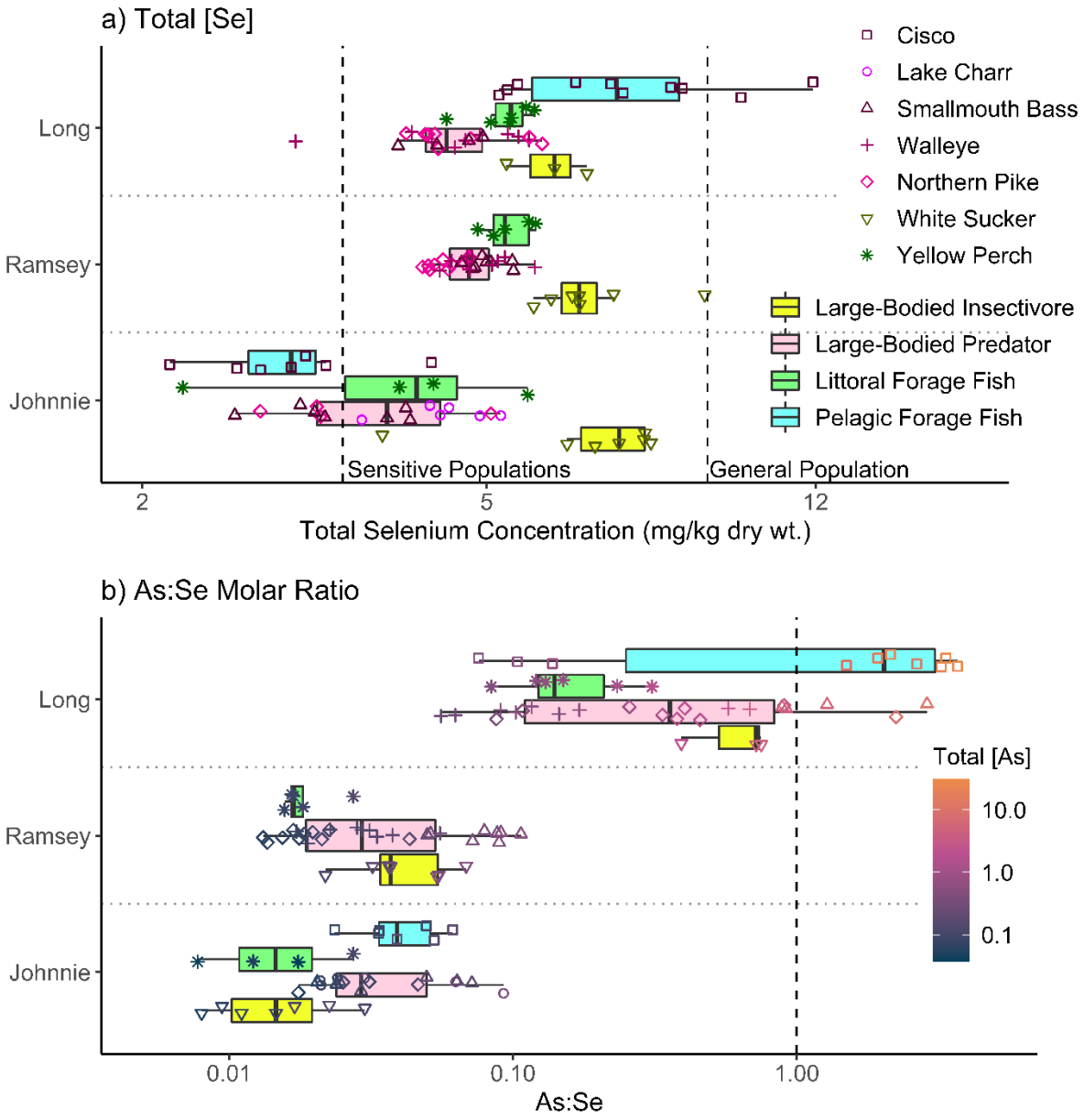


Figure SI-8. Total selenium concentrations (a) and arsenic:selenium molar ratios (b) in fish from 3 lakes near Sudbury, Ontario. Data are grouped by lake and functional group, with points representing individual fish, and species denoted by colour (a) and shape (a and b). In panel (b), colour represents total arsenic concentration. Boxes represent the 25th to 75th percentile of the data, the vertical line in each box represents the median, and the horizontal whiskers indicate the spread of the data within 1.5 times the interquartile distance from the 25th and 75th percentile. Dotted vertical lines represent consumption advisory benchmarks for total selenium in fish in Ontario for sensitive and general populations (panel a) or a 1:1 As:Se ratio (panel b).

