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Arsenic concentration and speciation in five freshwater fish species from Back Bay near Yellowknife, NT, CANADA

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Abstract The concentration of total arsenic and five different arsenic species [As(III), As(V), monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), and arsenobetaine (AsB)], were measured in the muscle, liver and gastrointestinal tract (GIT) of five different fish species [lake whitefish (*Coregonus clupeaformis*), walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*) and longnose sucker (*Catostomus catostomus*)] from Back Bay, Great Slave Lake, near the city of Yellowknife, NT, Canada. The total concentration (dry weight) of arsenic in muscle ranged from 0.57 to 1.15 mg/kg, in the liver from 0.42 to 2.52 mg/kg and in the GIT from 1.48 to 8.92 mg/kg. Among fish species, *C. commersoni* had significantly higher total arsenic concentrations in the GIT than *S. vitreum*, *E. lucius* and *C. clupeaformis*, and higher total arsenic concentrations in the liver than *C. clupeaformis*. The mean concentration of As(III) and As(V) in the muscle of all fish ranged from ≤0.01 to 0.05 mg/kg and ≤0.01 to 0.02 mg/kg, respectively, and together comprised ≤7.5% of the total arsenic measured in muscle. The concentrations of MMA were below detection in the muscle of all five fish species. However, AsB and DMA were measured in all fish species and nearly all fish tissues. The concentrations of AsB ranged from 0.01 to 0.13 mg/kg and the concentrations of DMA ranged from <0.02 to 0.45 mg/kg. The majority (>50%) of organic arsenic in almost all of the tissues from fish caught in Back Bay was not directly identified. Evidence from the literature suggests that most of these other organic arsenic species were likely trimethylated arsenic compounds, however, further analytical work would need to be performed to verify this hypothesis.

Keywords Arsenic · Arsenic speciation · Freshwater fish · Yellowknife

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Introduction

Operations of Giant Mine north of Yellowknife, NT, Canada, from the late 1940s to early 2000s have resulted in the release of large amounts of arsenic trioxide to the environment. Gold extraction practices used by the mine in the early 1950s resulted in atmospheric deposition of arsenic (2.6 million kg per year) to the surrounding environment and the release of arsenic contaminated effluent (25,000 kg/year) to Baker Creek (MacDonald 1997; SRK Consulting

2002). Improved extraction methods and filters implemented in the mid-1950s dramatically decreased the release of arsenic. By the late 1990s, aerial emissions of arsenic were estimated to be 5,700 kg/year and release of arsenic to Baker Creek was estimated to be 500 kg/year (MacDonald 1997; SRK Consulting 2002). In 2000, Giant Mine stopped milling practices but continued to mine gold from the area.

In 2002, it was estimated that Baker Creek continued to release approximately ~1,100 kg of arsenic into Back Bay, which is part of the larger Yellowknife Bay in Great Slave Lake (SRK Consulting 2002). Although Giant Mine has ceased milling practices, arsenic containing effluent is still released into Baker Creek (~430 kg As per year). Concentrations of arsenic in underground storage chambers and the Northwest Tailings Pond (NWTP) ranged from 100 to 4,000 mg/l and from 6 to 12 mg/l, respectively, in 2000 (Clark and Raven 2004). Seepage from storage chambers and the NWTP, run-off from contaminated soils and historic tailings spills, and slow release of arsenic from historically contaminated creek sediment contribute ~380 kg As per year to Baker Creek (SRK Consulting 2002; Clark and Raven 2004). In 2002, the concentration of arsenic upstream of Giant Mine was 50 μg/l (Dillon Consulting Ltd. 2002), which resulted in an additional release of ~290 kg As per year to Back Bay (SRK Consulting 2002). The concentration of arsenic at the mouth of Baker Creek in Back Bay measured ~224 μg/l in 2002 (Dillon Consulting Ltd. 2002).

The historic and current release of arsenic into Back Bay has been a concern for local residents. While historic concentrations of arsenic in Back Bay were as high as 740 μg/l in the 1970s and resulted in fishing for consumption, swimming and drinking water bans, the concentration of arsenic was reduced to 2.1 μg/l by the late 1990s (SRK Consulting 2002). As a result of this contamination, there was concern that arsenic could affect human health through the consumption of fish living in Back Bay that may have accumulated arsenic in their tissues.

Arsenic may exists in the environment in several forms. The toxic and biological effects of arsenic depend on the oxidation state and molecular form (speciation) in which it occurs. Arsenic can exist in the inorganic forms arsenite [As(III)] and arsenate [As(V)], or in organic forms such as monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AsB), arsenocholine (AsC) and a series of arsenolipids and arsenosugars (Feldman et al. 2004). Arsenite is more toxic than arsenate and both of these species are more toxic than organic arsenicals. Arsenic is a known carcinogen in humans, causing lung, liver, skin and bladder cancer (Bernstam and Nriagu 2000; Kapaj et al. 2006). Biomethylation of arsenic reduces the toxicity of arsenic to humans. For example, the trimethylated arsenical AsB is very stable due to full saturation of arsenic which masks its chemical properties; it is also rapidly excreted by humans (Feldman et. al. 2004). Monomethylated arsenic is more toxic than the dimethylated species and both arsenicals are enzyme inhibitors and cytotoxins (Bernstam and Nriagu 2000). Arsenosugars are significantly less toxic than MMA and DMA, but may cause damage to DNA (Andrewes et al. 2004).