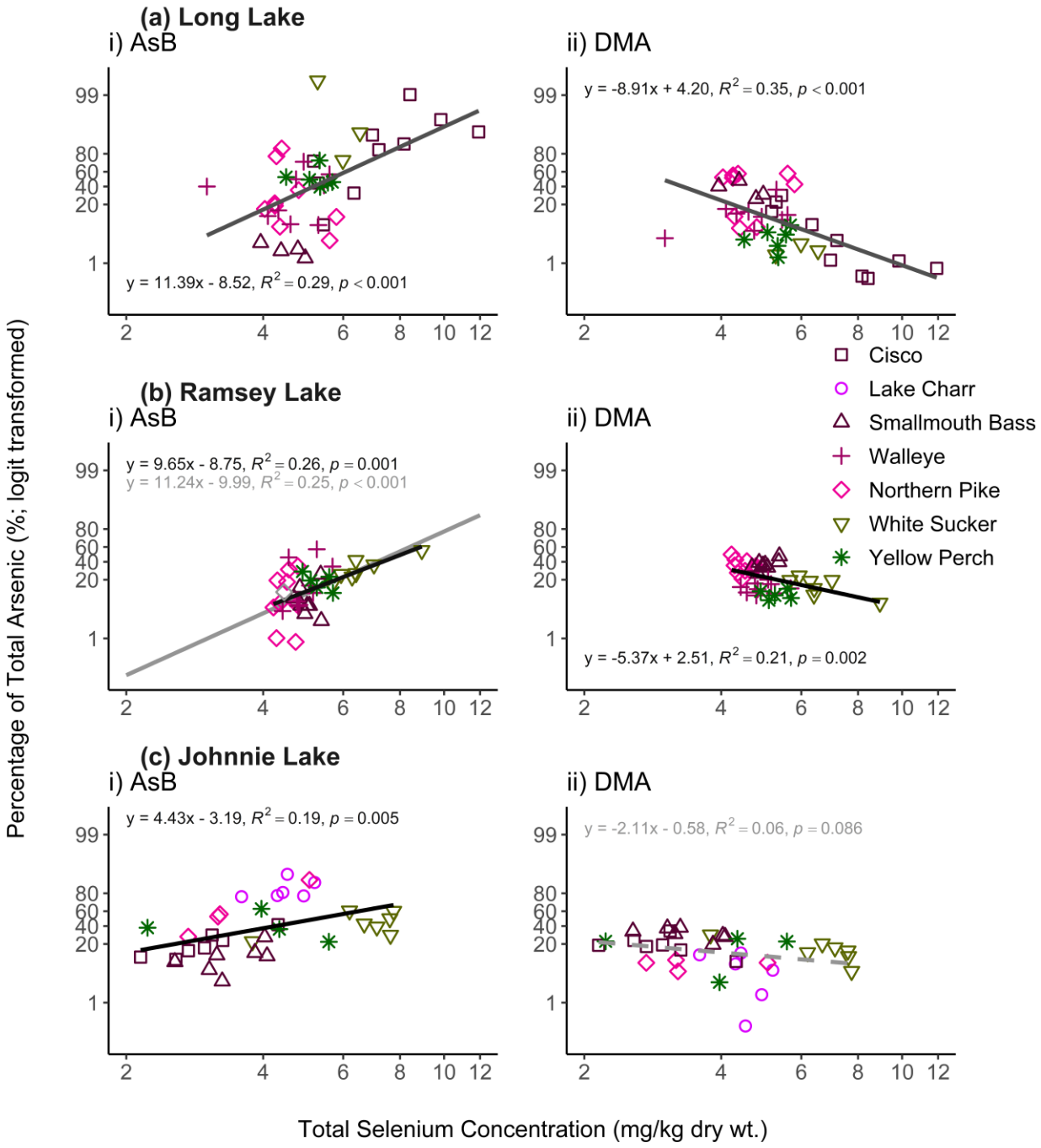


Figure SI-9. Relationships between %AsB (i) or %DMA (ii) and total Se concentration in fish from 3 lakes in the Sudbury area. Points are individual fish, with species denoted by colour and shape. Models shown in grey include outliers identified by Cook's Distance that were removed from the main model to pass normality assumptions. Solid lines indicate statistically significant relationships; dotted lines indicate statistically non-significant relationships.

Table SI-6. Results of analysis of covariance models assessing the effect of log10-transformed total selenium concentrations on logit-transformed %AsB and %DMA in fish from 3 lakes with lake as a class variable and total [Se] as the covariate. Bolded lines are significant.

a) %AsB

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**97.867**	**2**	**20.772**	**<0.001**
Total [Se]	**84.368**	**1**	**35.814**	**<0.001**
Interaction	**17.493**	**2**	**3.713**	**0.028**
Residuals	256.778	109		

b) %DMA

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**15.715**	**2**	**10.324**	**<0.001**
Total [Se]	**24.454**	**1**	**32.129**	**<0.001**
Interaction	**16.794**	**2**	**11.032**	**<0.001**
Residuals	78.395	103		

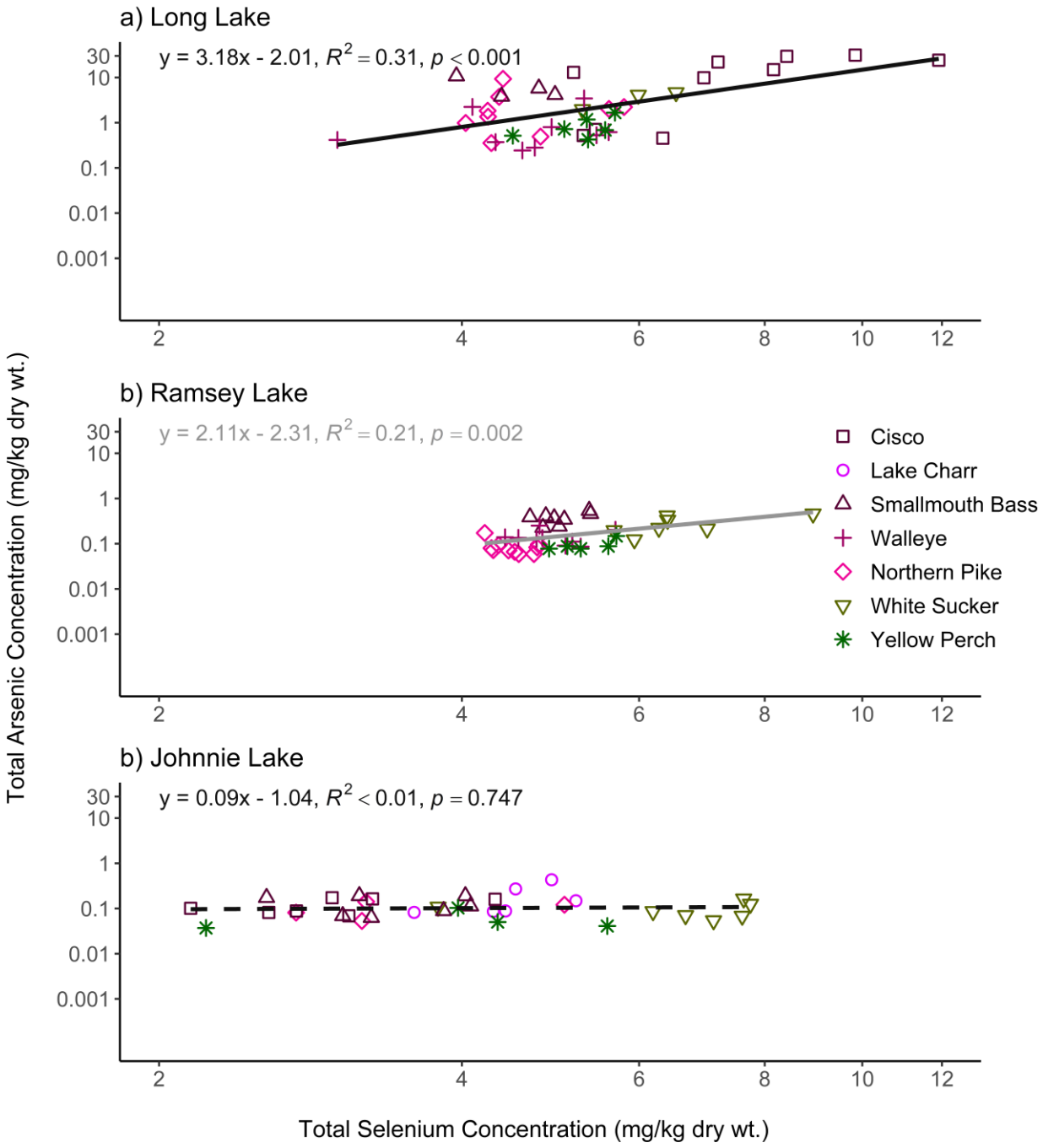


Figure SI-10. Relationships between total arsenic concentrations and total selenium concentration in fish from 3 lakes (a-c) near Sudbury, Ontario. Points are individual fish, with species denoted by colour and shape. Models shown in grey include outliers identified by Cook's Distance that were removed from the main model to pass normality assumptions. Model residuals in Ramsey Lake (b) were non-normal even after outlier removal. Solid lines indicate statistically significant relationships; dotted lines indicate statistically non-significant relationships.

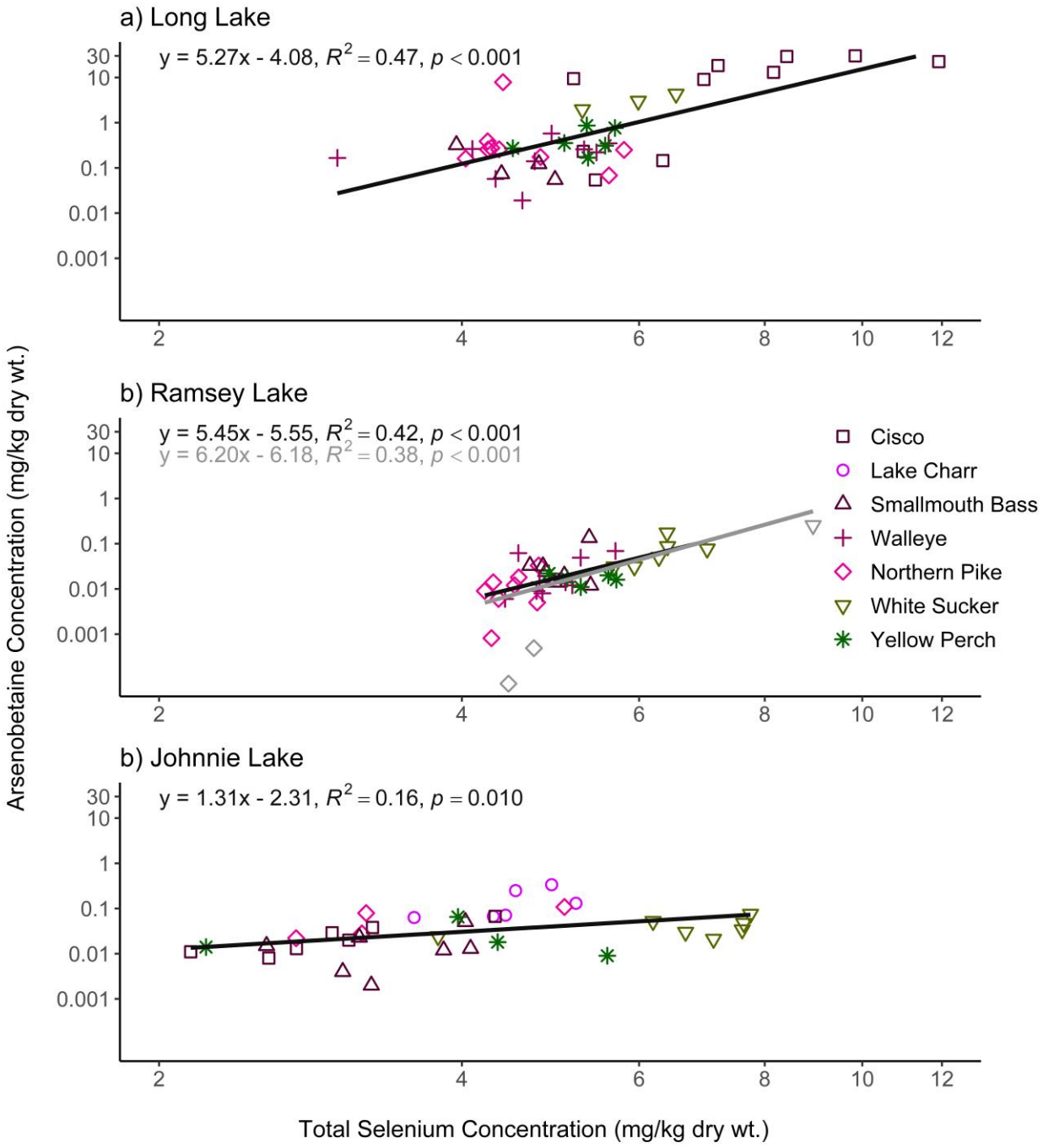


Figure SI-11. Relationships between arsenobetaine concentrations and total selenium concentration in fish from 3 lakes (a-c) near Sudbury, Ontario. Points are individual fish, with species denoted by colour and shape. Models shown in grey include outliers identified by Cook's Distance that were removed from the main model to pass normality assumptions. Solid lines indicate statistically significant relationships; dotted lines indicate statistically non-significant relationships.