A study conducted on Back Bay in 1997 by Koch (1998) found that the concentration of total arsenic ranged from 0.28 to 3.1 mg/kg dry weight (d.w.) in the muscle tissue of walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), lake whitefish (*Coregonus clupeaformis*), and white sucker (*Catostomus commersoni*). The concentrations of inorganic arsenic and MMA were below detection (<0.01 mg/kg) in most fish. However, AsB and DMA were present in nearly all samples. The concentration of AsB ranged from 0.05 to 0.28 mg/kg d.w in these fish and did not appear to be species-specific, whereas northern pike had higher concentrations of DMA (0.33 to 0.8 mg/kg d.w.) than all other fish species examined (Koch 1998). The results of the Koch (1998) study indicated that the concentrations of total arsenic and some arsenic species in fish from Back Bay could be of concern to human health. However, due several technical difficulties and the small number ($n \leq 3$ per species) of fish sampled, a second study was recommended to better evaluate the human health risk from consumption of fish living in Back Bay near Yellowknife, NT.

The objective of this study was to determine the concentrations and speciation of arsenic in different fish species common to Back Bay near Yellowknife, NT, in 2003. This information was then used in a separate assessment to evaluate if consumption of such fish could pose a risk to human health. In this study, the liver and gastro-intestinal tract (GIT) were chosen for analysis as these tissues are more likely to accumulate arsenic than muscle (Pedlar and Klaverkamp 2002; Pedlar et al. 2002a, b). Muscle was also

analyzed, since this is the most common and largest portion of fish consumed and, therefore, represents the most likely route of exposure for most consumers. The method used for arsenic speciation analysis in this study was able to determine the concentration of As (III), As(V), MMA, DMA and AsB simultaneously (Xie et al. 2002).

Materials and methods

Fish collection

Back Bay is located within the ~32 km^2 Yellowknife Bay (Lat. 62°29′N, Long. 114°22′W) in Great Slave Lake, Canada. Maximum water depth in Yellowknife Bay is 40 m, but near the outlet of Baker Creek measures 2 to 15 m (Mudroch et al. 1989). For the most part, water exiting Baker Creek flows in a southwesterly direction in Back Bay due to discharge from the Yellowknife River to the north. Higher concentrations of arsenic have been measured in sediments near the mouth and adjacent areas south of Baker Creek. Sediments and metals have been shown to accumulate in the shallow waters around Latham Island in Back Bay (Moore 1989; Mudroch et al. 1989).

Nets were mostly set south of Baker Creek and around Latham Island in Back Bay (Fig. 1). The aim was to catch fish that would most likely be used for human consumption and also represent the likely worst case scenario for arsenic contamination in Back

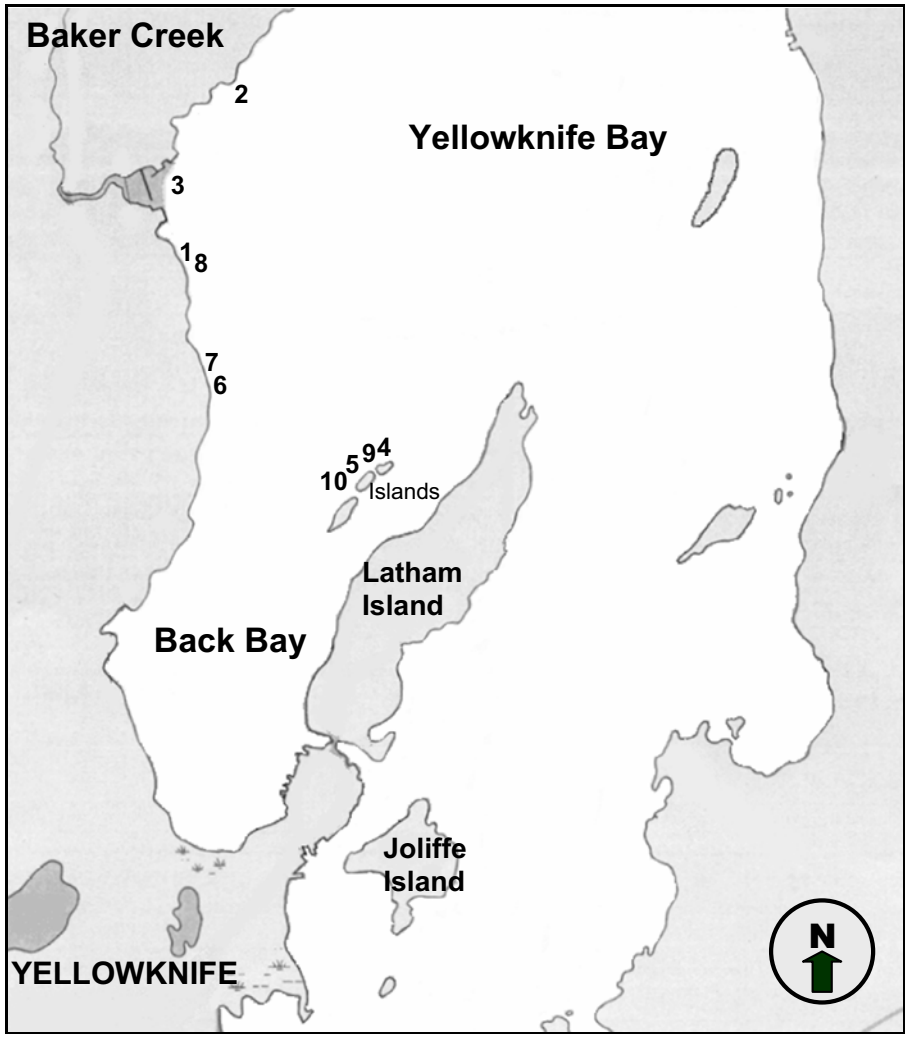

Fig. 1 Fish collection sites in Back Bay, Great Slave Lake, near the city of Yellowknife, NT, Canada (numbers indicate net location)