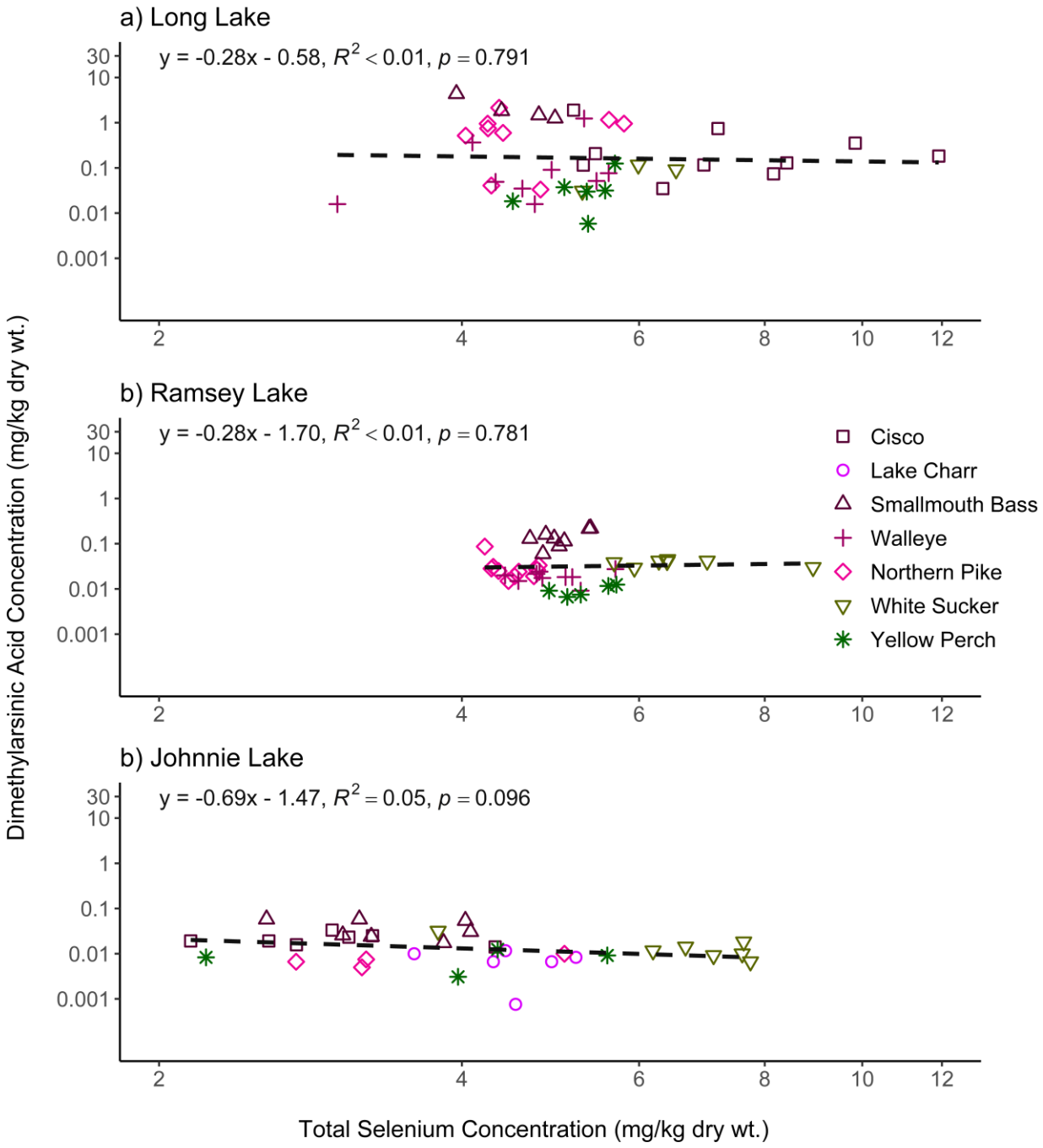


Figure SI-12. Relationships between dimethylarsinic acid concentrations and total selenium concentration in fish from 3 lakes (a-c) near Sudbury, Ontario. Points are individual fish, with species denoted by colour and shape. Solid lines indicate statistically significant relationships; dotted lines indicate statistically non-significant relationships.