Bay. Net-set locations were chosen on the basis of traditional fishing strategies, which were provided by local Yellowknife Dene resident Morris Martin, and as well as in areas that would most likely result in fish exposure to arsenic. Gill nets, with mesh sizes ranging from 5.0 to 12.7 cm, were set at ten different locations in Back Bay on August 26 and 27, 2003. Recommendations from Environment Canada's *Metal Mining Guidance Document for Environmental Effects Monitoring* (2002) were followed in order to ensure that enough fish were caught to conduct a human health risk assessment. In total, eight lake whitefish (*C. clupeaformis*), eight northern pike (*E. lucius*), eight walleye (*S. vitreum*), six white sucker (*C. commersoni*) and four longnose sucker (*C. catostomus*) were caught. The fish were killed by a blow to the head, individually wrapped in plastic bags, and immediately placed on ice in coolers. The fish were frozen to −17°C at the end of each day and transported to the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, on August 29, 2003. The fish remained frozen at −20°C until fish tissue samples were prepared for arsenic analysis.

Preparation of fish tissue samples

Frozen fish were thawed to room temperature (~20°C) in their plastic bags using a water bath. The gender, weight and fork length of each fish were recorded (Table 1). A sample of skeletal muscle was taken from the mid-lateral section dorsal to the abdominal cavity. The skin was removed and the muscle sample was rinsed with deionized water, weighed and frozen. The liver was removed from the abdominal cavity, separated from the gall bladder, rinsed with deionized water, weighed and frozen. The entire GIT was cut at both extremities and removed from the abdominal cavity. The stomach, pyloric caeca, and intestine were separated and the contents gently squeezed out. The GIT of white and longnose sucker did not consist of a stomach or pyloric caeca, only of a long intestine. Each section was rinsed with deionized water and, if necessary, scraped clean of debris. The GIT and gall bladder were combined, weighed and frozen. Samples were stored at −20 °C until all of the samples had been prepared for freeze-drying. A commercial lyophilizer (Virtis Model 50-SRC-6, Gardiner, NY, USA) was used to freeze-dry the samples. The lyophilized fish samples were stored at −80 °C until they could be homogenized. The dried liver samples were ground and homogenized into a powder using an agate mortar and pestle. The dried muscle and GIT samples were homogenized using a motorized grinder (Model DCG-20BKNC, Cuisinart, Brampton, ON, Canada). Two separate methods were used to digest the dried fish tissues and extract arsenic; one for total arsenic and another for arsenic speciation.

Determination of total arsenic

Sample digestions for total arsenic analysis were conducted at the Toxicology Centre. Sub-samples of lyophilized fish tissue (~100 mg) were digested in 15-ml Teflon® vials using a combination of double-distilled 16 N nitric acid (2–3 ml) and hydrogen peroxide (0.25 ml; [As] <0.005 μg/l). The Teflon® vials were covered with glass and samples were digested on a hot plate at a temperature of 40–50°C for 1–2 h. Small amounts (~1 ml) of nitric acid and 5–10 drops of hydrogen peroxide were added to the samples during digestion. Once a clear, light-yellow solution was obtained, the glass was removed and the solutions were evaporated to ≤0.25 ml. The remaining solution was diluted with 1 ml

Table 1 Sample size, gender and the mean weight and fork length of five different fish species caught in Back Bay, Great Slave Lake, near Yellowknife, NT, in August 2003

Species	Common name	Sample size	Number of		Mean (range)	
			Males	Females	Wet weight (g)	Fork length (cm)
Coregonus clupeaformis	Lake whitefish	8	4	4	957 (671–1,150)	40.3 (36.3–43.8)
Stizostedion vitreum	Walleye	8	3	5	850 (338–1,222)	40.1 (30.0–45.7)
Esox lucius	Northern pike	8	2	6	1,869 (1,156–2,612)	61.5 (53.2–70.0)
Catostomus commersoni	White sucker	6[a]	2	3	759 (569–900)	36.8 (33.5–38.8)
Catostomus catostomus	Longnose sucker	4	2	2	1,136 (1,016–1,265)	42.5 (40.0–43.8)

[a] One fish was too immature to identify the gender

of 16 N nitric acid and 19 ml of ultra-pure (18.1Ω) water. Solutions were stored in pre-cleaned 30-ml high-density polyethylene bottles at 4°C until analysis for total arsenic could be completed by the Worsfold Water Quality Centre, Trent University, Peterborough, ON. The concentration of total arsenic in fish tissues was determined using inductively coupled plasma-mass spectrometry (ICP-MS). The instrument used was a Micromass platform collision cell ICP-MS (Micromass, Manchester, UK).

Determination of different arsenic species

Tissue digestions for arsenic speciation analysis were conducted at the Worsfold Water Quality Centre. A sub-sample (~500 mg) of freeze-dried tissue was vortexed with 5 ml of acetone in a 15-ml plastic centrifuge tube. The solution was sonicated for 30 min followed by 10 min of centrifuging at 4,000 rpm. The residue was re-suspended in 5 ml of acetone and vortexed, sonicated, and centrifuged as described above twice more. The supernatant from each extraction was decanted and combined in a clean 15-ml plastic centrifuge tube and saved for future reference. The remaining tissue residue was suspended in 5 ml of a 50:50 (*m*/*m*) methanol/ultra-pure water solution, sonicated, and centrifuged at 4,000 rpm. This procedure was repeated twice. Each time, the supernatant was collected and combined in a clean 15-ml plastic centrifuge tube. The supernatant was evaporated for 3–4 h to ambient dryness using a Speed VacPlus at a temperature of 65°C. The dried residue was re-dissolved in 5 ml ultra-pure water and centrifuged at 4,000 rpm for 10 min. The supernatant was collected and 1 ml of this solution filtered through a 0.45 μm cellulose nitrate filter and used for arsenic speciation analysis. The concentration of As(III), As(V), MMA, DMA and AsB were determined simultaneously using a multi-mode ion exchange column that allowed near baseline separation of the five different arsenic species with a nitrate mobile phase. Arsenic speciation analysis was conducted using high performance liquid chromatography (HPLC, Waters-2690, Waters Corporation, Milford, MA, USA) coupled to the Micromass platform ICP-MS equipped with a hexapole collision cell. This method, previously described by Xie et al. (2002), reduced the polyatomic interferences commonly encountered by a conventional ICP-MS and resulted in improved detection limits. Xie et al. (2002) was able to obtain the following detection limits in water samples: As(III) 0.006 μg/l, As(III) 0.05 μg/l, MMA 0.4 μg/l, DMA 0.1 μg/l, and AsB 0.02 μg/l.

The procedure was checked by analysis of nine samples in duplicate and a standard reference material, DORM-2 (dog fish muscle, NRC, Ottawa, ON, Canada). The mean (±SD) difference in total arsenic concentration between duplicate samples was 11.6± 8.0%. Two procedure blanks were analyzed and found to have <0.015 μg As/l. The precision and accuracy of the method was checked by repeated analysis of DORM-2 for total arsenic and AsB. Eight repeated analyses of DORM-2 gave a mean concentration of 19.7 mg As/kg with a precision of 5.4%, which agreed, within error, with the certified concentration of total arsenic in DORM-2 (18.0±1.1 mg/kg). DORM-2 was analyzed 19 times for AsB and had a mean concentration of 15.3 mg AsB/kg with a precision of 5.4%. This was also in agreement with the certified AsB concentration in the DORM-2 reference material (16.4±1.1 mg/kg) and recent results by other researchers (McSheehy and Mester 2004). The reproducibility of sample replicates was slightly poorer than that reported for the DORM-2 standard. This may have been due to sample heterogeneity and/or minor variability during sample preparation. The limits of quantification, established using repeated analysis of blanks and based on the blank equivalent concentrations in a tissue mass of 500 mg, were: total As 0.005 mg/kg, As(III) 0.01 mg/kg, As(V) 0.01 mg/kg, MMA 0.08 mg/kg, DMA 0.02 mg/kg, and AsB 0.004 mg/kg.

Statistical analysis

Differences in the concentration of total arsenic among the liver, muscle and GIT for each fish species (tissue-specific differences) were determined using one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test. Differences in the concentration of total arsenic in the liver, muscle, or GIT among the five fish species (species-specific differences) were determined using separate one-way ANOVAs, each followed by a Tukey's test. Where the test for normality failed, a Kruskal-Wallis one-way ANOVA on ranks followed by Dunn's *t*-test was performed. The difference between the sum of all arsenic species measured in this study and the total

arsenic measured is assumed to be other species that were not identified in the HPLC analysis. The unidentified arsenic is referred to as other asenic species (OAS). Differences in the concentrations of As(III), As(V), MMA, DMA, AsB, and other OAS in the muscle, liver, and GIT (tissue-specific differences) among the five fish species were determined for each arsenic species separately using two-way ANOVA followed by a Tukey's multiple comparison test. The concentration of other OAS were estimated by subtracting the sum of the concentration of identified arsenic species [As (III), As(V), MMA, DMA, and AsB] from the total concentration of arsenic in each tissue of individual fish. Statistical analyses were performed using SigmaStat® Version.03 (1997) with a 95% level of confidence (α=0.05). If the concentration of arsenic species in samples were below the detection limit, a value of one-half the detection limit was used to calculate the mean concentration and for associated statistical analyses reported in Table 2.

Results

Total arsenic concentrations

The mean (±standard deviation) total arsenic concentrations in the liver, muscle and GIT of the five fish species are presented in Table 2. The mean concentration of total arsenic ranged from 0.57 to 1.15 mg/kg in the muscle, 0.42 to 2.52 mg/kg in the liver, and 1.48 to 8.92 mg/kg in the GIT. There were significant differences in the total arsenic concentrations among the three tissue types in walleye, northern pike and white sucker, but not in lake whitefish and longnose sucker. In walleye, total arsenic concentrations in the liver and GIT were significantly higher than in the muscle. In northern pike, total arsenic concentration in the GIT was significantly higher than in the liver and muscle. In white sucker, the total arsenic concentration in the GIT was significantly higher than in the muscle, but not in the liver. Across fish species, white sucker and longnose sucker had the greatest total arsenic concentrations in their tissues (Table 2). The total arsenic concentration in the liver of white sucker was significantly higher than in northern pike, but not significantly different from other fish species. White sucker also had significantly higher concentrations of total arsenic in the GIT compared to lake whitefish, walleye, and northern pike.