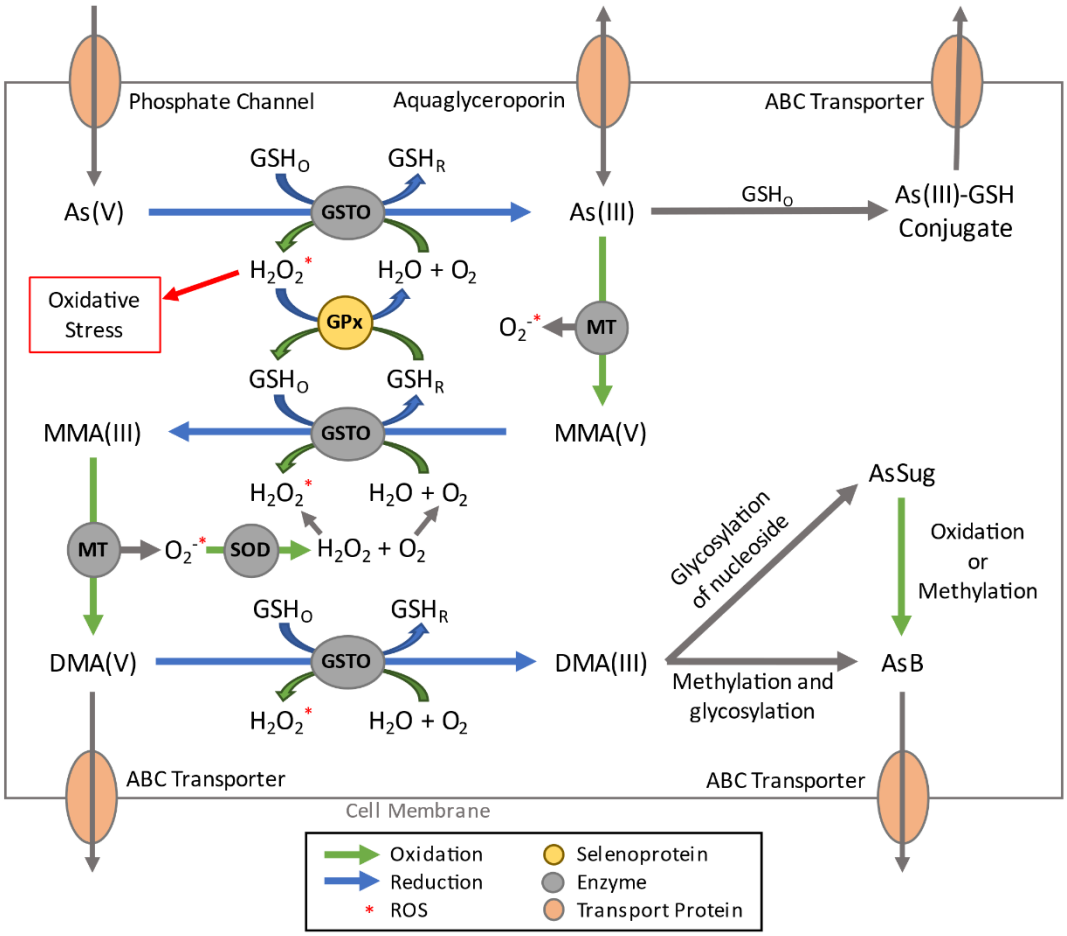


Figure SI-13. One potential metabolic pathway of arsenic in aquatic organisms, adapted from (Byeon et al. 2021) to highlight the role of the selenoprotein glutathione peroxidase (Arteel and Sies, 2001) in preventing oxidative stress from H_2O_2 as well as recycling GSHR, two by-products of arsenic metabolism. To visually simplify the diagram, some interactions are only shown in one place, although they occur throughout. MMA(III) and DMA(III) are also likely excreted as GSH conjugates (Leslie, 2012). Abbreviations: GSTO: glutathione S-transferase omega; MT: arsenic methyltransferase; GPx: glutathione peroxidase; SOD: superoxide dismutase; GSH_O: oxidized glutathione; GSH_R: reduced glutathione; As(V): arsenate; As(III): arsenite; MMA(V): monomethylarsonic acid; MMA(III): monomethylarsonous acid; DMA(V) dimethylarsinic acid; DMA(III): dimethylarsenous acid; AsB: arsenobetaine; AsSug: arsenosugars; ROS: reactive oxygen species.

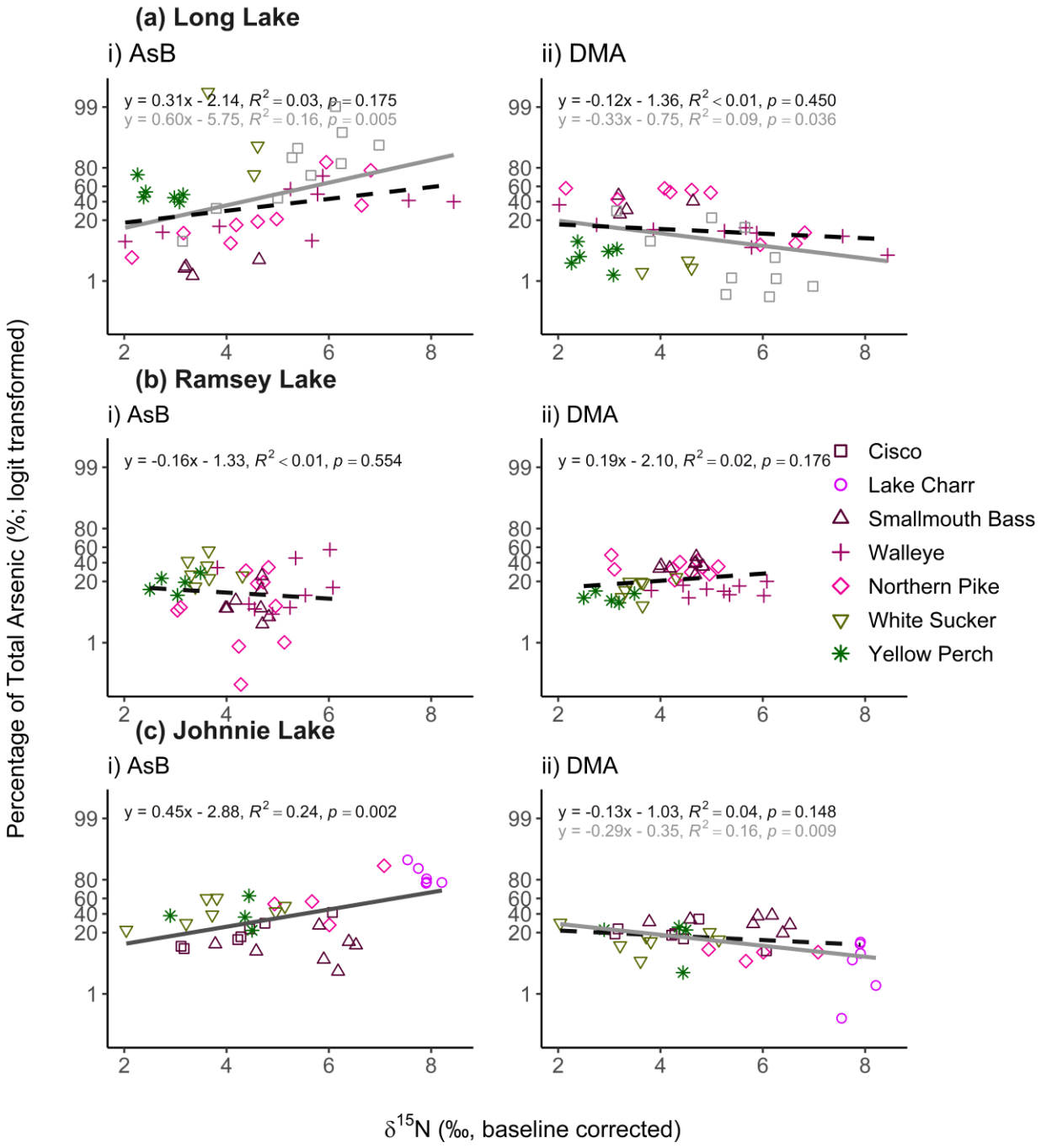


Figure SI-14. Relationships between the percentage of total arsenic made up by AsB (i) or DMA (ii) and baseline corrected δ^{15}N in freshwater fish in 3 lakes in a mining impacted region. Points are individual fish, with species denoted by shape and colour. Models shown in grey include cisco from Long Lake, which were removed due to their separation in δ^{13}C from other taxa indicating they are not being consumed in large quantities by other taxa, as well as any outliers identified by Cook's Distance which were removed to pass residual normality assumptions. Solid lines indicate statistically significant relationships; dashed lines indicate statistically non-significant relationships.