Arsenic speciation

The mean concentrations of As(III), As(V), MMA, DMA, AsB, and other OAS in the muscle, liver, and GIT of all five fish species are presented in Table 3. The proportion (%) of individual arsenic species [As(III), As (V), MMA, DMA, AsB] relative to total arsenic in muscle, liver and GIT tissue samples from the five fish are presented in Table 4. The mean concentration of As (III) and As(V) in the muscle of all fish ranged

Table 2 Mean (±standard deviation) total arsenic concentration in the muscle, liver and gastrointestinal tract (GIT) of five different fish species caught in Back Bay near Yellowknife, NT, in August 2003

Species	Sample size	Total arsenic concentration (mg/kg dry wt.)		
		Muscle	Liver	GIT
Lake whitefish (*Coregonus clupeaformis*)	8	0.77±0.59	1.07±0.58	2.07±1.86
Walleye (*Stizostedion vitreum*)	8[a]	0.57±0.19	1.22±0.35[b]	1.48±0.31[b]
Northern pike (*Esox lucius*)	8	0.97±0.54	0.42±0.13	1.82±0.61[c]
White sucker (*Catostomus commersoni*)	6	0.91±0.22	2.52±2.12[d]	8.92±7.51[b,e]
Longnose sucker (*Catostomus catostomus*)	4	1.15±0.16	1.33±0.86	3.30±2.84

[a] For the GIT n=7

[b] Significantly different from the total arsenic concentration in the muscle within that species

[c] Significantly different from the total arsenic concentration in the muscle and liver within that species

[d] Significantly different from total arsenic concentrations in the liver of northern pike

[e] Significantly different from total arsenic concentrations in the GIT of lake whitefish, walleye and northern pike

from ≤0.01 to 0.05 mg/kg and ≤0.01 to 0.02 mg/kg, respectively (Table 3). In all fish species, the proportion of As(III) and As(V) in muscle was ≤7.5% and ≤1.6%, respectively, of the total arsenic measured. The concentration of inorganic arsenic was lower in the muscle than in the liver and GIT; As(III) and As(V) comprised 5.5 to 22.3% of the total arsenic in the liver and <0.1 to 6.0% of the total arsenic in GIT (Table 4).

The majority of arsenic in fish caught in Back Bay was organic arsenic (Table 3). In all fish species, the concentration of MMA in the muscle was below detection (<0.08 mg/kg), and only measured in the liver and GIT of white and longnose suckers at low concentrations (≤0.8 mg/kg). The concentration of AsB in the muscle and liver was ≤0.15 mg/kg and in the GIT was ≤0.05 mg/kg in all fish species (Table 3). There were no significant differences in the concentrations of MMA and AsB among tissues of individual fish species or among fish species. Statistical analysis of As(III), As(V), MMA and AsB showed that the concentration of these arsenic species were not significantly different among muscle, liver and GIT of individual fish species, nor were there fish species related differences in the concentration of these arsenicals.

Dimethylarsenic acid was measured in all fish species and tissue types, except the GIT of walleye where it was below detection (Table 3). In all fish species, the concentration of DMA ranged from 0.02 to 0.18 mg/kg in the muscle, from 0.10 to 0.30 mg/kg in the liver and from <0.02 to 0.45 mg/kg in the GIT. Among fish species, northern pike had the highest proportion of DMA in the liver (73.7%), followed by lower proportions in muscle (23.3%) and GIT (22.5%; Table 4). The only significant differences in tissue concentrations of DMA were observed in northern pike, where the concentration was significantly higher in the GIT compared to the muscle and liver (Table 3). In northern pike, the concentration of DMA in the GIT was significantly higher than the concentration of all other arsenic species analyzed, and in the liver significantly higher than As(V). In all

Table 3 The mean (±standard deviation) concentration of arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AsB), and other organic arsenic species (OAS) in the muscle, liver and gastrointestinal tract (GIT) of five different fish species caught in Back Bay near Yellowknife, NT, in August 2003

Species	Sample size	Arsenic species (mg/kg dry wt.)						
		Tissue	As(III)	As(V)	MMA	DMA	AsB	Other OAS[a]
Lake whitefish	8	Muscle	< 0.01	< 0.01	< 0.08	0.02±0.04	0.08±0.09	0.66±0.54[b]
(*Coregonus clupeaformis*)	8	Liver	0.07±0.08	< 0.01	< 0.08	0.14±0.17[c]	0.08±0.17	0.75±0.49[b]
	8	GIT	< 0.01	0.01±0.01	< 0.08	0.10±0.18	0.01±0.04	1.95±1.68[b,e]
Walleye	8	Muscle	0.05±0.08	< 0.01	< 0.08	0.06±0.08	0.05±0.07	0.41±0.30[b]
(*Stizostedion vitreum*)	8	Liver	0.07±0.08	0.01±0.02	< 0.08	0.10±0.05	0.03±0.06	1.00±0.27[b,f]
	7	GIT	< 0.01	< 0.01	< 0.08	< 0.02	0.02±0.04	1.46±0.28[b,e]
Northern pike	8	Muscle	0.01±0.01	< 0.01	< 0.08	0.18±0.14	0.13±0.09	0.63±0.56[b]
(*Esox lucius*)	8	Liver	0.07±0.08	< 0.01[d]	< 0.08	0.30±0.16	0.06±0.08	0.04±0.06[f]
	8	GIT	0.16±0.32[d]	< 0.01[d]	< 0.08[d]	0.45±0.33[e]	0.05±0.05[d]	1.17±0.23[b,e]
White sucker	6	Muscle	0.05±0.07	0.02±0.01	< 0.08	0.07±0.07	0.09±0.07	0.63±0.28
(*Catostomus commersoni*)	6	Liver	0.31±0.28	0.30±0.47	0.80±1.75	0.17±0.23	0.13±0.19	0.65±0.72
	6	GIT	0.10±0.15	0.12±0.18	0.49±0.74	0.02±0.02	0.04±0.05	8.18±6.48[b,e]
Longnose sucker	4	Muscle	0.05±0.09	0.01±0.01	< 0.08	0.05±0.07	0.12±0.10	0.89±0.24
(*Catostomus catostomus*)	4	Liver	0.23±0.25	0.09±0.10	0.13±0.11	0.18±0.19	0.15±0.23	0.55±0.36
	4	GIT	0.01±0.01	0.03±0.06	0.13±0.18	0.02±0.02	0.05±0.06	3.08±2.63[b,e]