Table SI-7. Results of analysis of covariance models assessing the effect of trophic elevation (inferred from $\delta^{15}N$) on logit-transformed %AsB and %DMA in fish from 3 lakes with lake as a class variable and $\delta^{15}N$ as the covariate. Bolded lines are significant.

a) AsB

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**22.603**	**2**	**5.942**	**0.004**
δ15N	**20.593**	**1**	**10.827**	**0.001**
Interaction	**12.551**	**2**	**3.299**	**0.041**
Residuals	182.591	96		

b) DMA

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	5.985	2	2.572	0.081
δ15N	**4.892**	**1**	**4.205**	**0.043**
Interaction	5.176	2	2.225	0.113
Residuals	115.166	99		

ii) Main Effects Model (Type III SS)

	Sum Sq.	Df	F-value	p-value
Intercept	**6.908**	**1**	**5.797**	**0.018**
Lake	5.985	2	2.511	0.086
δ15N	**4.892**	**1**	**4.106**	**0.045**
Residuals	120.342	101		

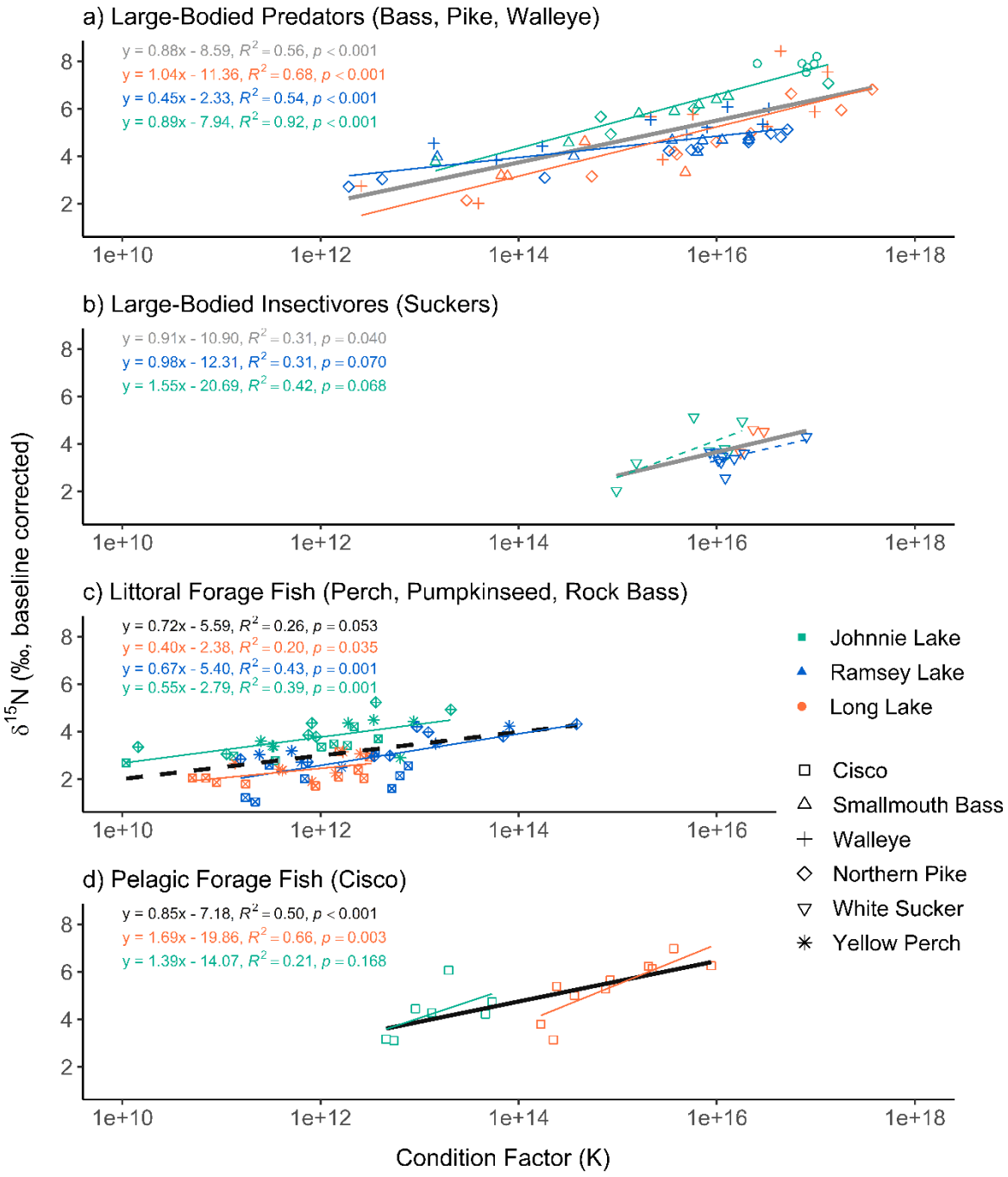
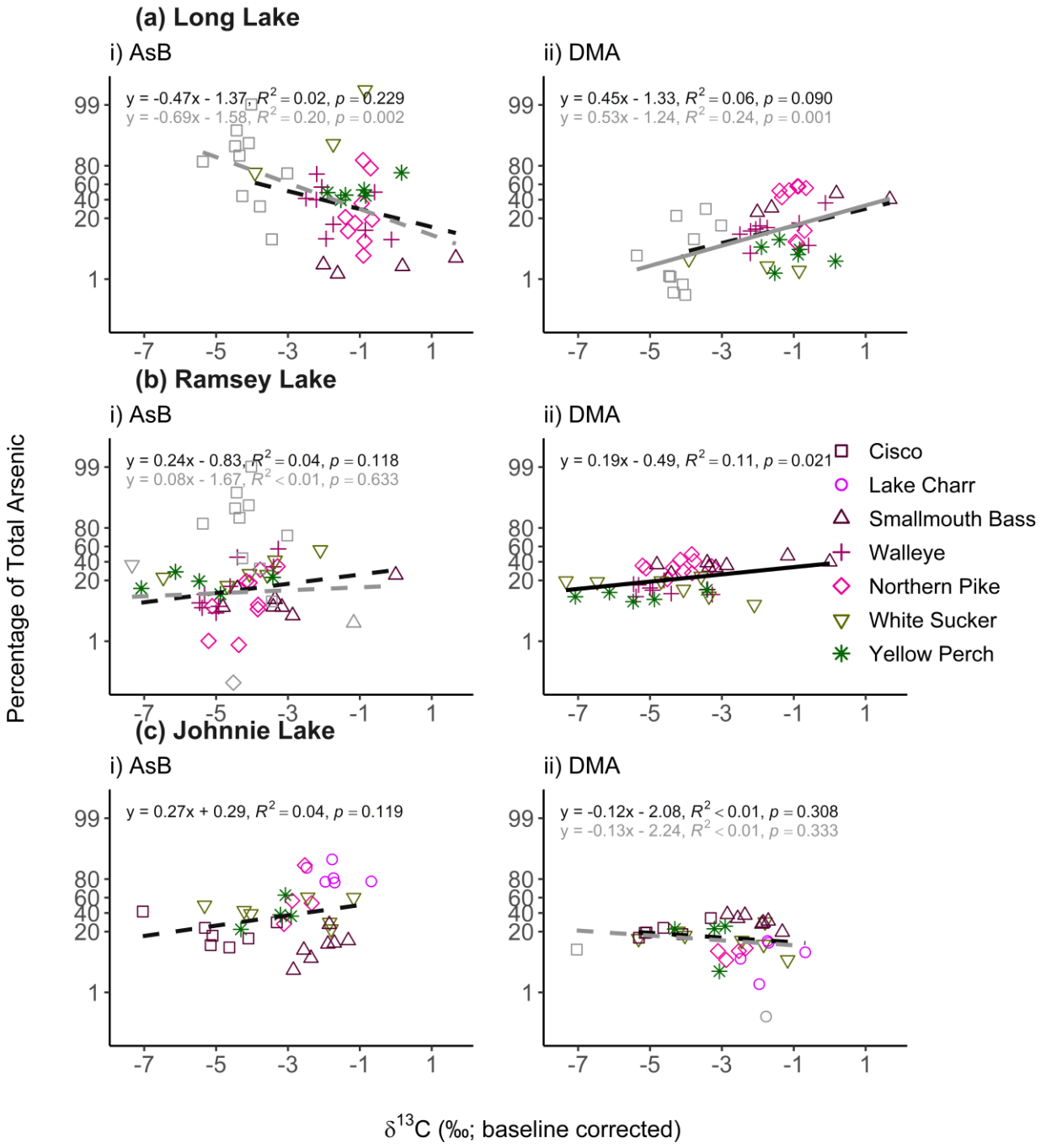


Figure SI-15. Relationships between baseline corrected δ^{15}N and fish condition factor in 3 lakes near Sudbury, Ontario. Points are individual fish, grouped by functional group (a-d) with species denoted by shape and lake denoted by colour. Models shown in grey did not pass normality assumptions, even after removal of outliers by Cook's Distance. Solid lines indicate statistically significant relationships; dashed lines indicate statistically non-significant relationships.



2019

2020 **Figure SI-16.** Relationships between the percentage of total arsenic made up by AsB (i) or DMA
2021 (ii) and baseline corrected $\delta^{13}C$ in freshwater fish in 3 lakes (a-c) in a mining impacted region.
2022 Points are individual fish, with species denoted by shape and colour. Models shown in grey include
2023 cisco from Long Lake, which were removed due to their separation in δ13C from other taxa
2024 indicating they are not being consumed in large quantities by other taxa, as well as any outliers
2025 identified by Cook's Distance which were removed to pass normality assumptions. Solid lines
2026 indicate statistically significant relationships; dashed lines indicate statistically non-significant
2027 relationships.

Table SI-8. Results of analysis of covariance models assessing the effect of dietary carbon source (inferred from $\delta^{13}C$) on logit-transformed %AsB and %DMA in fish from 3 lakes with lake as a class variable and $\delta^{13}C$ as the covariate. Bolded lines are significant.

a) AsB

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**30.990**	**2**	**5.660**	**0.005**
$\delta^{13}C$	0.732	1	0.267	0.606
Interaction	11.584	2	2.116	0.126
Residuals	271.031	99		

ii) Main Effects Model (Type III SS)

	Sum Sq.	Df	F-value	p-value
Intercept	1.410	1	0.504	0.480
Lake	**30.990**	**2**	**5.537**	**0.005**
$\delta^{13}C$	0.732	1	0.262	0.610
Residuals	282.615	101		

b) DMA (4 outliers removed)

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**10.838**	**2**	**5.795**	**0.004**
$\delta^{13}C$	**4.796**	**1**	**5.129**	**0.026**
Interaction	**9.490**	**2**	**5.074**	**0.008**
Residuals	88.838	95		

Table SI-9. Results of analysis of covariance models assessing the effect of trophic elevation (inferred from $\delta^{15}N$) on logit-transformed %AsB and %DMA in fish and invertebrates from 3 lakes with lake as a class variable and $\delta^{15}N$ as the covariate. Bolded lines are significant.

a) AsB (14 outliers removed)

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**20.949**	**2**	**83.656**	**<0.001**
δ15N	**10.459**	**1**	**83.531**	**<0.001**
Interaction	**4.963**	**2**	**19.818**	**<0.001**
Residuals	22.788	182		

b) DMA (13 outliers removed; residuals still non-normal)

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**27.700**	**2**	**68.134**	**<0.001**
δ15N	**2.359**	**1**	**11.603**	**0.001**
Interaction	0.018	2	0.043	0.958
Residuals	36.996	182		

ii) Main Effects Model (Type III SS)

	Sum Sq.	Df	F-value	p-value
Intercept	**73.300**	**1**	**364.382**	**<0.001**
Lake	**27.700**	**2**	**68.850**	**<0.001**
δ15N	**2.359**	**1**	**11.725**	**0.001**
Residuals	37.014	184		