[a] Other OAS = other organic arsenic species; calculated by subtracting the sum of the concentration of As(III), As(V), MMA, DMA and AsB from the concentration of total arsenic (Table 1)

[b] Significantly different from the concentration of As(III), As(V), MMA, DMA and AsB within each tissue of individual fish species

[c] Not significantly different from the concentration of other OAS in lake whitefish

[d] Significantly different from the concentration of DMA within that tissue of northern pike

[e] Significantly different from the arsenic concentration in the other tissues within that species of fish

[f] Significantly different from the arsenic concentration in the muscle within that species of fish

Table 4 The mean (±standard deviation) proportion of arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AsB), and other organic arsenic species (OAS) relative to the concentration of total arsenic in the muscle, liver and gastrointestinal tract (GIT) of five different fish species caught in Back Bay, near Yellowknife, NT, in August 2003

Species	Sample size	Tissue	Percent of total arsenic concentration[a]					
			As(III)	As(V)	MMA	DMA	AsB	Other OAS
Lake whitefish	8	Muscle	BDL	BDL	BDL	3.4±8.5	9.4±11.6	87.8±15.9
(*Coregonus clupeaformis*)	8	Liver	8.0±13.5	BDL	BDL	12.5±15.7	5.5±10.5	69.3±13.6
	8	GIT	BDL	0.1±0.1	BDL	2.7±4.7	1.0±2.6	95.6±6.1
Walleye	8	Muscle	7.5±14.5	BDL	BDL	14.2±23.2	16.2±25.8	63.8±44.9
(*Stizostedion vitreum*)	8	Liver	4.8±7.0	0.7±1.4	BDL	7.8±4.5	2.3±4.3	82.8±11.0
	7	GIT	BDL	BDL	BDL	BDL	0.7±1.8	98.8±2.0
Northern pike	8	Muscle	0.5±1.1	BDL	BDL	23.3±17.8	16.5±15.5	54.0±23.7
(*Esox lucius*)	8	Liver	12.9±18.3	BDL	BDL	73.7±44.9	11.1±15.3	10.6±18.5
	8	GIT	6.0±11.0	BDL	BDL	22.5±9.8	2.8±3.2	68.7±17.9
White sucker	6	Muscle	5.1±1.1	1.6±1.4	BDL	8.9±9.7	9.0±7.6	67.7±20.0
(*Catostomus commersoni*)	6	Liver	12.5±10.5	8.7±11.2	15.6±24.7	5.9±3.5	6.5±10.0	51.2±34.2
	6	GIT	0.8±0.6	0.9±0.8	2.8±3.6	0.3±0.4	0.7±1.1	94.6±4.2
Longnose sucker	4	Muscle	4.1±8.1	0.9±1.3	BDL	3.8±7.1	10.7±8.8	77.3±14.4
(*Catostomus catostomus*)	4	Liver	17.6±15.9	4.7±3.8	9.8±2.7	15.0±12.2	7.1±9.3	45.8±30.2
	4	GIT	0.3±0.4	0.5±0.8	1.9±2.5	1.2±1.4	2.6±3.0	93.6±1.2

OAS, organic arsenic species; the concentration other OAS was calculated by subtracting the sum of the concentration of As(III), As (V), MMA, DMA and AsB from the concentration of total arsenic (Table 2); BDL = below detection limit; based on values in Table 3, where the mean concentration was less than the detection limit

[a] The proportion (%) of each arsenic species was calculated by dividing the arsenic species' concentration by the concentration of total arsenic

other fish species, the proportion of DMA in the muscle, liver and GIT were lower than in northern pike and ranged from below detection to <15.0%, and the concentration of DMA was not significantly different from that of other arsenicals measured (Table 4).

A large proportion of organic arsenic in fish remained unidentified (Table 3). In all fish species, the concentration of OAS ranged from 0.41 to 0.89 mg/kg in the muscle, from 0.04 to 1.00 mg/kg in the liver, and from 1.17 to 8.18 mg/kg in the GIT. Most importantly, the concentration of other OAS was significantly higher in the GIT than in the muscle and liver of all five fish species. In walleye, the concentration of other OAS in the muscle was significantly lower than in the liver, whereas in northern pike the concentration of other OAS was higher in the muscle than in the liver. In lake white fish and walleye, the concentrations of other OAS were significantly higher than those of all individual arsenic species measured in the muscle, liver and GIT; with the exception of DMA in whitefish which was not significantly different from other OAS. In northern pike muscle and GIT, the concentrations of other OAS were significantly higher than those of all other arsenic species measured. In white and longnose suckers, the concentrations of other OAS were significantly higher than other arsenicals in the GIT, but not in muscle or liver. The percent contribution of other OAS to the total arsenic concentration ranged from 68.7 to 98.8% in the GIT, 10.6 to 82.8% in the liver, and 54.0 to 87.8% in the muscle (Table 4).

Discussion

High variation in the concentration of arsenic among individual fish made it somewhat difficult to determine significant differences in the concentrations of total arsenic and different arsenic species among the different fish species and tissues analyzed. However, it is clear that white sucker had higher concentrations of total arsenic than lake whitefish, northern pike and walleye. The small sample size of longnose sucker (n=4) made it

difficult to determine if the concentration of total arsenic was significantly different from northern pike, lake whitefish and walleye. Furthermore, in all fish species the concentrations of total arsenic and most arsenic species were highest in the GIT compared to concentrations in muscle and liver.