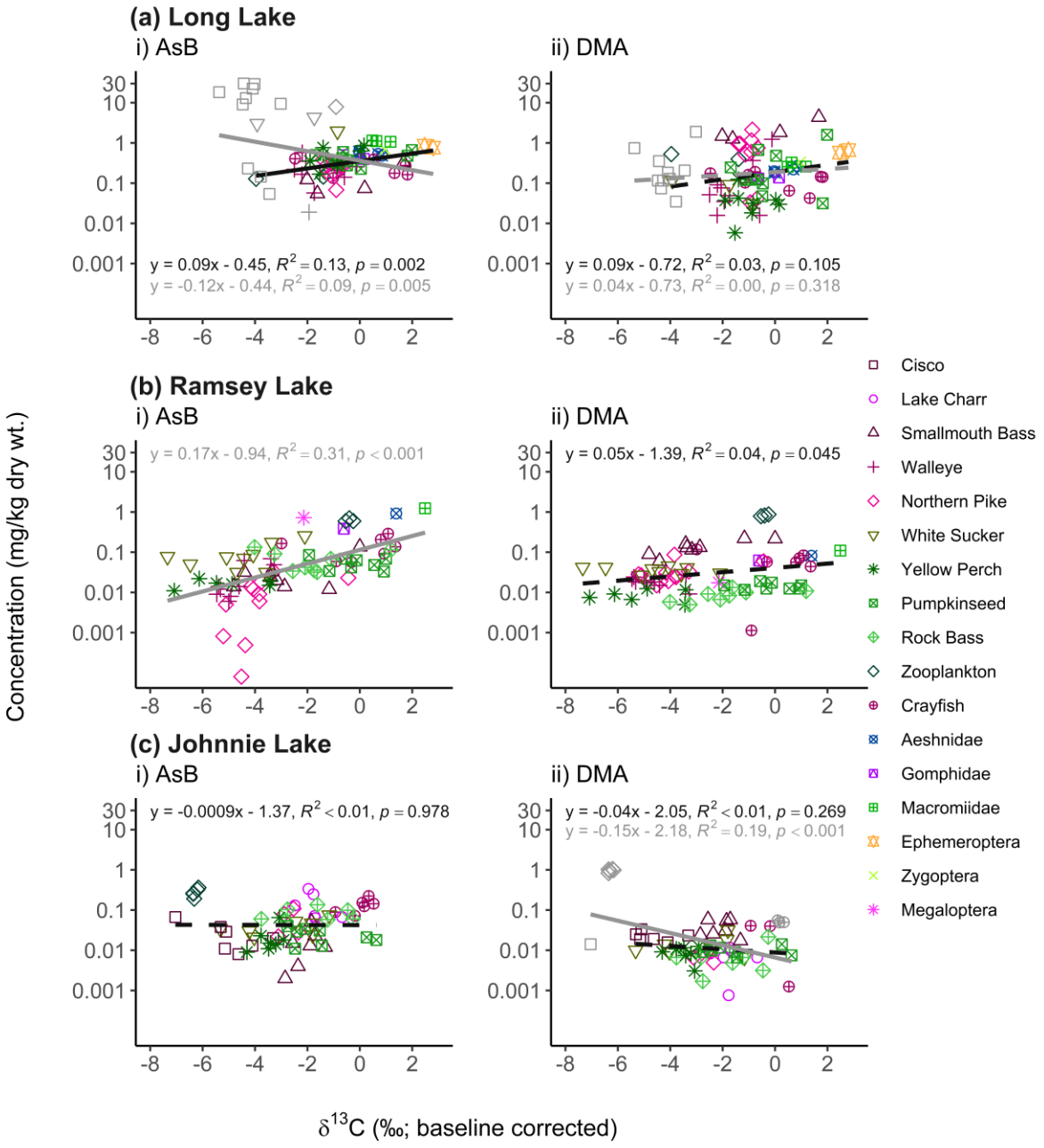


Figure SI-17. Relationships between AsB (i) and DMA (ii) concentrations and baseline corrected $\delta^{13}C$ values in freshwater fish and invertebrates in 3 lakes (a-c) in a mining impacted region. Points are individual fish, with species denoted by shape and colour. Solid lines indicate statistically significant relationships; dashed lines indicate statistically non-significant relationships. Models shown in grey include cisco from Long Lake, which were removed due to their separation in $\delta^{13}C$ from other taxa indicating they are not being consumed in large quantities by other taxa, as well as any outliers identified by Cook's Distance which were removed to improve model normality; model residuals for [AsB] in Ramsey Lake (panel b-i) were still non-normally distributed after outlier removal.

Table SI-10. Results of analysis of covariance models assessing the effect of dietary carbon source (inferred from $\delta^{13}C$) on logit-transformed %AsB and %DMA in fish and invertebrates from 3 lakes with lake as a class variable and $\delta^{13}C$ as the covariate. Bolded lines are significant.

a) AsB (16 outliers removed)

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**13.741**	**2**	**45.101**	**<0.001**
δ13C	**9.130**	**1**	**59.933**	**<0.001**
Interaction	0.549	2	1.803	0.168
Residuals	27.420	180		

ii) Main Effects Model (Type III SS)

	Sum Sq.	Df	F-value	p-value
Intercept	**47.782**	**1**	**310.927**	**<0.001**
Lake	**13.741**	**2**	**44.707**	**<0.001**
δ13C	**9.130**	**1**	**59.409**	**<0.001**
Residuals	27.969	182		

b) DMA (19 outliers removed)

i) Interaction Model

	Sum Sq.	Df	F-value	p-value
Lake	**29.539**	**2**	**73.866**	**<0.001**
δ13C	0.523	1	2.615	0.108
Interaction	0.960	2	2.400	0.094
Residuals	35.392	177		

ii) Main Effects Model (Type III SS)

	Sum Sq.	Df	F-value	p-value
Intercept	**124.820**	**1**	**614.627**	**<0.001**
Lake	**29.539**	**2**	**72.728**	**<0.001**
δ13C	0.523	1	2.575	0.110
Residuals	36.352	179		