The concentrations of total arsenic in the muscle (≤1.15 mg/kg d.w.) of fish from this study were similar to those measured in the other freshwater fish exposed to low concentrations of arsenic (Koch 1998; Chen and Folt 2000; Soeroes et al. 2005; Jankong et al. 2007). Koch (1998) reported similar total arsenic concentrations in the muscle tissue of lake whitefish (0.28 to 3.10 mg/kg), walleye (0.46 to 0.85 mg/kg), northern pike (1.30 to 1.40 mg/kg), and sucker (0.98 to 1.24 mg/kg) also collected from Back Bay. Soeroes et al. (2005) found that carp (*Cyprinus carpio*) cultured in Hungarian lakes with low arsenic contamination (0.7 to 13.2 μg/l) had concentrations of total arsenic ranging from 0.062 to 0.363 mg/kg d.w. in muscle. Striped snakehead (*Channa striata*), a carnivorous fish from Thailand, exposed to arsenic concentrations of 1.4 μg As/l had 1.9±1.4 mg/kg d.w. total arsenic in the muscle tissue and 1.6±1.0 mg/kg d.w total arsenic in the liver (Jankong et al. 2007).

Differences in the accumulation of arsenic in different tissues and fish species may be related to trophic status and feeding behavior, tissue-specific differences and fish species-specific physiology. Aquatic organisms at lower trophic levels, such as zooplankton and benthic invertebrates, tend to accumulate more arsenic than organisms at higher trophic levels, such as fish (Chen and Folt 2000; Mason et al. 2000; Suhendrayatna et al. 2001). Furthermore, bottom feeding fish tend to have higher body burdens of total arsenic than forage fish, and predatory fish tend to have the lowest body burdens overall (Chen and Folt 2000; Suhendrayatna et al. 2001; Kirby and Maher 2002). White and longnose sucker are both bottom feeders with a diet consisting mostly of benthic invertebrates. Lake whitefish are bottom feeders as well, but their diet also consists of small fishes. Walleye are largely predatory fish, but will feed on emerging benthic invertebrates, and adult northern pike are solely predatory fish (Scott and Crossman 1998). Although only white sucker had significantly higher concentrations of total arsenic in the GIT, both species of sucker generally had elevated concentrations of total arsenic in the muscle, liver and GIT compared to other fish analyzed, supporting the general findings that trophic status may influence the accumulation of arsenic.

The general trend in arsenic tissue distribution for all fish species in this study was GIT > liver > muscle, which is generally similar to results from other studies of freshwater fish. For example, arsenic has previously been shown to accumulate differentially in organs of fish exposed to dietary arsenate. Pedlar and Klaverkamp (2002) found that lake whitefish fed a diet of 100 μg/g arsenic containing food for 30 days accumulated arsenic in the pyloric caeca> liver>intestine≫stomach (muscle was not analyzed). In lake whitefish and lake trout (*Salvelinus namaycush*) also fed diets containing arsenic, the highest concentrations of arsenic were found in the pyloric caeca and liver, and lower concentrations in the muscle (Pedlar et al. 2002b). Higher concentrations of total arsenic in the liver compared to muscle have also been shown to occur in fish living in arsenic-contaminated freshwater ecosystems (Jankong et al. 2007).

Arsenic tends to accumulate in the GIT largely because it is the major route of dietary arsenic exposure and uptake. Once absorbed by the GIT, arsenic is distributed to organs throughout the body via the circulatory system. The liver readily absorbs arsenic and is the site where arsenic is biotranformed to mono- and di-methylated forms (Pedlar et al. 2002a). Higher arsenic concentrations in the GIT may also be due to constant re-exposure through the bile (Pedlar et al. 2002a). Biotransformed arsenic is released from the liver into circulation and also into the bile, where it re-enters the intestine and thus hepatoportal vein (Cockell and Bettger 1993). The majority of absorbed arsenic may persist in this entero-hepatic loop for a relatively long time and may also account for higher concentrations observed in the liver compared to other tissues (Cockell and Bettger 1993).

Fish species-specific differences in GIT function and structure may also affect the accumulation of arsenic in different organs of the body. In the present study, the highest concentration of total arsenic was observed in the GIT of white sucker. The GIT structure of sucker is different from that of the other fish species studied, which may have caused significantly more arsenic to accumulate here. Lake whitefish and walleye have highly defined stomach, pyloric caeca and intestine, whereas northern pike do

not have a pyloric caeca, but do have a large stomach and a long, undifferentiated intestine (Scott and Crossman 1998). White and longnose sucker do not have a pyloric caeca or stomach, rather the GIT consists of a long intestine which coils four or five times and is closely associated with the liver (Scott and Crossman 1998). The lack of a pyloric caeca and stomach may have resulted in higher arsenic accumulations in the intestine of sucker due to its rudimentary nature compared to the highly differentiated GIT of the other species in this study.

Accumulation of arsenic may also be related to other differences in arsenic absorption among fish species. Lake whitefish fed diets containing 100 μg As/g food had the following distribution of arsenic concentrations: pyloric caeca > liver > muscle > kidney > intestine > stomach. Lake trout, on the other hand, fed the same diet had arsenic distributions: pyloric caeca = liver > kidney > intestine > muscle > stomach (Pedlar et al. 2002a). The somewhat different distribution of arsenic in these two fish species was thought to be due to differences in arsenic absorption through the GIT. Dietary exposure to arsenic has been shown to disrupt the mucosal lining of the GIT of lake whitefish, which causes mucosal sloughing followed by increased mucosal production (Pedlar et al. 2002a, b). The continuous production and shedding of mucus layers in the GIT may substantially reduce the absorption of arsenic (Pedlar et al. 2002a). However, mucosal sloughing of the GIT was not observed in lake trout (Pedlar et al. 2002a,b). Walleye has a similar GIT to lake trout (Scott and Crossman 1998), which may explain the distribution of total arsenic observed in this study (GIT ≈ liver > muscle).

The HPLC-ICP-MS method was capable of identifying simultaneously the five most common arsenic species (As(III), As(V), DMA, MMA, AsB; Xie et al. 2002). The data suggest that fishes from the study area have majority of arsenic in molecular forms that are different from the five most common species. The only exception was the liver of northern pike, which contained nearly 90% identifiable arsenic species. Likewise, the technique used by Koch (1998) was not able to measure the majority of arsenic in fish muscle, although the concentration of arsenosugars and two unknown species of arsenic were quantified. Regardless, the concentrations of As(III), As(V), DMA, MMA, AsB, and other OAS in the muscle tissue of lake whitefish, northern pike, walleye and white sucker in this study were similar to concentrations previously measured in fish from Back Bay (Koch 1998). Furthermore, it is hypothesized that some of the other OAS species in white sucker and longnose sucker may have been arsenosugars, which were previously measured in this fish species by Koch (1998).

At the time of this study, little was known about the arsenic species that accumulate in freshwater fish, while arsenic speciation in marine fish had been widely studied (US EPA 2003). The majority of arsenic (80 to 90%) found in marine fish is organic arsenic primarily in the form of AsB, AsC, and DMA and the remaining 10% was inorganic arsenic (US EPA 2003). Other organic arsenic species commonly found in minor amounts in marine fish include MMA, and a series of trimethylated arsenic (TMA) compounds and arsenoribosides or arsenosugars (Le 2001; Kirby and Maher 2002; US EPA 2003). Kirby and Maher (2002) determined the concentrations of different arsenic species in eight tissues from three different species of marine fish and found that the majority of arsenic was AsB (59–100%) in all tissues except the intestine. Dimethylarsenic acid was present in smaller amounts (<39% of total arsenic) in most tissues, while trimethylarsine oxide (TMAO), tetramethylarsonium ion, AsC and MMA were present in very low amounts (≤8% of total arsenic, respectively; Kirby and Maher 2002).

It has recently been suggested that the majority of organic arsenic in freshwater fish are dimethylated and trimethylated arsenic species, not AsB or AsC (US EPA 2003). Kaise et al. (1997) found that the major arsenic species present in four different species of freshwater fish were TMA compounds (55.5–78.4% of total arsenic) followed by DMA (0.8.–43.2% of total arsenic). Slejkovec et al. (2004) investigated nine different freshwater fish species belonging to four different families and found the major organic arsenic species in salmonids was AsB (92–100% of extractable arsenic) and in burbot (*Lota lota*) was DMA (75% of extractable arsenic). However, it is also noted DMA and TMAO were not detected or detected at very low concentrations (≤32 and 4.4 ng/g, respectively) and most of the arsenic species in silurids and cyprinids were unidentified (Slejkovec et al. 2004). Recent investigations on four different freshwater fish species, including pike, suggest that 10 to 35% of the arsenic in the muscle are tetramethylated arsenic compounds, and only a

small fraction (<7%) is trimethylated arsenic oxide (Zheng and Hintelmann 2004).

The accumulation and distribution of organic arsenicals in different tissues and different freshwater fish species may be related to differences in exposure route (water only versus food chain transfer), type of exposure and trophic status of fish (Kuroiwa et al. 1994; Suhendrayatna et al. 2002a, b; Zheng and Hintelmann 2004). In water only exposure, the freshwater fish *Oryzias latipes* mostly accumulated MMA, but in food chain experiments *O. latipes* accumulated mostly trimethylated arsenic species (Kuroiwa et al. 1994). Aqueous exposure to As(III) and As(V) resulted in a smaller proportion of methylated arsenic species, whereas aqueous exposure to MMA and DMA resulted in a greater proportion of the methylated arsenic species, including trimethylarsenic compounds (Suhendrayatna et al. 2002a, b). Zheng and Hintelmann (2004) found that 25.3% of the identified arsenic in the muscle of *E. lucius* was AsB and 46.0% was DMA. In yellow perch (*Perca flavescens*) and pumpkinseed sunfish (*Lepomis gibbosus*), which are lower trophic level fish than pike, AsB and DMA were less than 6.0 and 3.9%, respectively, of the total arsenic identified in muscle (Zheng and Hintelmann 2004).

In the present study, the low body burdens (<8%) of inorganic arsenic in muscle and GIT suggest that fish from Back Bay accumulate arsenic largely through the food chain rather than the water. It is likely that the higher concentrations of inorganic arsenic in the liver and GIT of white sucker and longnose sucker compared to the other species of fish are due to trophic status. The bottom feeding behavior of sucker may expose them to higher concentrations of inorganic arsenic found in the sediment of Back Bay. In Back Bay, near the outlet of Baker Creek, the concentrations of As(III) in sediment and pore water have previously been measured at 19.1 mg/kg and 0.296 mg/l, respectively, which is considerably higher than concentrations in the surface water (<0.0025 mg/l; Koch 1998).

Conclusion

Ideally, more research could be done to determine the concentration and type of arsenic species in fish tissues from Back Bay. Unfortunately, the analytical method used in this study was not able to quantify other tri- and tetra-methylated arsenic species. Future research should focus on determining the concentration and speciation of unidentified arsenic species, as well as determining if the mono- and dimethylated forms of arsenic are in the trivalent or pentavalent form. Regardless, it is believed that this study has generated sufficient data on the concentration of key arsenic species in fish from Back Bay to inform a human health risk assessment on consumption of locally caught fish.

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